Nanoproducts in Medical Application

Risks at the Cellular Level

Prof. Dr. Ing. Jörg Vienken
BioSciences, Fresenius Medical Care
Bad Homburg

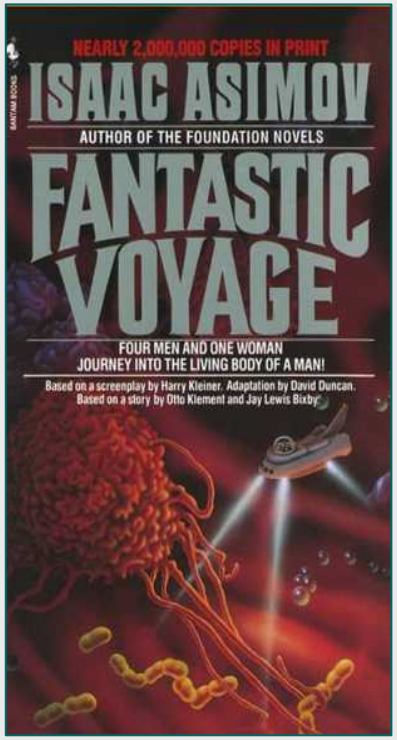
Nanoproducts in Medical Application

Risks at the Cellular Level

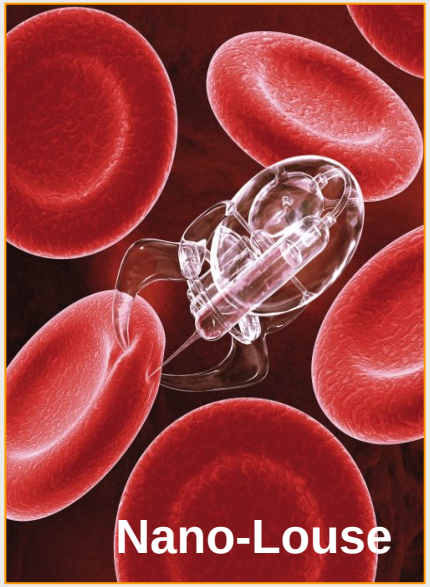
- **Nano-compounds in medical devices**
  General observations
- **Possible mechanisms of cellular interaction**
- **Observed effects of nano-compounds at the cellular level**
- **Conclusion**

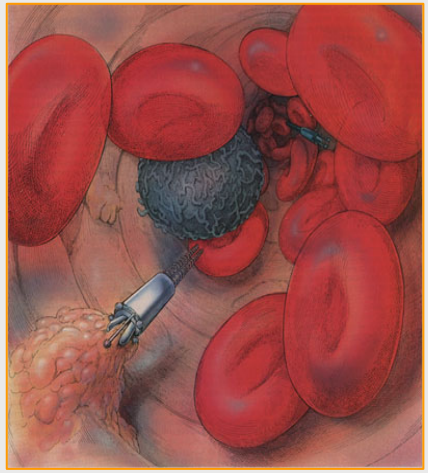
Nano - Bio – Medicine - Technology

What is realistic?



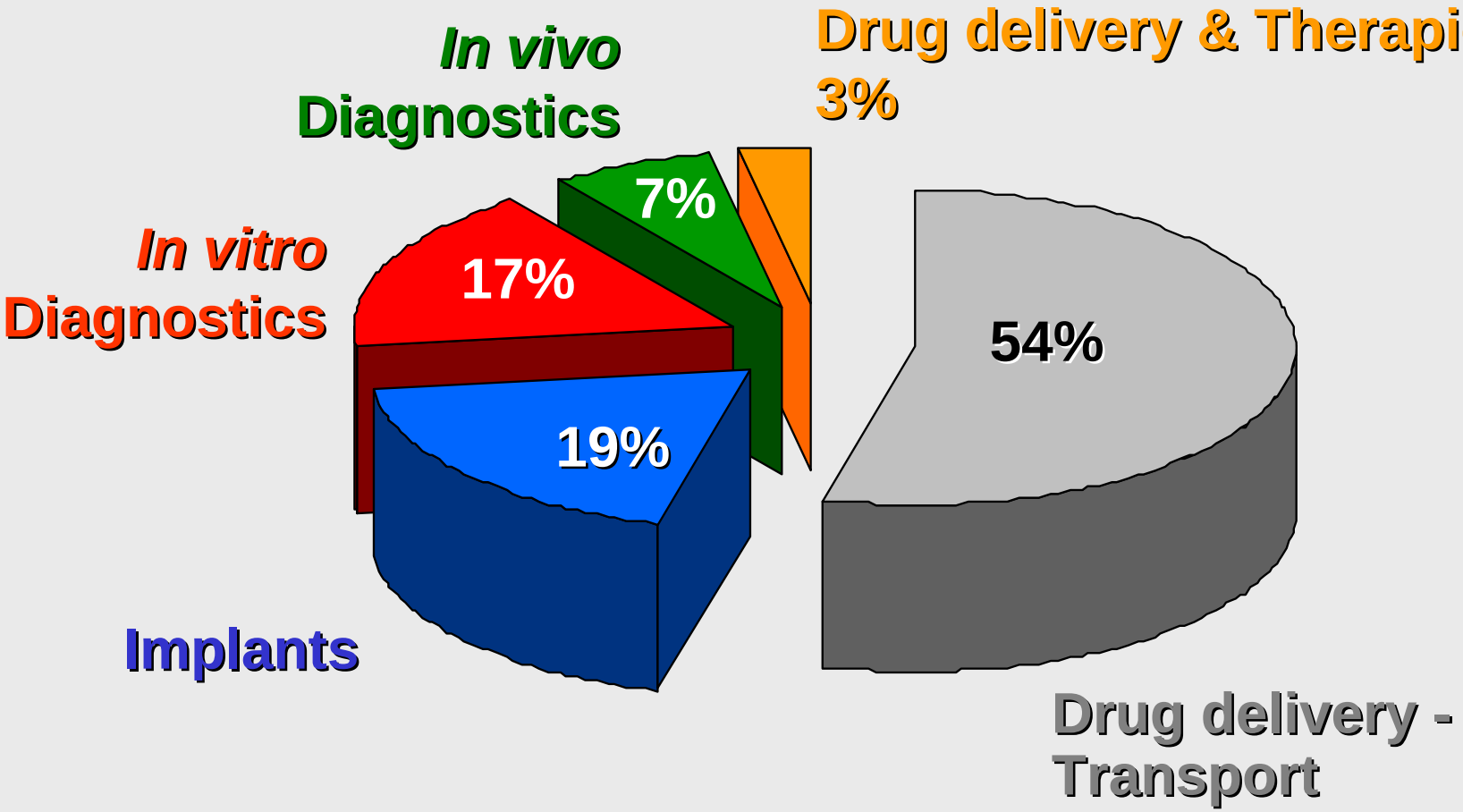
Stern 1999





Nano-Hoover against fat

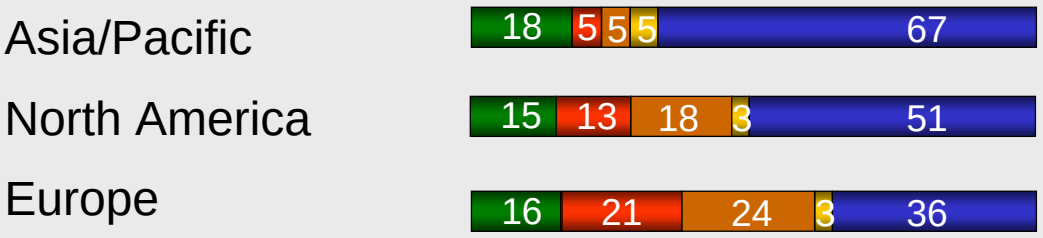
MedTech & Nanotechnology - Activities - World 2005 -



Re: VDI Technologiezentrum GmbH 2005

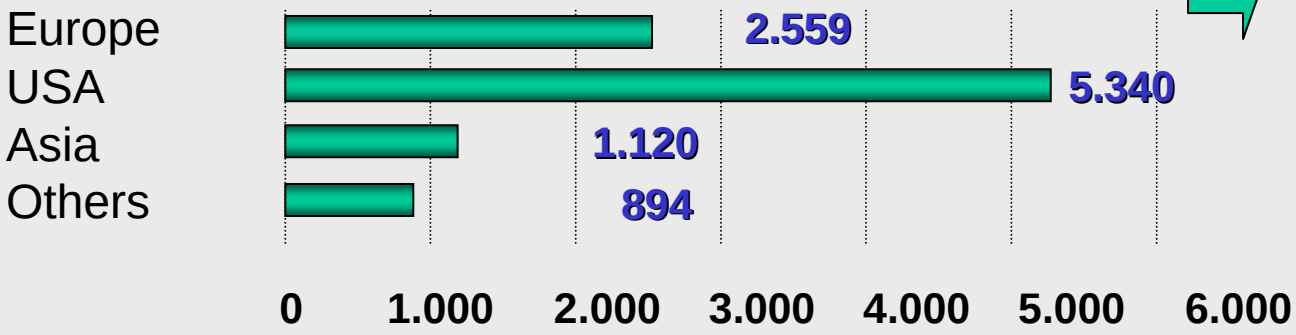
Nanotechnology in Medical Application 2007

Areas of Nanomedical Application in % of Products



Analyt, Material & Instruments
Diagnostics
Med. Material & Implantate
Therapeutics
Drug-delivery systems

Patents (cumulative until 2004)



308 Comp.in Life Science
Inflow Capital:
534 Mio € worldwide
210 Mio € by VC

Re: N Dunzweiler, Ernest & Young
CHEManager, 18/2007, p.3

Nanoproducts in Medical Application

Risks at the Cellular Level

- **Nano-compounds in medical devices**
  General observations
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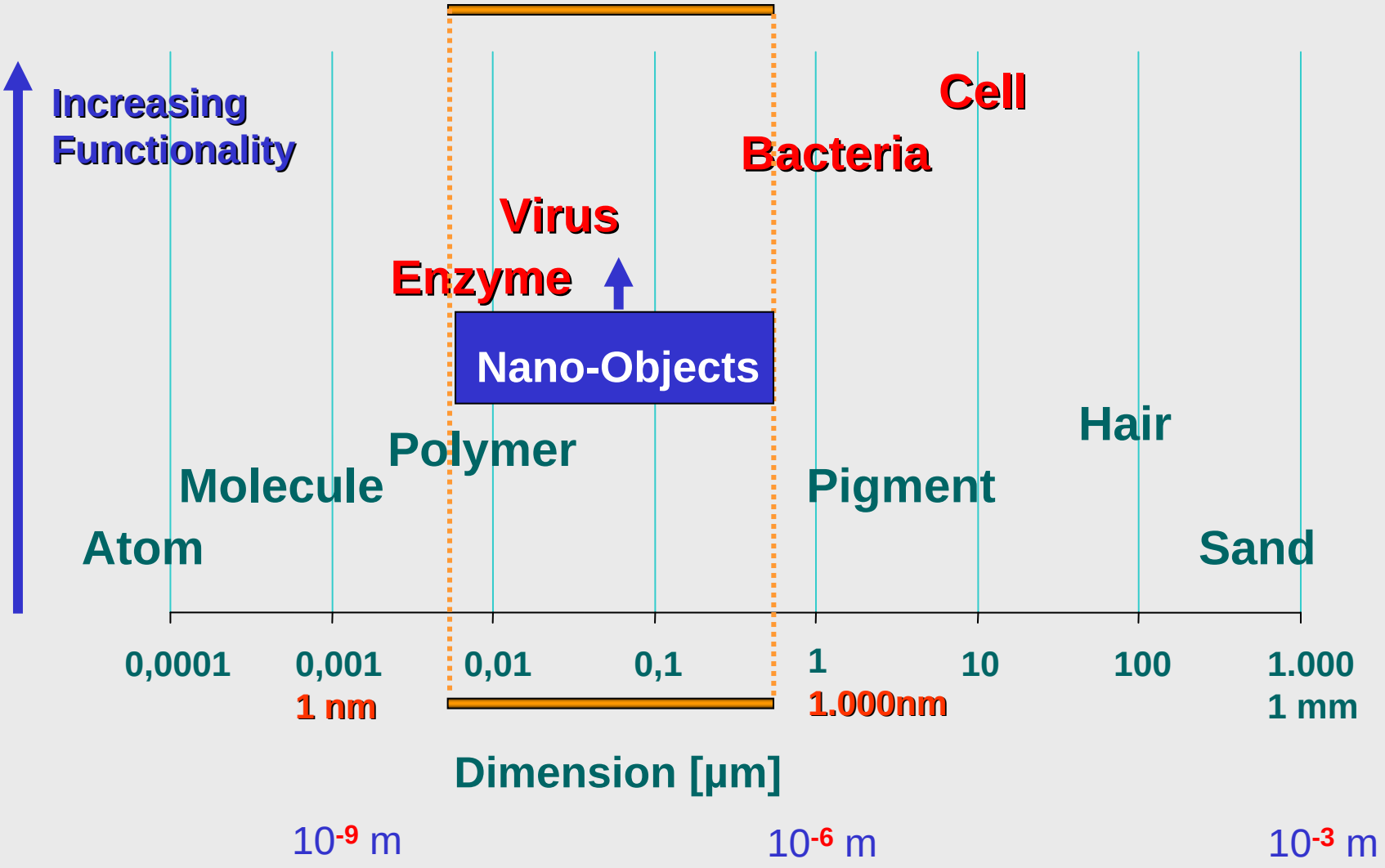
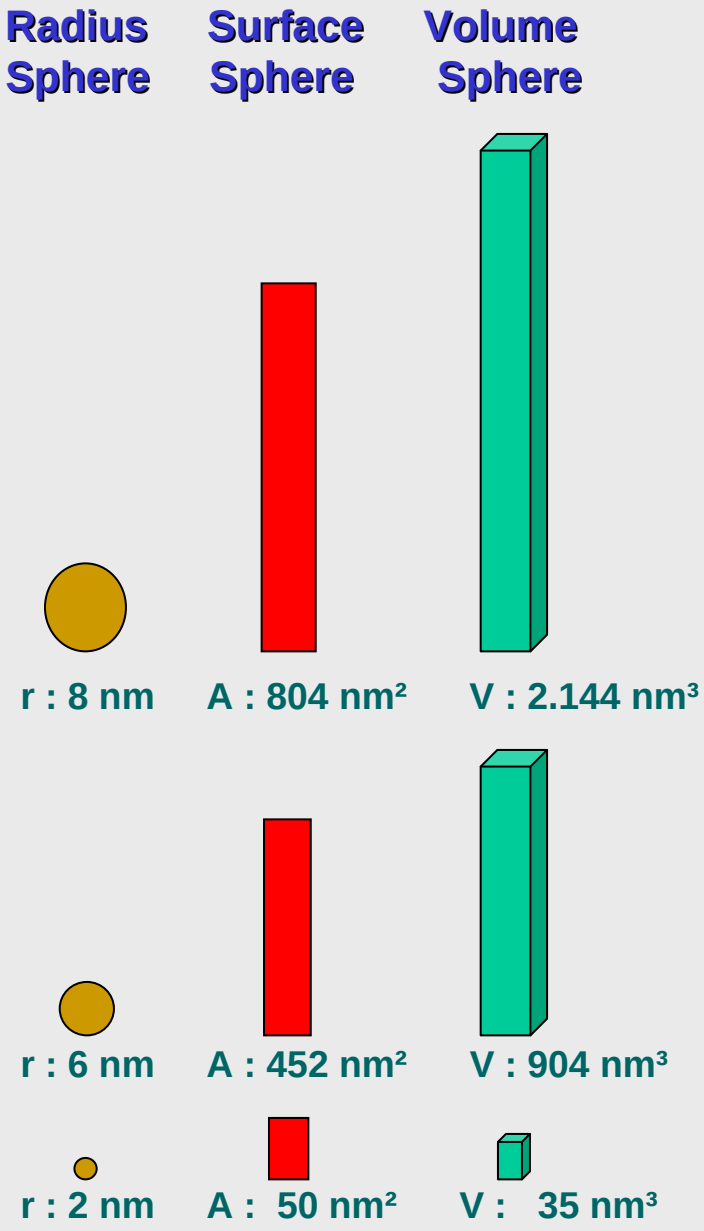
To be kept in mind:
The nanoscale – A biological scale



Nanoparticles and Dimensions



With decreasing radius volume values drop faster than area values!

Radius dependency: r^3 *vs* r^2

Nanoeffects are Surface-associated effects

Nanoscaled Products in MedTech

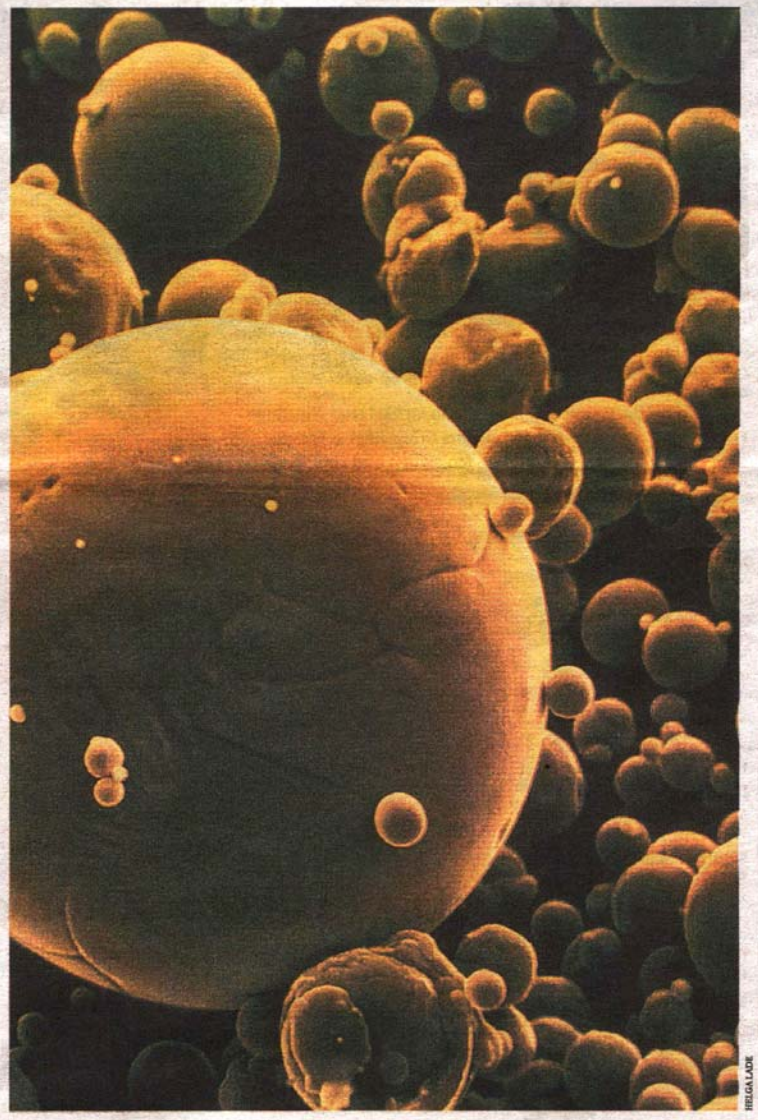
Does it hold true that…

The leaner the meaner?

(Je kleiner, je gemeiner?)

Frankfurter Rundschau

vom 22.10.2009



Gefährliche Winzlinge

Nanotechnologie steckt in Socken und Sonnencremes. Manche Partikel schaden der Gesundheit.

Schärfere Gesetze zum Schutz der Verbraucher fordert jetzt das Umweltbundesamt. **Seiten 2/3**

Expectations and Risk Assessement of Nanotechnology not in Line!

COMMENTARY

Scientists worry about some risks more than the public

DIETRAM A. SCHEUFELE[1]*, ELIZABETH A. CORLEY[2], SHARON DUNWOODY[3], TSUNG-JEN SHIH[3], ELLIOTT HILLBACK[3] AND DAVID H. GUSTON[4]

are in [1]the Department of Life Sciences Communication, University of Wisconsin–Madison, 440 Henry Mall, Madison, Wisconsin 53706, USA; [2]the School of Public Affairs, Arizona State University, 411 North Central Avenue, Phoenix, Arizona 85004, USA; [3]the School of Journalism & Mass Communication, University of Wisconsin–Madison, 821 University Avenue, Madison, Wisconsin 53706, USA; [4]the Department of Political Science, Arizona State University, PO Box 874401, Tempe, Arizona 85287, USA.
*e-mail: scheufele@wisc.edu

A comparison between two recent national surveys among nanoscientists and the general public in the US shows that, in general, nanoscientists are more optimistic than the public about the potential benefits of nanotechnology. However, for some issues related to the environmental and long-term health impacts of nanotechnology, nanoscientists were significantly more concerned than the public.

Re: Nature Nanotechnology, 2:732-734 (2007)

Soiled: Nanotech's Reputation

Nanotech experts agree that the health risks of nanotechnology need more study, but the British Soil Association, which sets standards for organic farming, isn't waiting for the data. This week the group launched a preemptive "ban" announcing that it would not allow engineered nanoparticles smaller than 200 nanometers in the products that it certifies as organic. "Companies are going to face greater pressure to clarify risks and benefits," says David Rejeski, who directs the Project on Emerging Nanotechnologies of the Woodrow Wilson International Center for Scholars.

–ERIK STOKSTAD

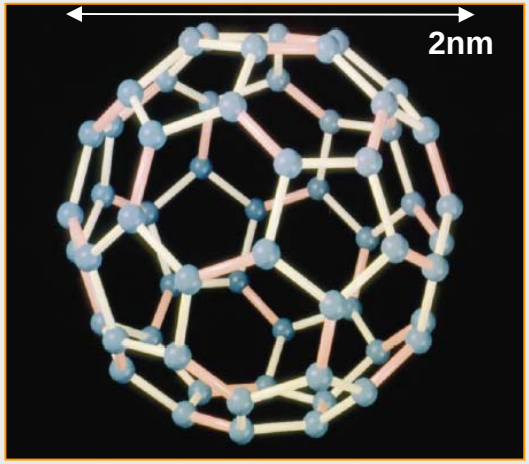
Re: Science, 319:271 (2008)

Toys or Tools?

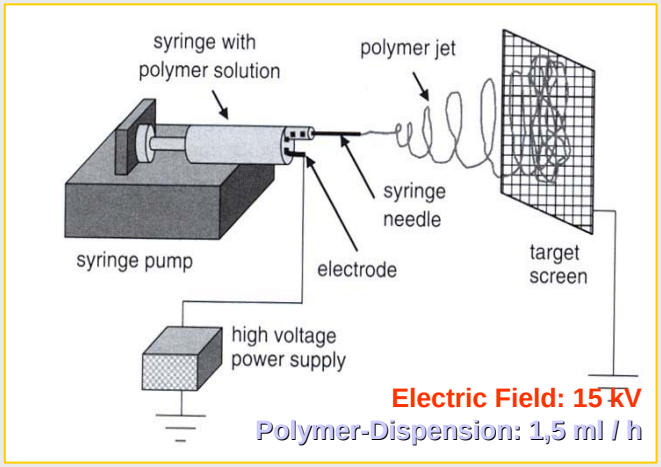
Nanocages
Nanopores

Nanofibres, - tubes
Cantilever

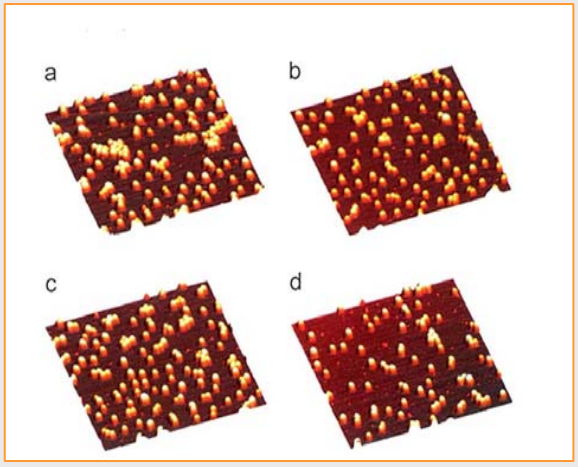
Surfaces, particles
Nanomachines



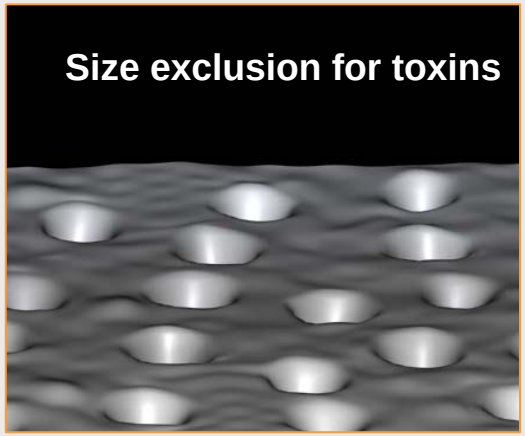
Fulleren cages
B. Fuller (1895-1983)



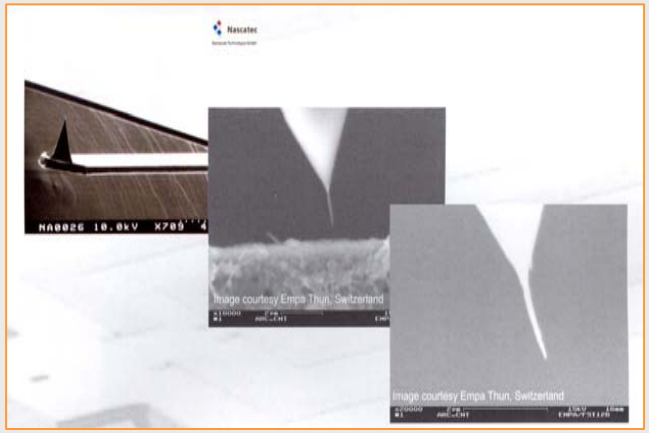
A Badami et al.,
Biomaterials, 27:596-606 (2006)



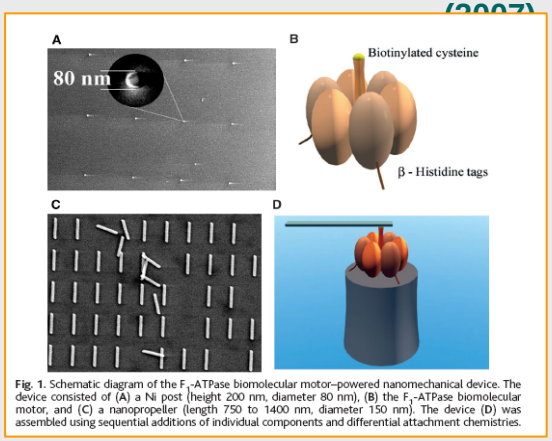
T Kunzler et al.,
Biomaterials, 28:5000-5006 (2007)



Membrane pores
Fresenius Medical Care,
Bad Homburg, Germany 2007



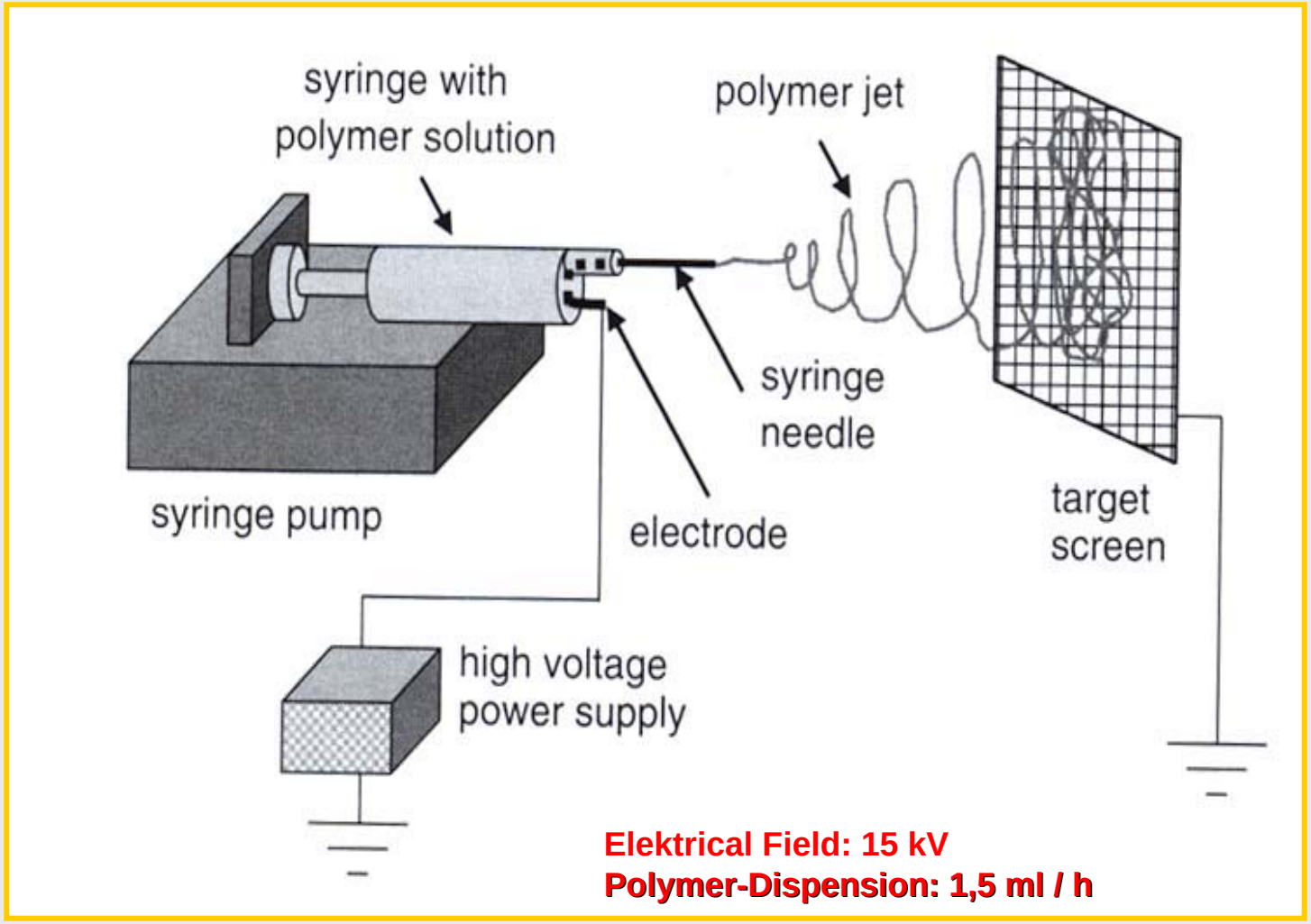
Cantilever
Nascatec,
Stuttgart, Germany 2007



Nanomachines
Science, 290:1555-58 (2000)

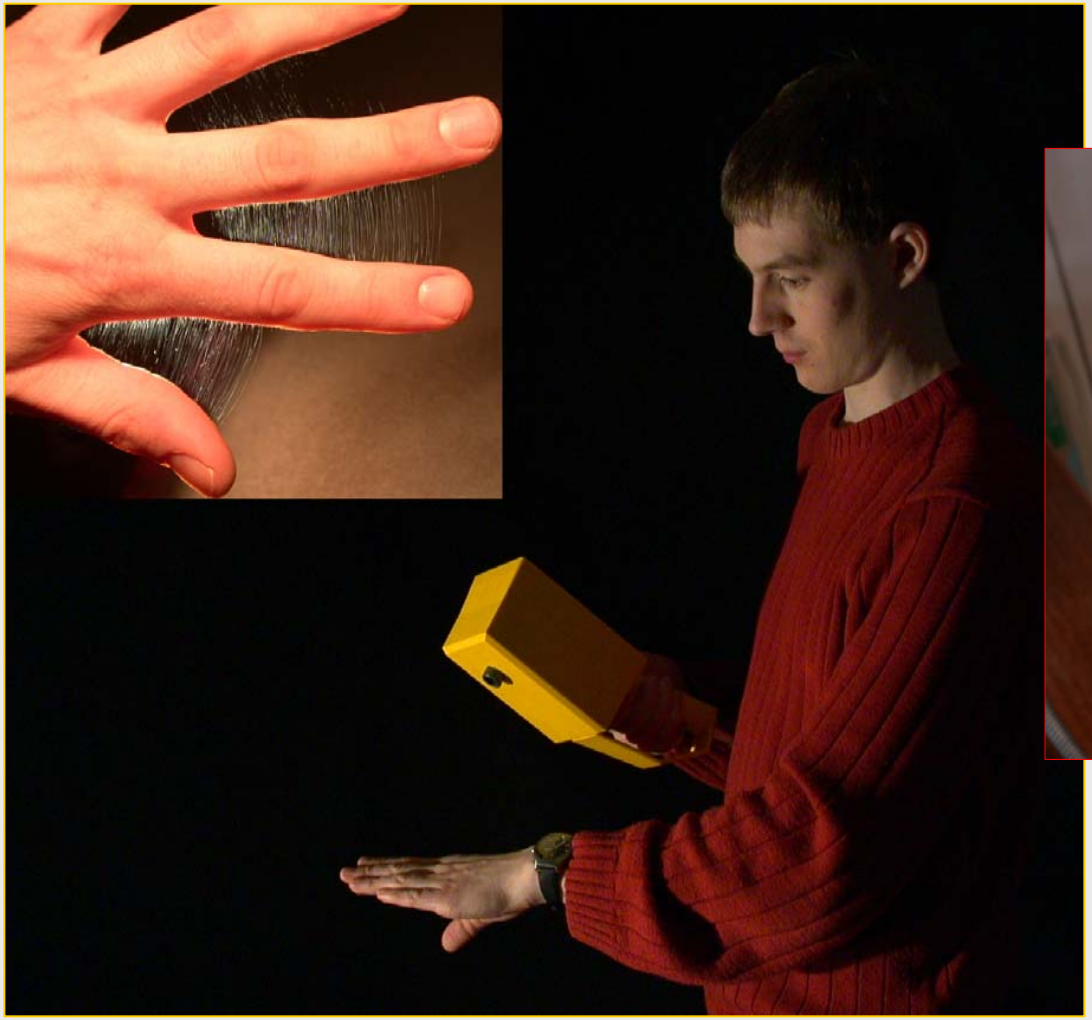
The tools
Nanofibres

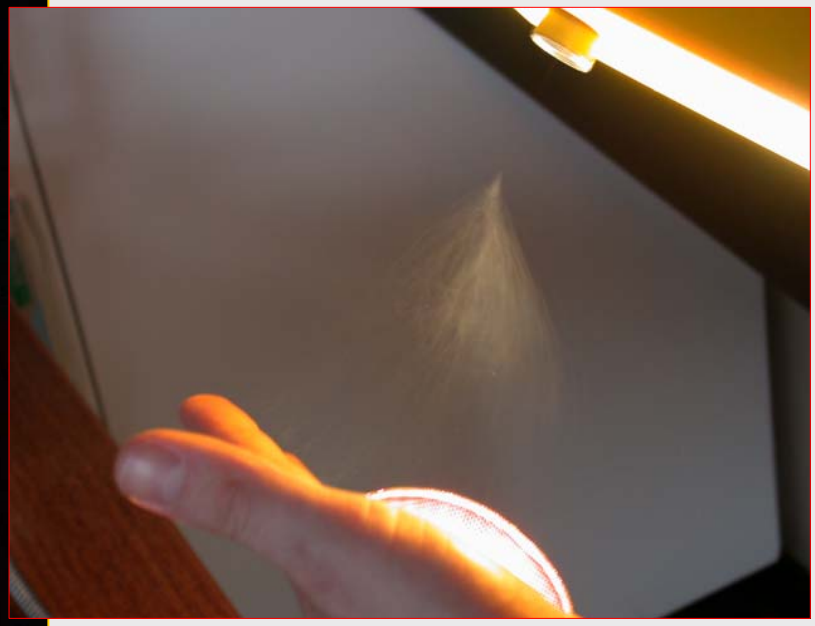
How to produce Nanofibres?
- Elektrospinning – Technology -



Modifiziert nach: A Badami et al., Biomaterials, 27:596-606 (2006)

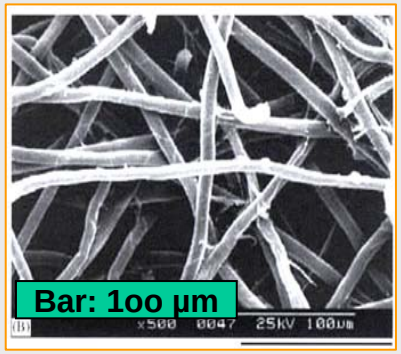
Wearable Electrospinning Device
for wound-healing therapies



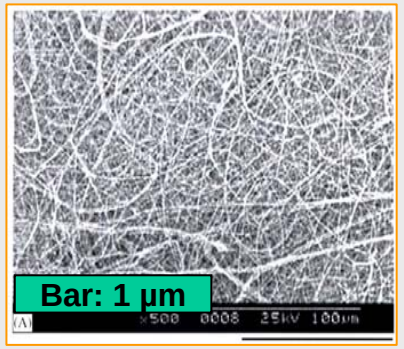


J Wendorf,
Uni Marburg 2005

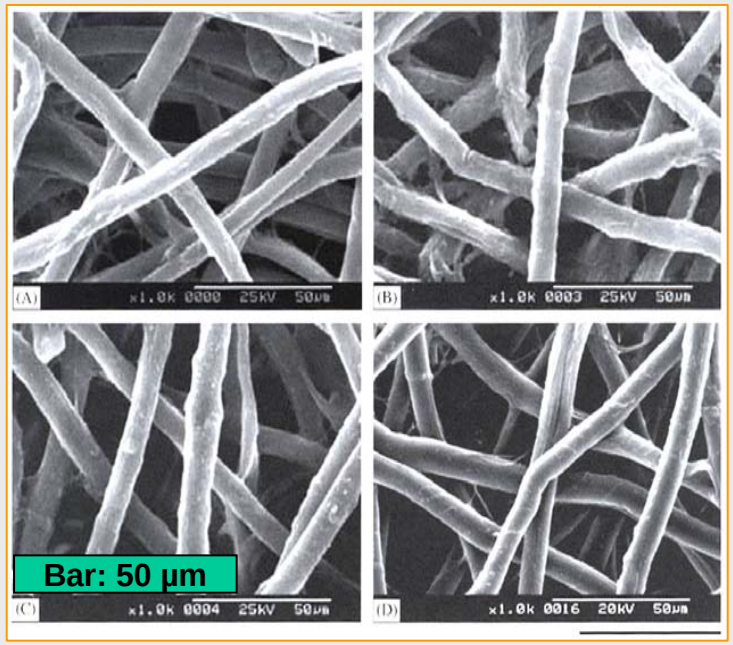
Degradation of Chitinfibres and Chitinnanofibers

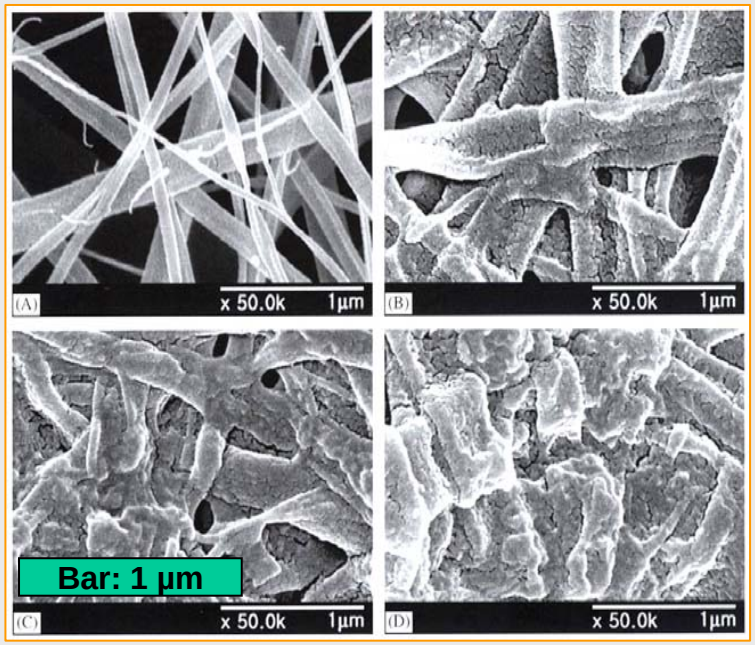


Commercial Chitinfibres



Nanofibers

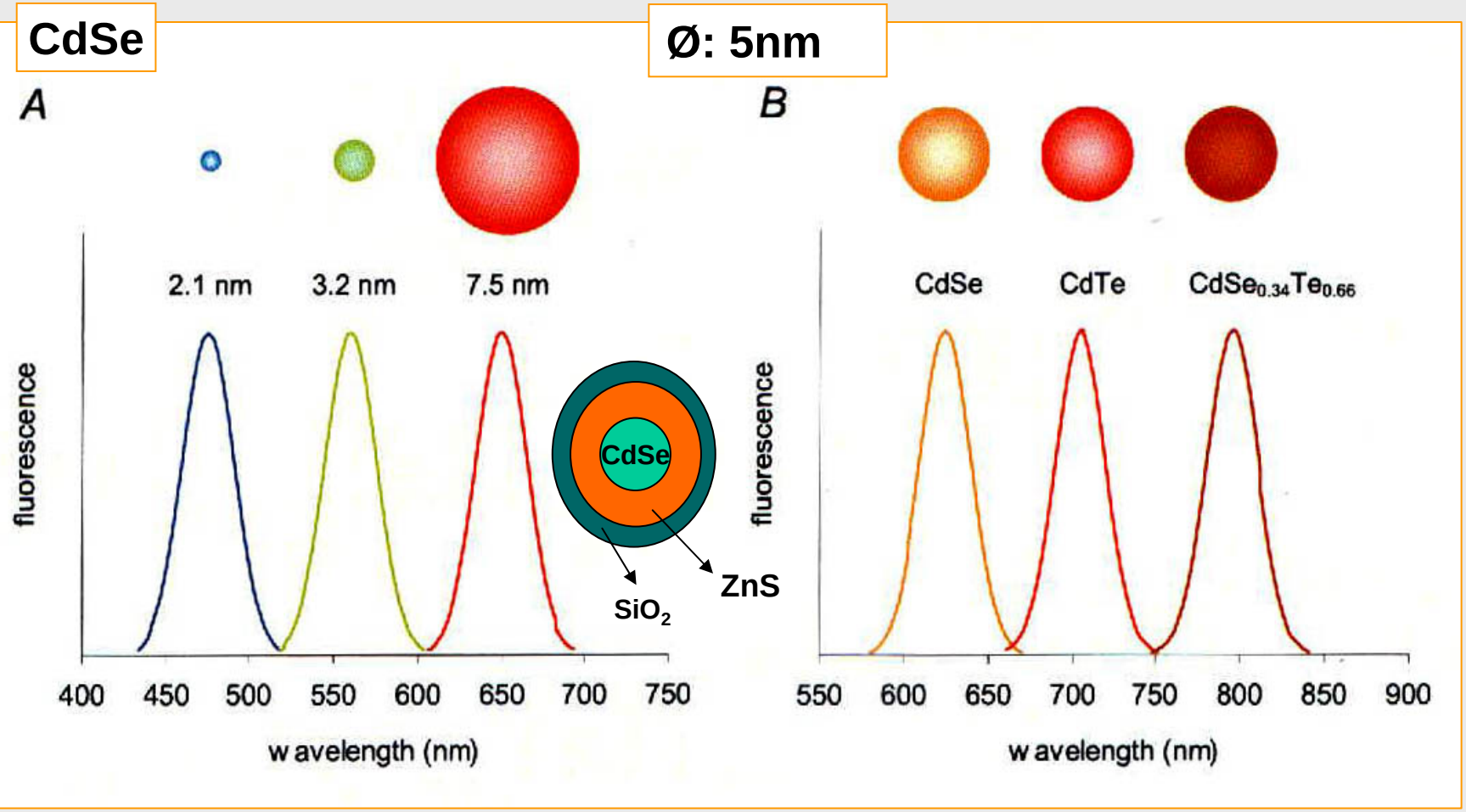




in vitro degradation after
0, 3, 15, 25 days

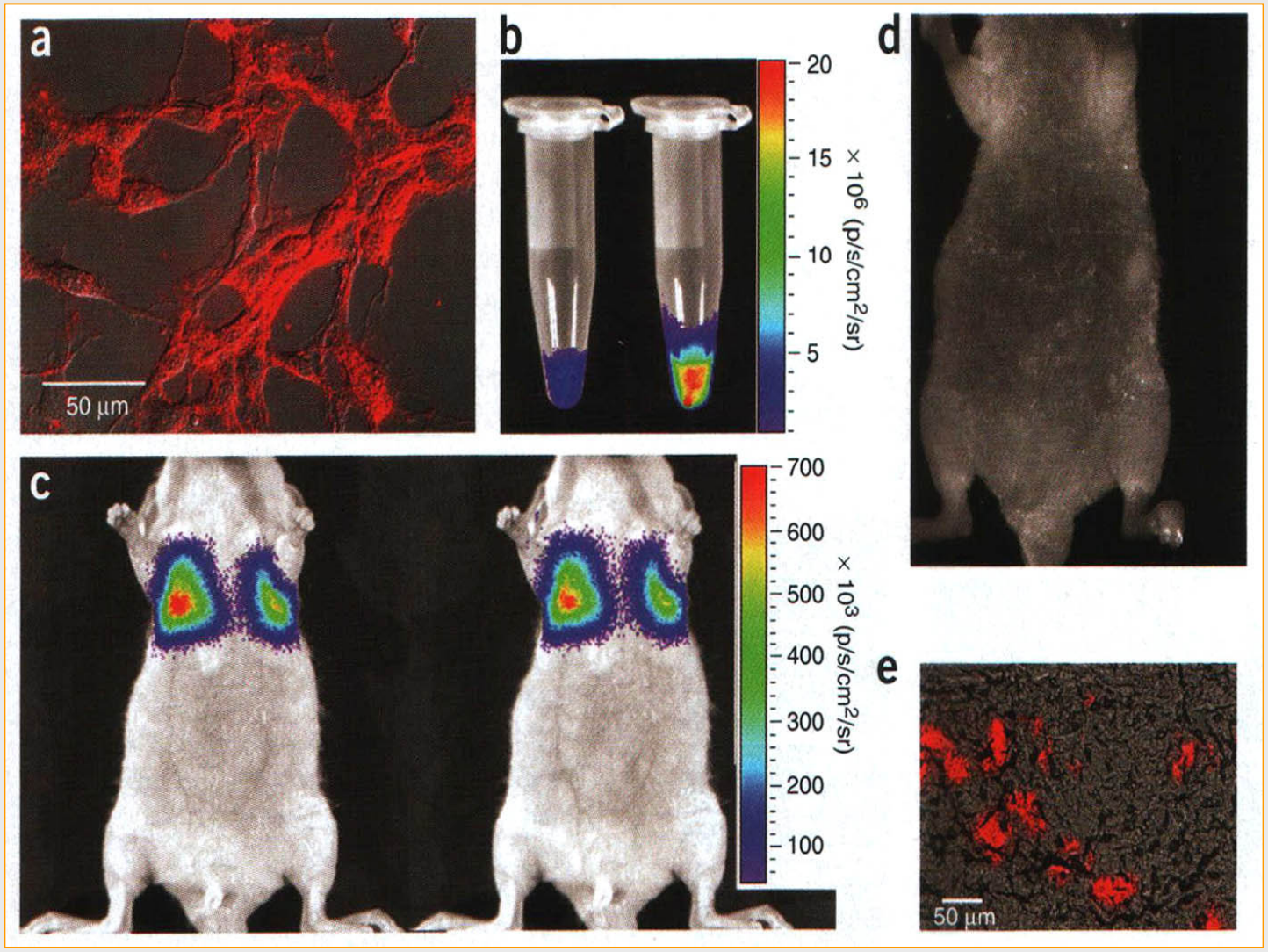
HK Noh et al.,
Biomaterials, 27:3934-3944 (2006)

Application and tools
Quantum dots



B Roszek et al,
RIVM Report 2650001001/2005

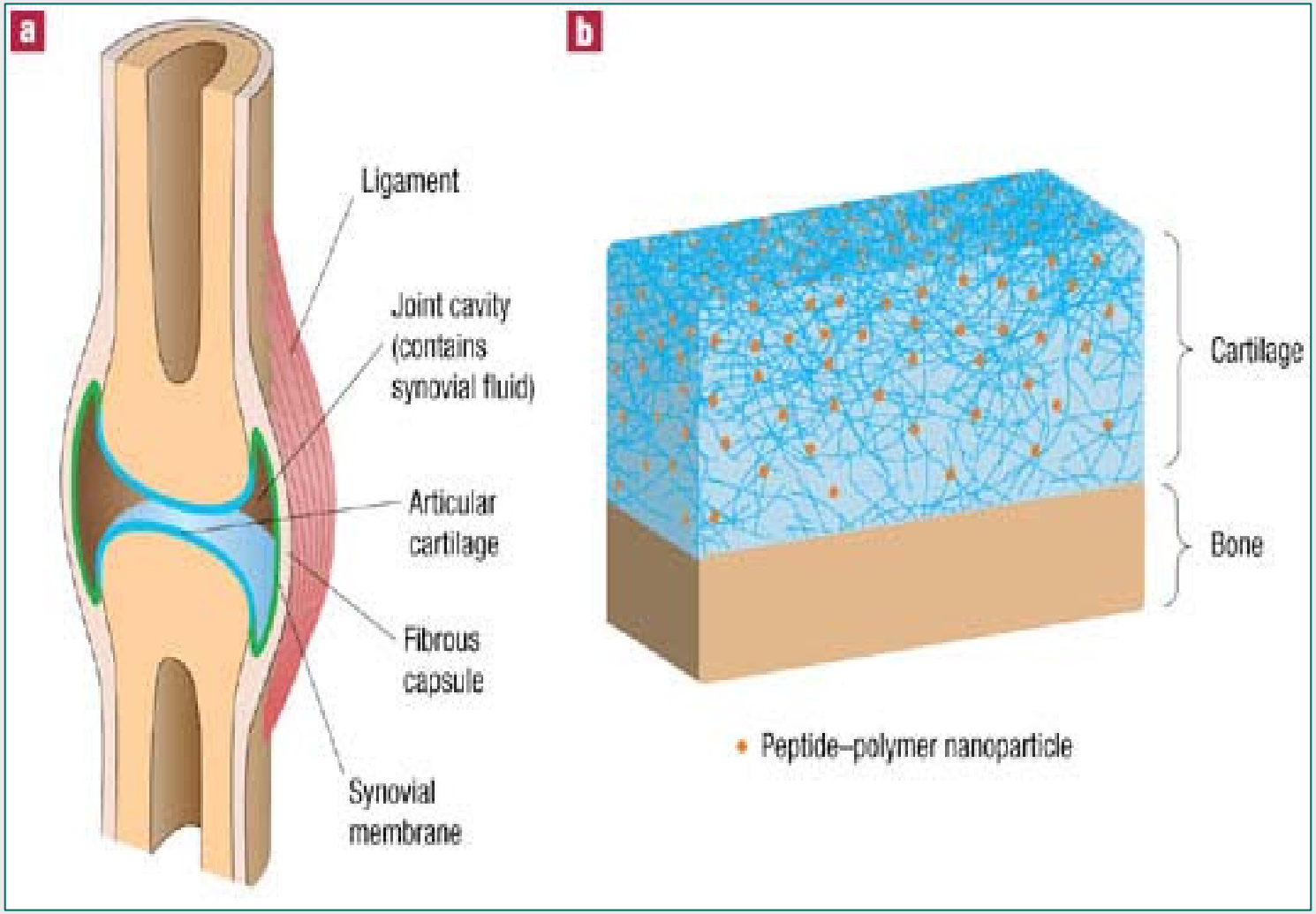
Imaging of BRET-QD´s in Nude Mice
- after Injection of Gliomacells with BRET-QDs -



MK So et al.,
Nature Biotech, 24:339-343 (2006)

Applications

Nanoparticles for controlled drug release



Nature Materials 7: 172-174 (2008)

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Risks at the Cellular Level

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- **Possible mechanisms of cellular interaction**
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Blood, the perfect Extraktionmedium
5 - 6 l in humans

Plasma

> 1.300 Components

1. 8% Proteins

Albumin	**60%**
Immunglobulins	**35%**
Fibrinogen	**5%**

2. Water

3 Anorganic Salts

4. Transport compounds

Hormon
Fat
Carbohydrates
Enzymes

Cells

1. Erythrocytes **4-5 x 10^{12} /l**

2. Platelets **200-300 x 10^{9} /l**

3. Leukocytes **6-8 x 10^{9} /l**

> 17 different Types
u. a.Th-, Ts-Cells
B-Cells
Monocytes
Killercells
and: The cellular Internet
(Cytokines for communication)

Biostability

Property of a substance
to remain unchanged
in a given biological environment.

INTERNATIONAL STANDARD

ISO 10993-1

Fourth edition
2009-10-15

Biological evaluation of medical devices —

Part 1:
Evaluation and testing within a risk management process

Évaluation biologique des dispositifs médicaux —

Partie 1: Évaluation et essais au sein d'un processus de gestion du risque

4.3 The following shall be taken into account for their relevance to the overall biological evaluation of the device:

a) the material(s) of manufacture;

b) intended additives, process contaminants and residues (see ISO 10993-7 for ethylene oxide residues);

c) leachable substances (see ISO 10993-17);

d) degradation products (see ISO 10993-9, for general principles and 10993-13, 10993-14 and 10993-15 for degradation products from polymers, ceramics and metals, respectively);

e) other components and their interactions in the final product;

f) the performance and characteristics of the final product;

g) physical characteristics of the final product, including but not limited to, porosity, particle size, shape and surface morphology.

Identification of material chemical constituents and consideration of chemical characterization (see ISO 10993-18) shall precede any biological testing (see Figure 1).

Cave with Extractables and Leachables, why?

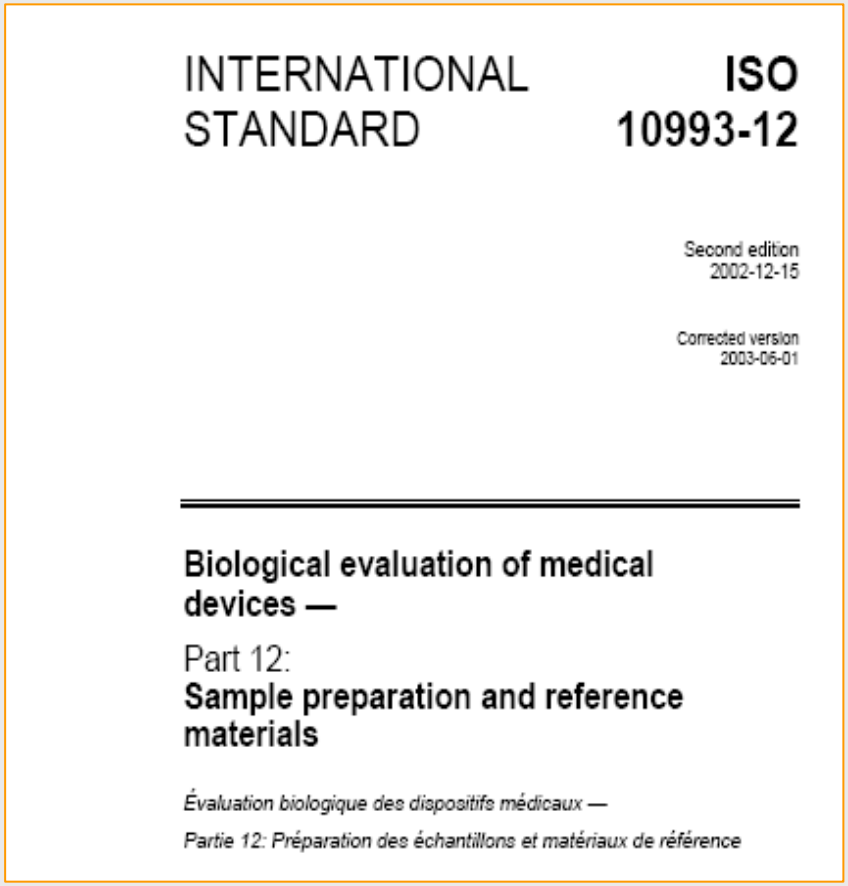
Example: Chronic Dialysis Patients in Japan

Therapy duration Dialysis patients	Total	%
Shorter than 5 years	133.627	49,0
5 - 9 years	66.617	25.1
10 - 14 years	33.696	12.3
15 - 19 years	17.265	6.3
20 - 24 years	**9.815**	**3.6**
25 years and longer	**10.017**	**3.7**
Rate per Million population	2.219 Patients	
Longest Dialysis treatment	40 years, 8 Months	

>50%

Total number of Dialysis Patients in Japan 2008 : 273.237

S Nakai et al., Ther Apher Dial, 14:505- 540 (2010)


INTERNATIONAL STANDARD

ISO 10993-12

Second edition
2002-12-15

Corrected version
2003-06-01

Biological evaluation of medical devices —

Part 12:
Sample preparation and reference materials

Évaluation biologique des dispositifs médicaux —

Partie 12: Préparation des échantillons et matériaux de référence

Extraction: media and procedures

The solvents selected as extractants shall

a) be suitable for use in the specific biological test systems.

b) simulate the extraction which occurs during clinical use of the device and/or

c) maximize the amount of extract

Extraction media:

1. **Polar solutions:** **Water, Saline (0.9%) Culture media without serum**
2. **Unpolar solutions:** **Vegetable oil (e.g. Sesamoil)**
3. **Additionally:** **Ethanol/Water (17,5% w/w), PEG 400, DMSO, culture media & Serum**

INTERNATIONAL STANDARD

ISO 10993-17

First edition
2002-12-01

Biological evaluation of medical devices —

Part 17: Establishment of allowable limits for leachable substances

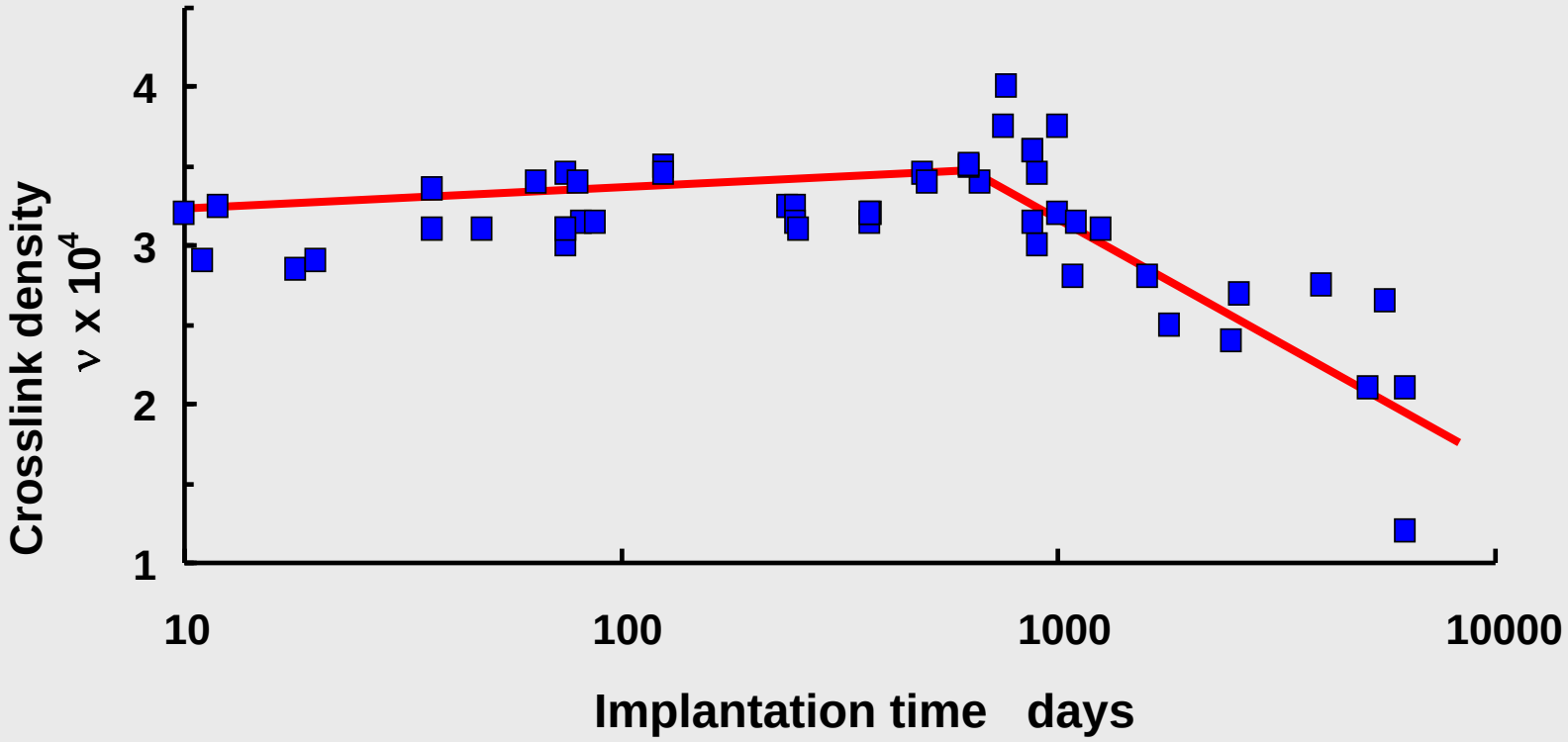
Évaluation biologique des dispositifs médicaux —

Partie 17: Établissement des limites admissibles des substances relargables

Reference number
ISO 10993-17:2002(E)

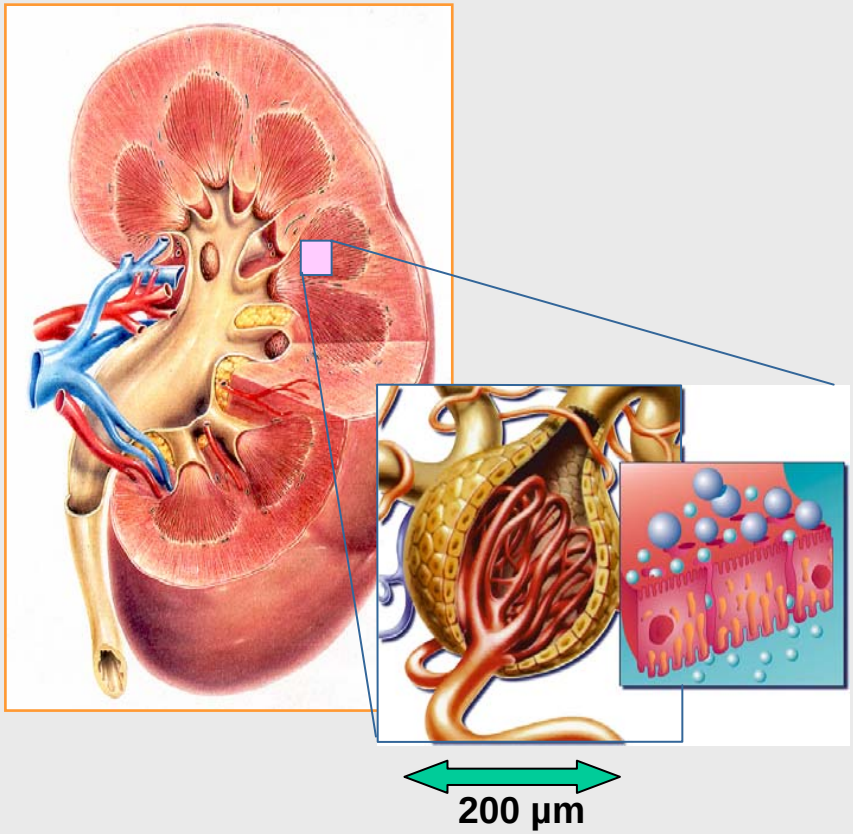
In vivo Polymer degradation

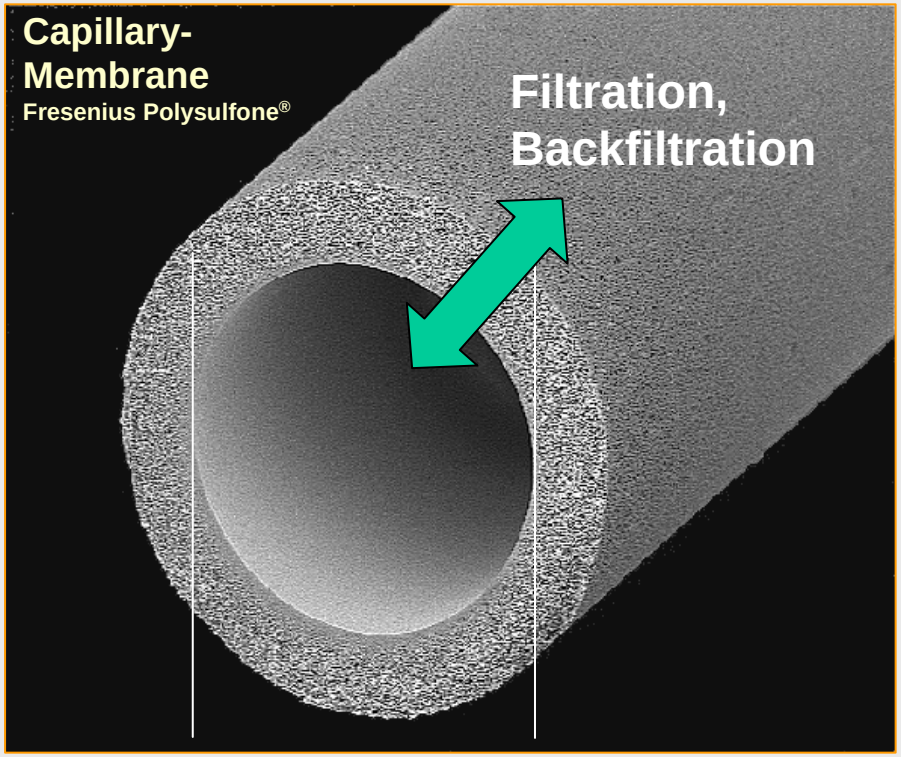
Isolation material from Silicone for the longterm use in Pacemakers



Dolezel et al.,
Biomaterials, 10: 387- 392 (1989)

Current dialysis treatment based on the removal of water and matter!



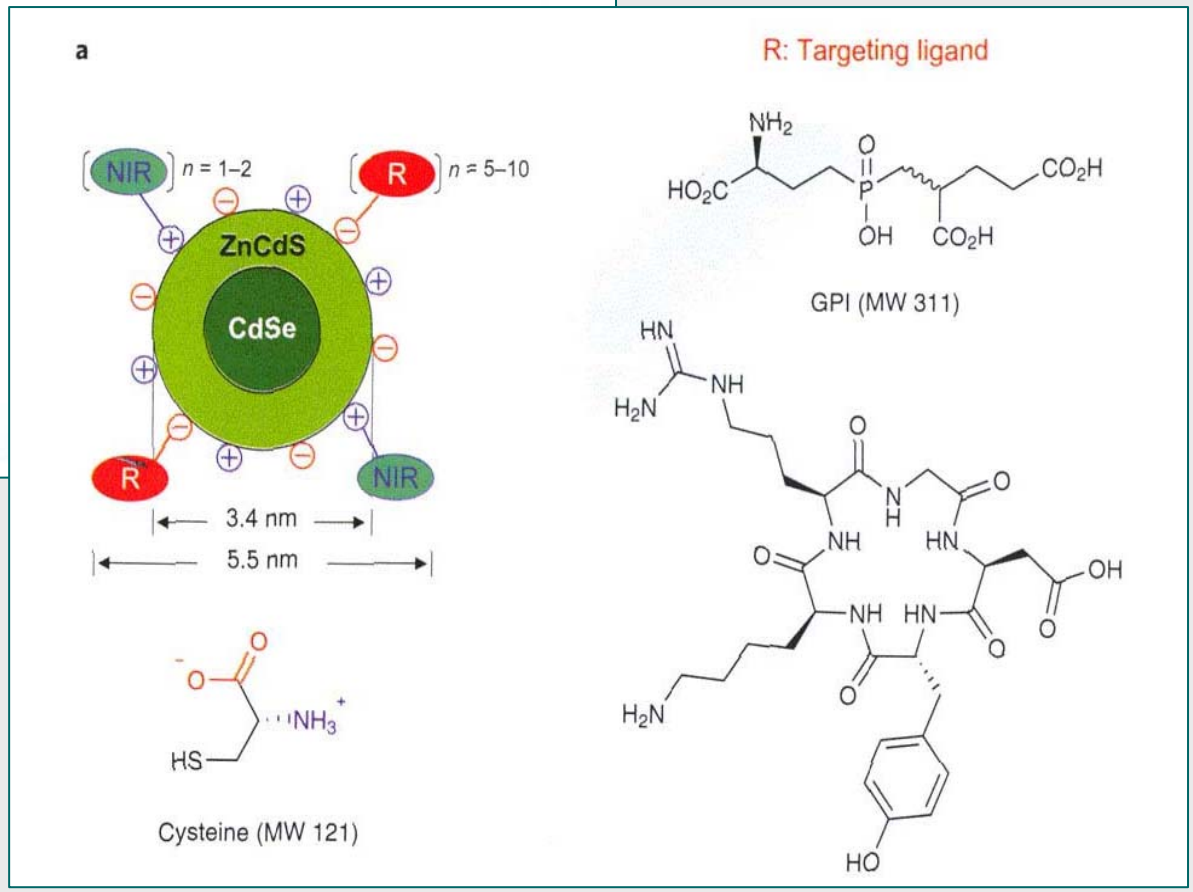


LETTERS
PUBLISHED ONLINE: 1 NOVEMBER 2009 | DOI: 10.1038/NNANO.2009.314
nature nanotechnology

Design considerations for tumour-targeted nanoparticles

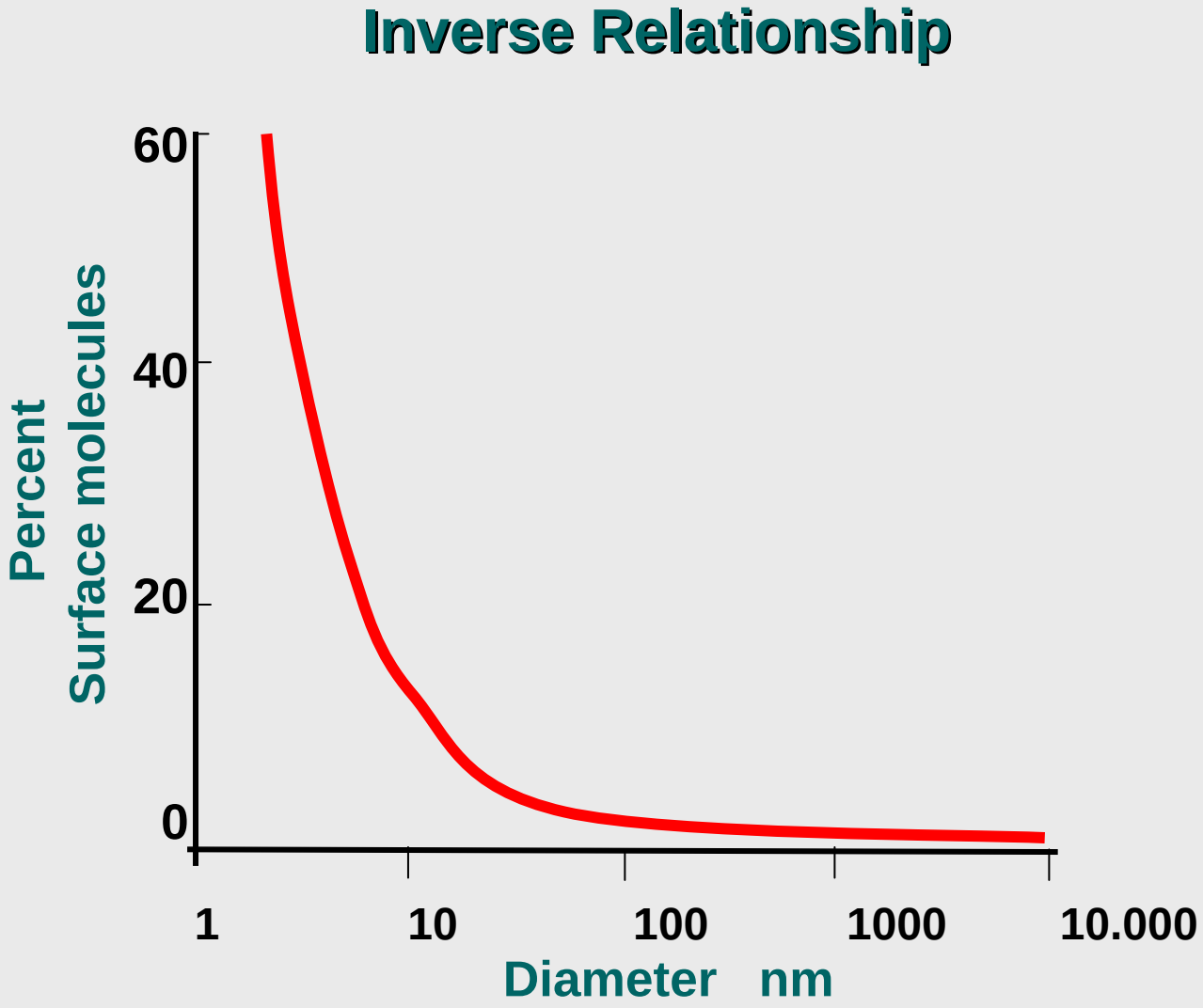
Hak Soo Choi[1], Wenhao Liu[2], Fangbing Liu[1], Khaled Nasr[1], Preeti Misra[1], Moungi G. Bawendi[2] and John V. Frangioni[1,3]*

Inorganic/organic hybrid nanoparticles are potentially useful in biomedicine, but to avoid non-specific background fluorescence and long-term toxicity, they need to be cleared from the body within a reasonable timescale[1]. Previously, we have shown that rigid spherical nanoparticles such as quantum dots can be cleared by the kidneys if they have a hydrodynamic diameter of approximately 5.5 nm and a zwitterionic surface charge[2]. Here, we show that quantum dots functionalized with high-affinity small-molecule ligands that target tumours can also be cleared by the kidneys if their hydrodynamic diameter is less than this value, which sets an upper limit of 5–10 ligands per quantum dot for renal clearance. Animal models of prostate cancer and melanoma show receptor-specific imaging and renal clearance within 4 h post-injection. This study suggests a set of design rules for the clinical translation of targeted nanoparticles that can be eliminated through the kidneys.



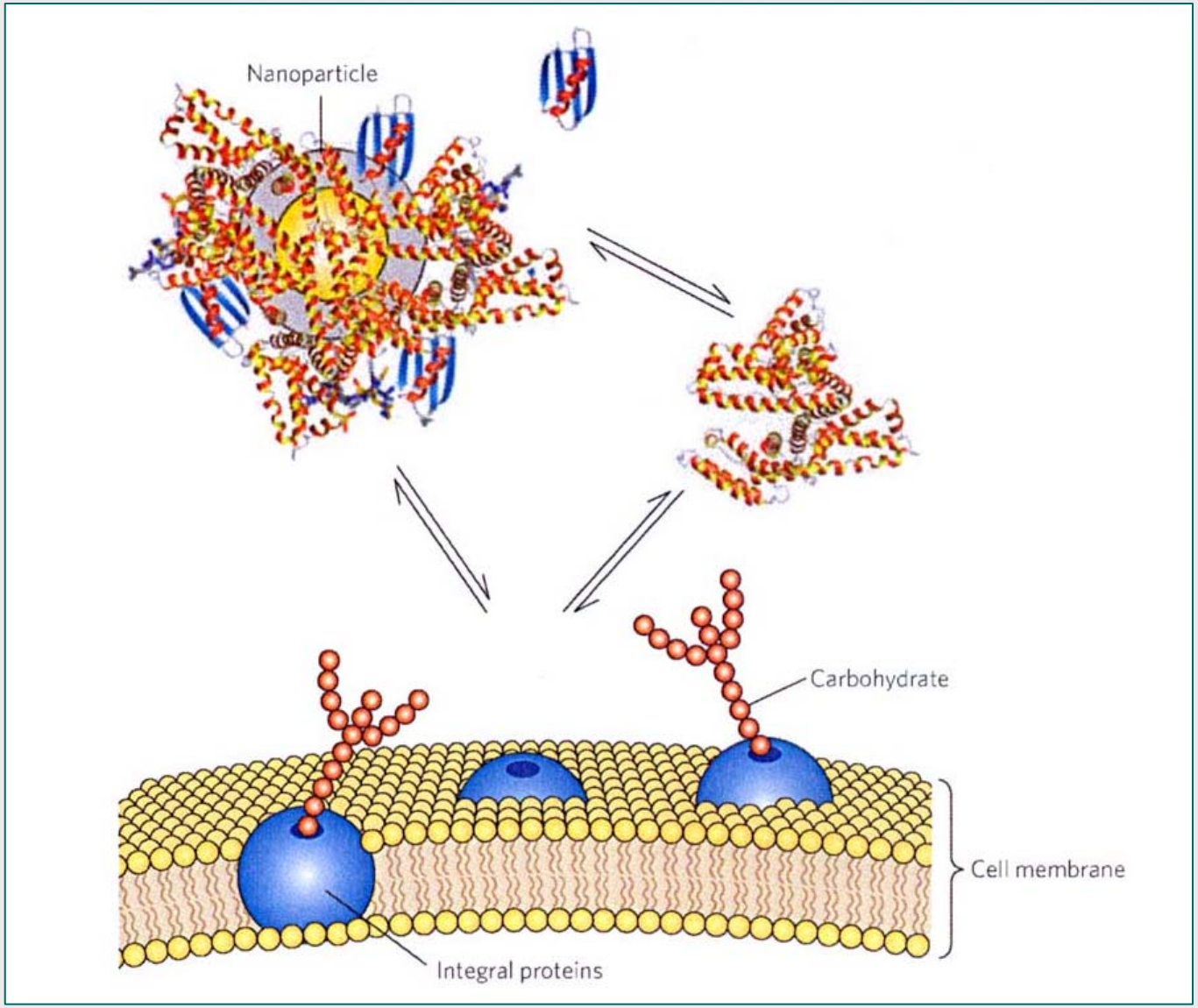
Re:
Nature Nanotechnology,
5:42- 47 (2010)

Particle size and number expressed molecules at a surface



Nach: A Nel et al., Science, 311:622 (2006)

Interaction of Nanoparticles with biological cells determined by protein adsorption



Re: I Lynch
Nature Nanotechn, 4:546-547 (2009)

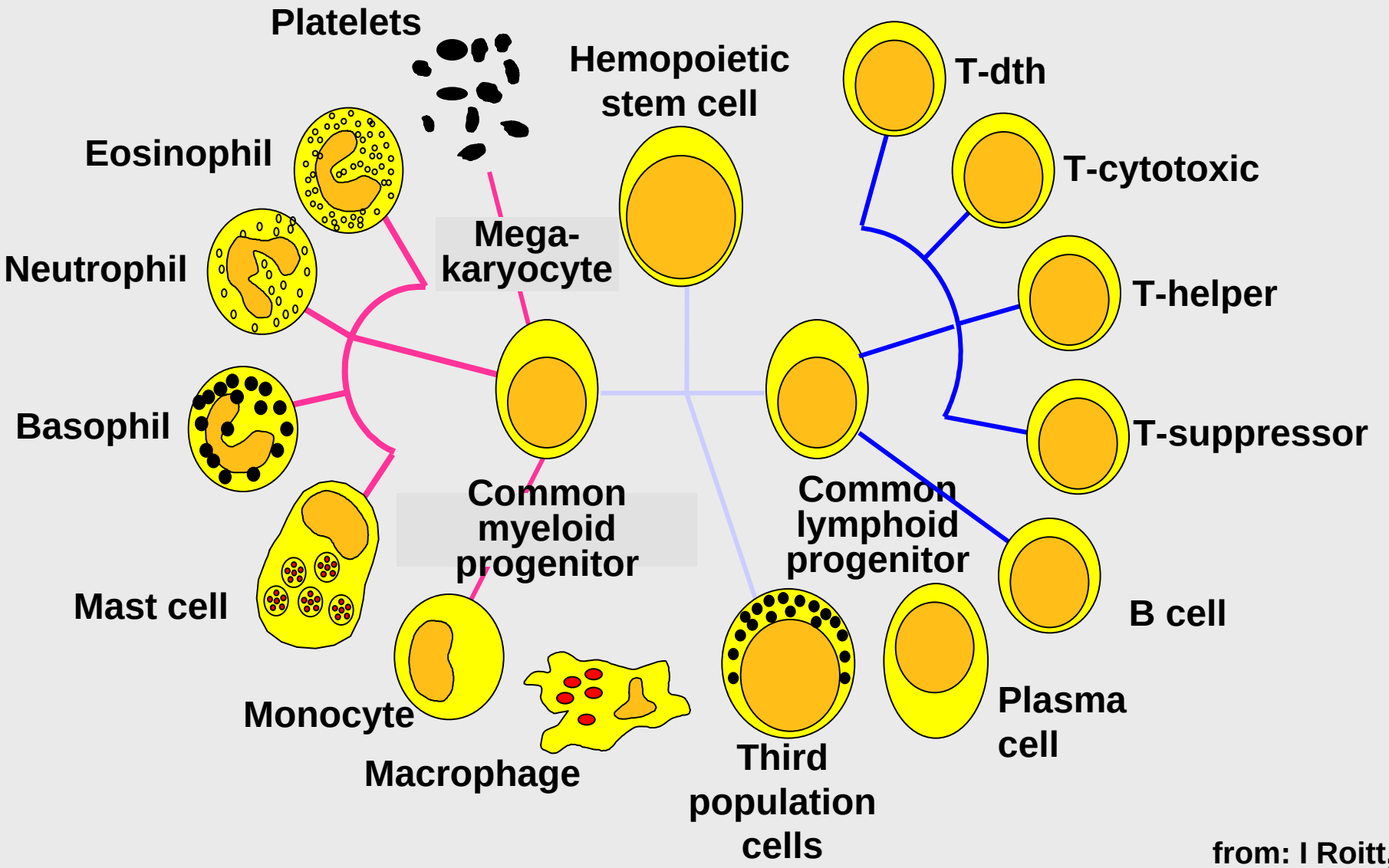
Protein deposition on Biomaterials

Sequence under Flow conditions

	Deposition time [s] a=50%	**Timefactor for formation of boundary layer [s]**
Albumin	0,050	9
Fibrinogen	7,4	13
Factor XII	140,0	8
HMWK	68,0	8
Platelets	260,0	18

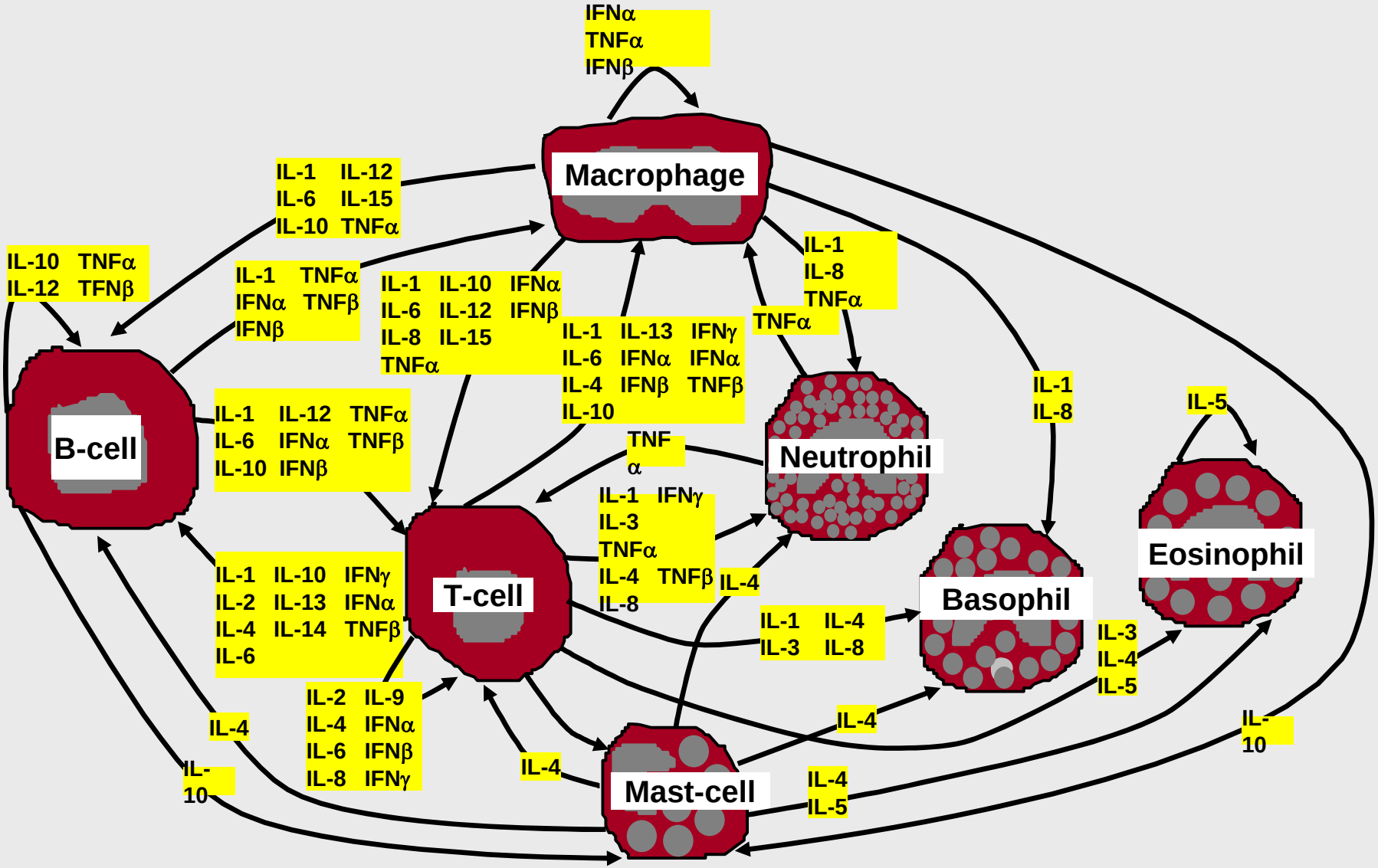
D Basmadjian et al., Biomaterials, 18: 1511-1522 (1997)

Family Tree of Blood Cells



from: I Roitt, Immunology 1985

The Cellular Internet

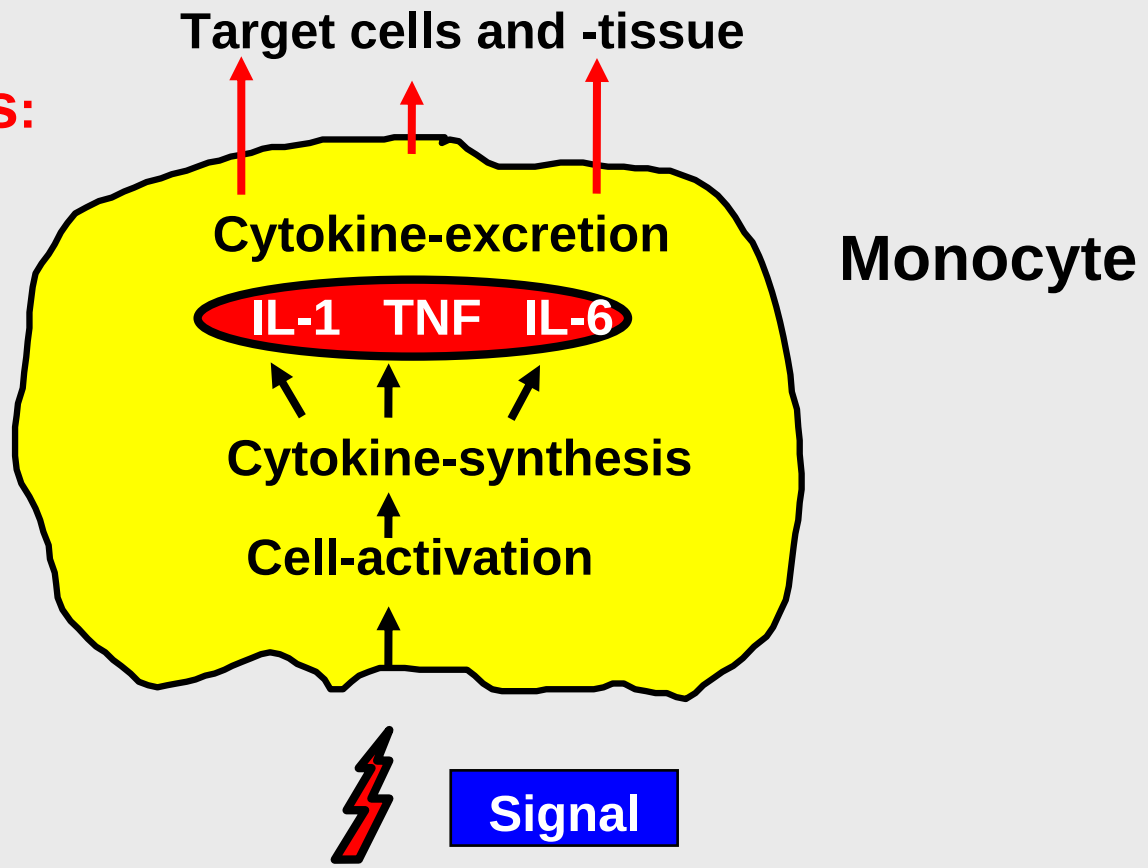


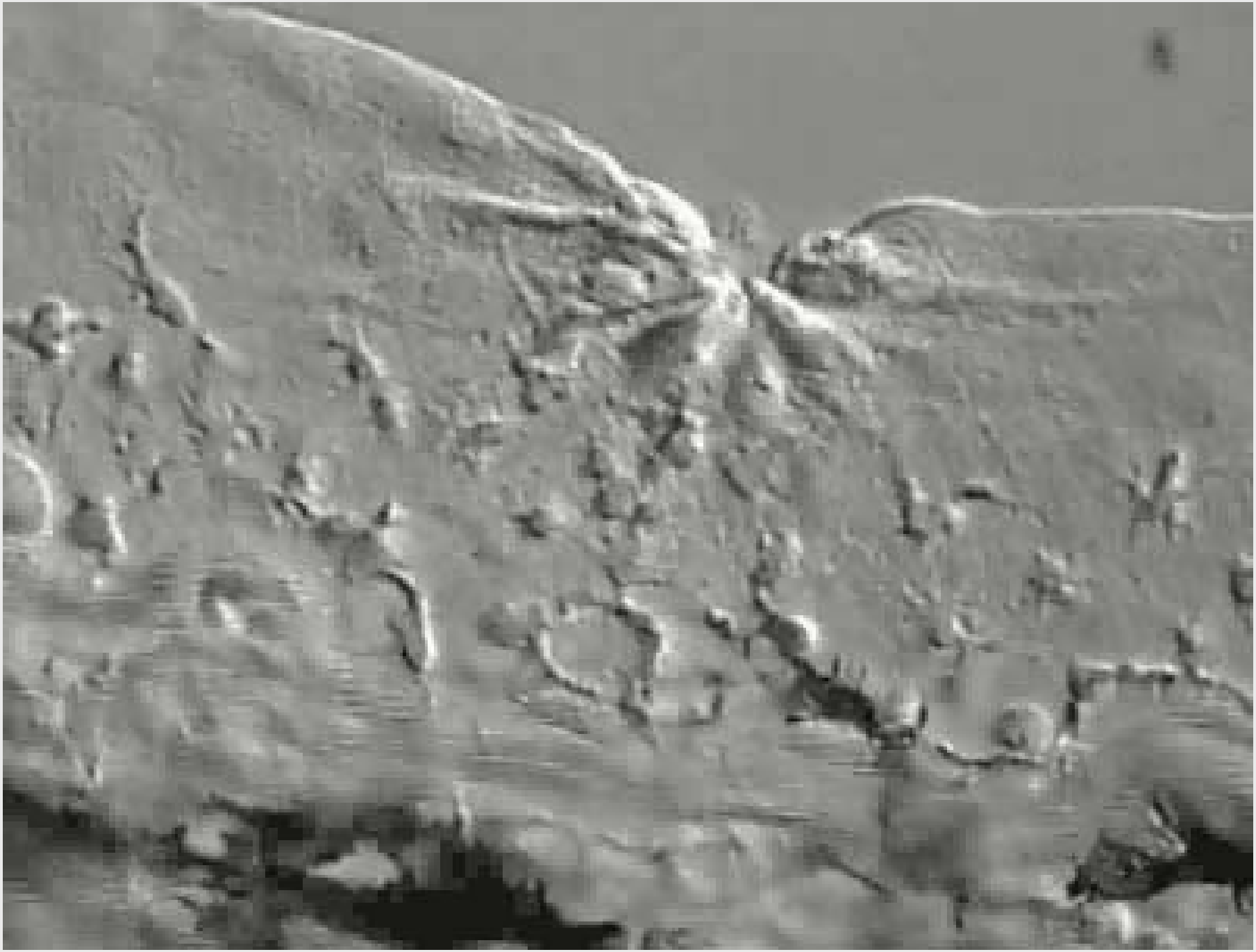
0648-1

Production of Cytokines Follows Cell-Activation after an Appropriate Signal

Inflammatory Mediators:

Interleukin - 1 (IL-1)
Interleukin - 6 (IL-6)
Tumor Necrosis Factor (TNFα)

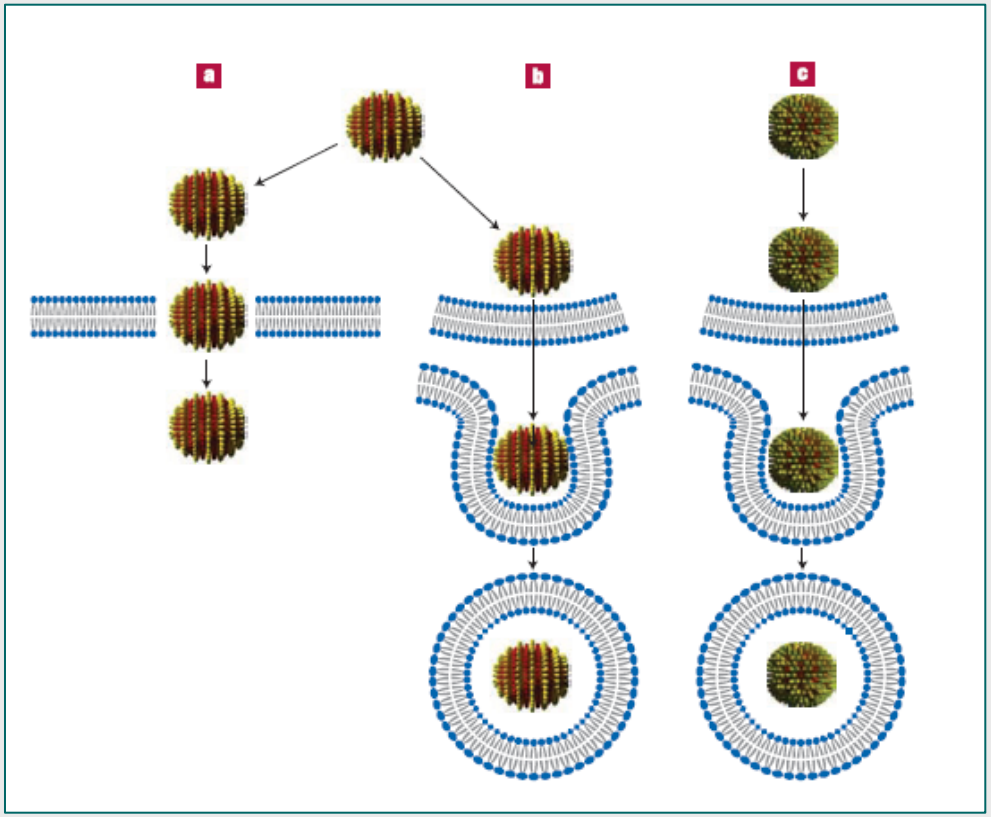




NANOBIOLOGY

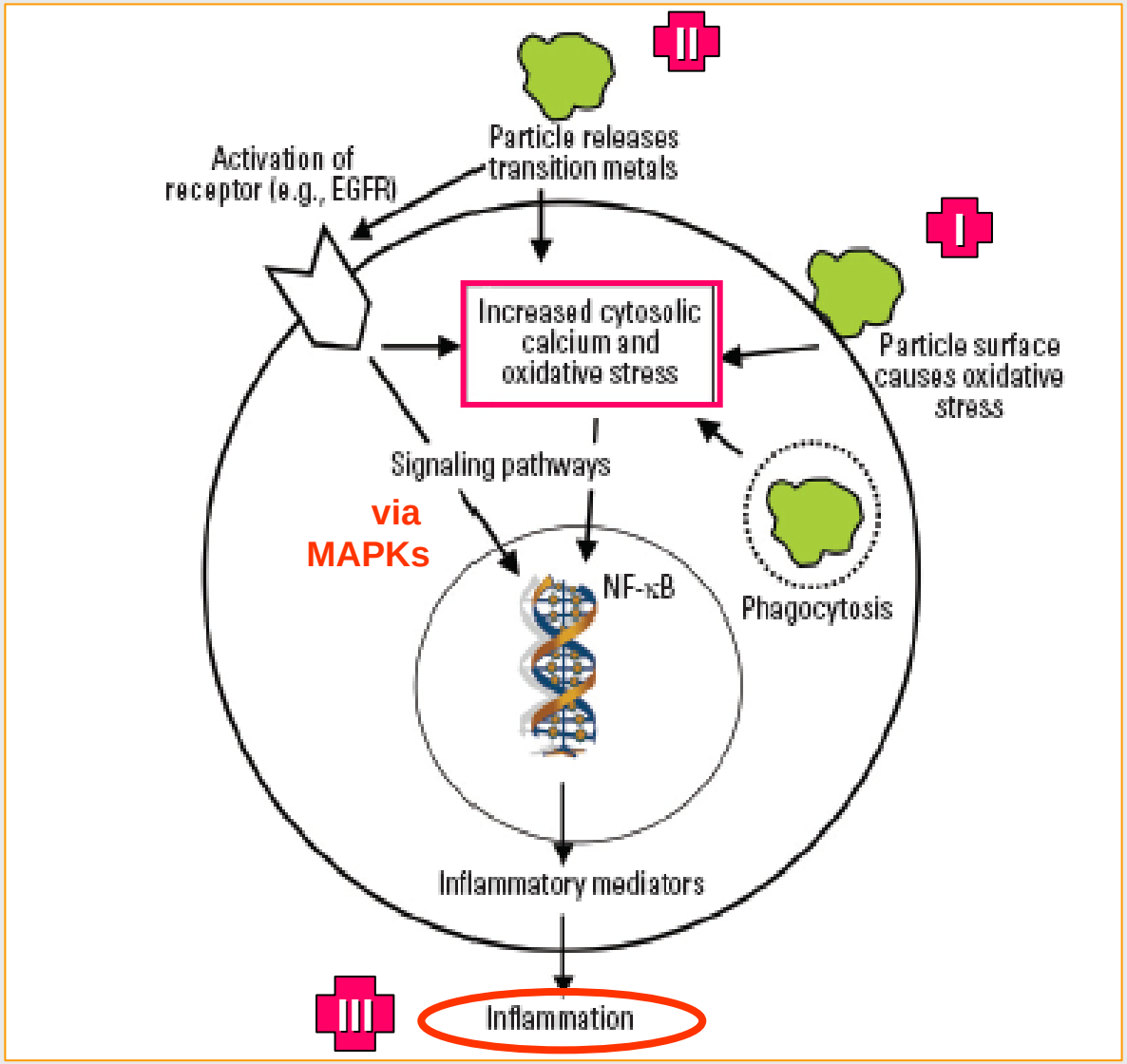
Particles slip cell security

Nanoparticles with alternating striations of hydrophobic and hydrophilic ligands cross the cell membrane by a direct mechanism — a route that delivers them to the main compartment of the cell while leaving the membrane undisrupted.



Re: T Xia et al.,
Nature Materials, 7:519-520 (2008)

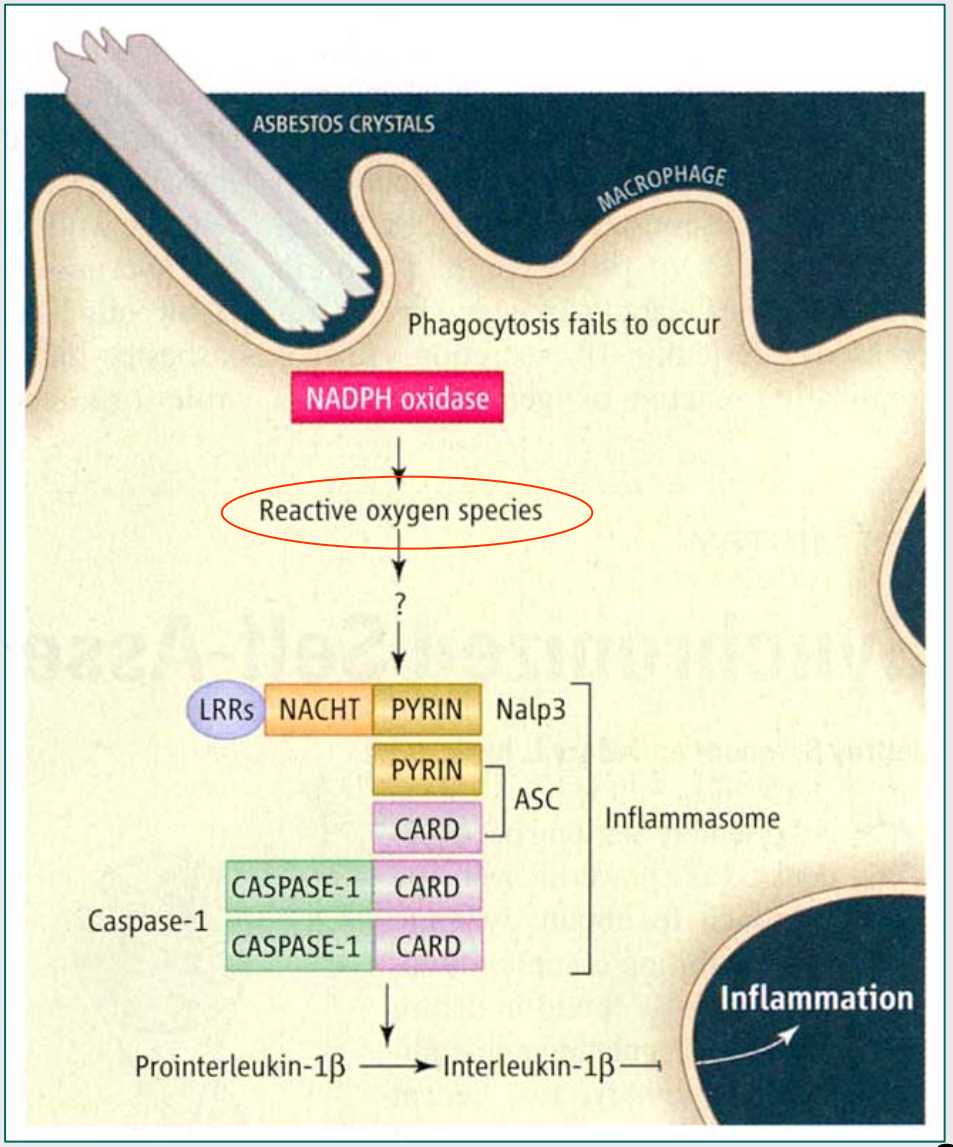
Inflammatory Reactions after Cellexposition of Nanoparticles: Postulated Mechanism



Oberdörster et al., Environ Health Perspect, 113:823-839 (2005)

The Asbestos Case

- Frustrated phagocytosis followed by inflammation -

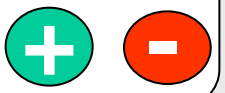


Re: L O´Neill
Science, 320:619-620 (2008)

Nanoproducts in Medical Application

Risks at the Cellular Level

- **Nano-compounds in medical devices**
  General observations
- **Possible mechanisms of cellular interaction**
- **Observed effects of nano-compounds at the cellular level**



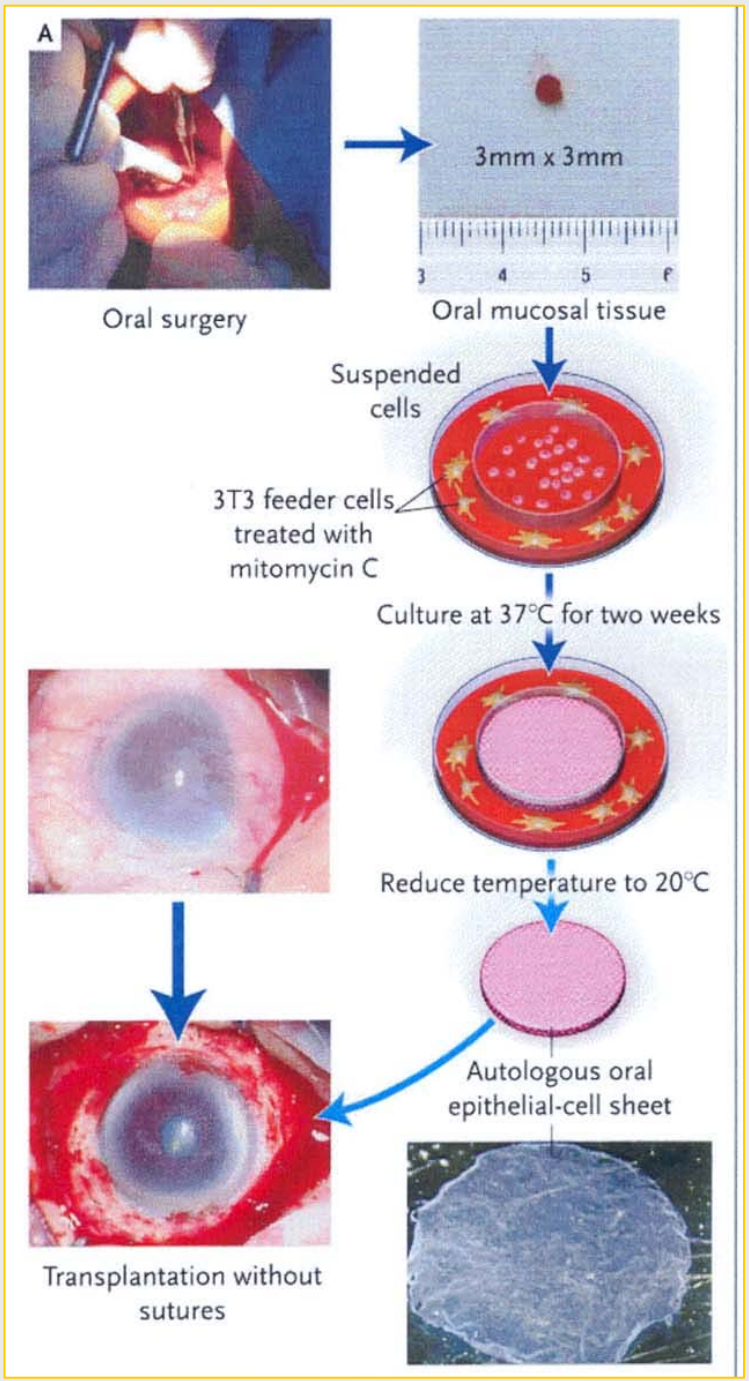
- **Conclusion**

Transplantation of Cornea - Epithel

Use of temperature-sensitive PIPAAM – Polymers

(PIPAAM – poly-N-isopropylacrylamide)

Hydratation and Swelling of PIPAAM by reducing the temperature (37° - 20°C), thereby cell-detachment from surface due to a change of surface properties (hydrophobic to hydrophilic).



NEJM, 351:1187 – 1196 (2004)

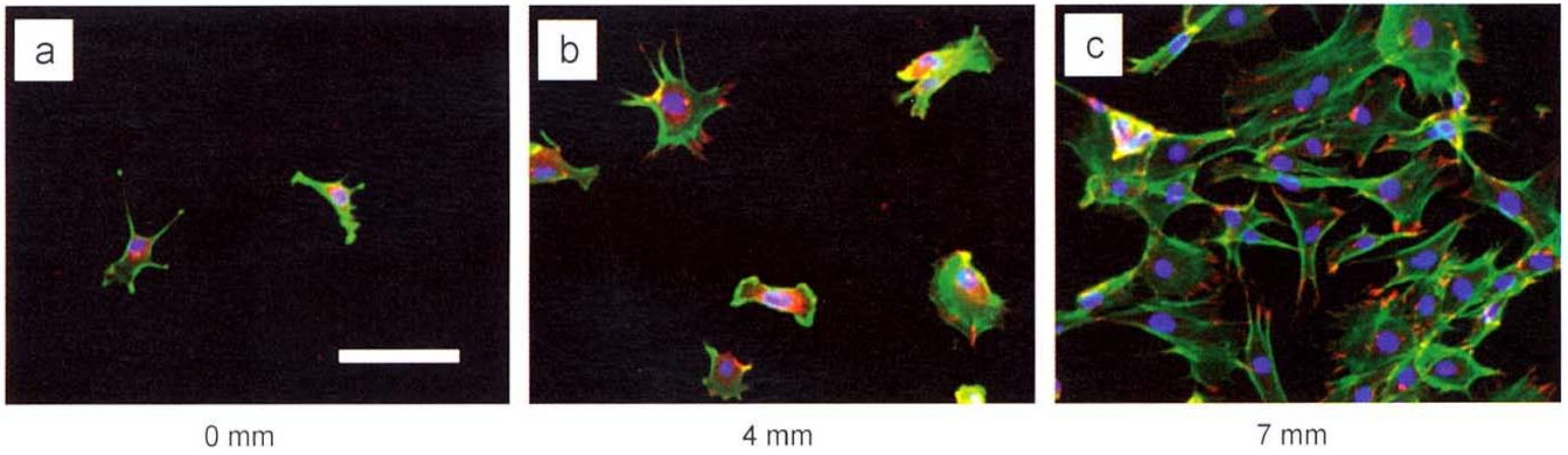
The NEW ENGLAND JOURNAL of MEDICINE

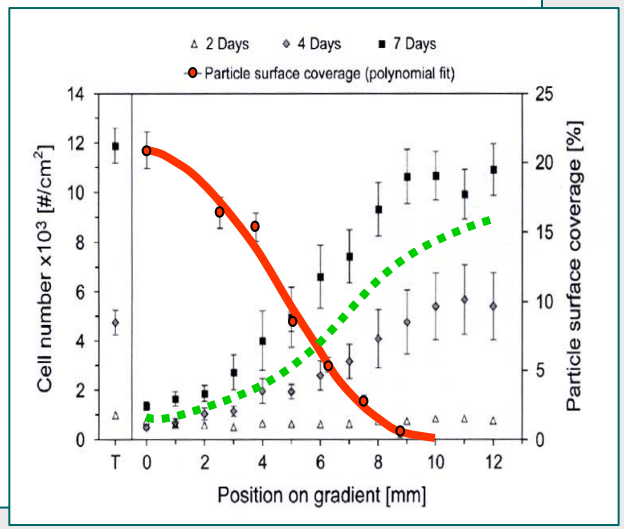
ORIGINAL ARTICLE

Corneal Reconstruction with Tissue-Engineered Cell Sheets Composed of Autologous Oral Mucosal Epithelium

Kohji Nishida, M.D., Ph.D., Masayuki Yamato, Ph.D., Yasutaka Hayashida, M.D., Katsuhiko Watanabe, M.Sc., Kazuaki Yamamoto, M.Sc., Eijiro Adachi, M.D., Ph.D., Shigeru Nagai, M.Sc., Akihiko Kikuchi, Ph.D., Naoyuki Maeda, M.D., Ph.D., Hitoshi Watanabe, M.D., Ph.D., Teruo Okano, Ph.D., and Yasuo Tano, M.D., Ph.D.

Optimization of Surfaces with Nanoparticles - Attachment of Osteoblasts -





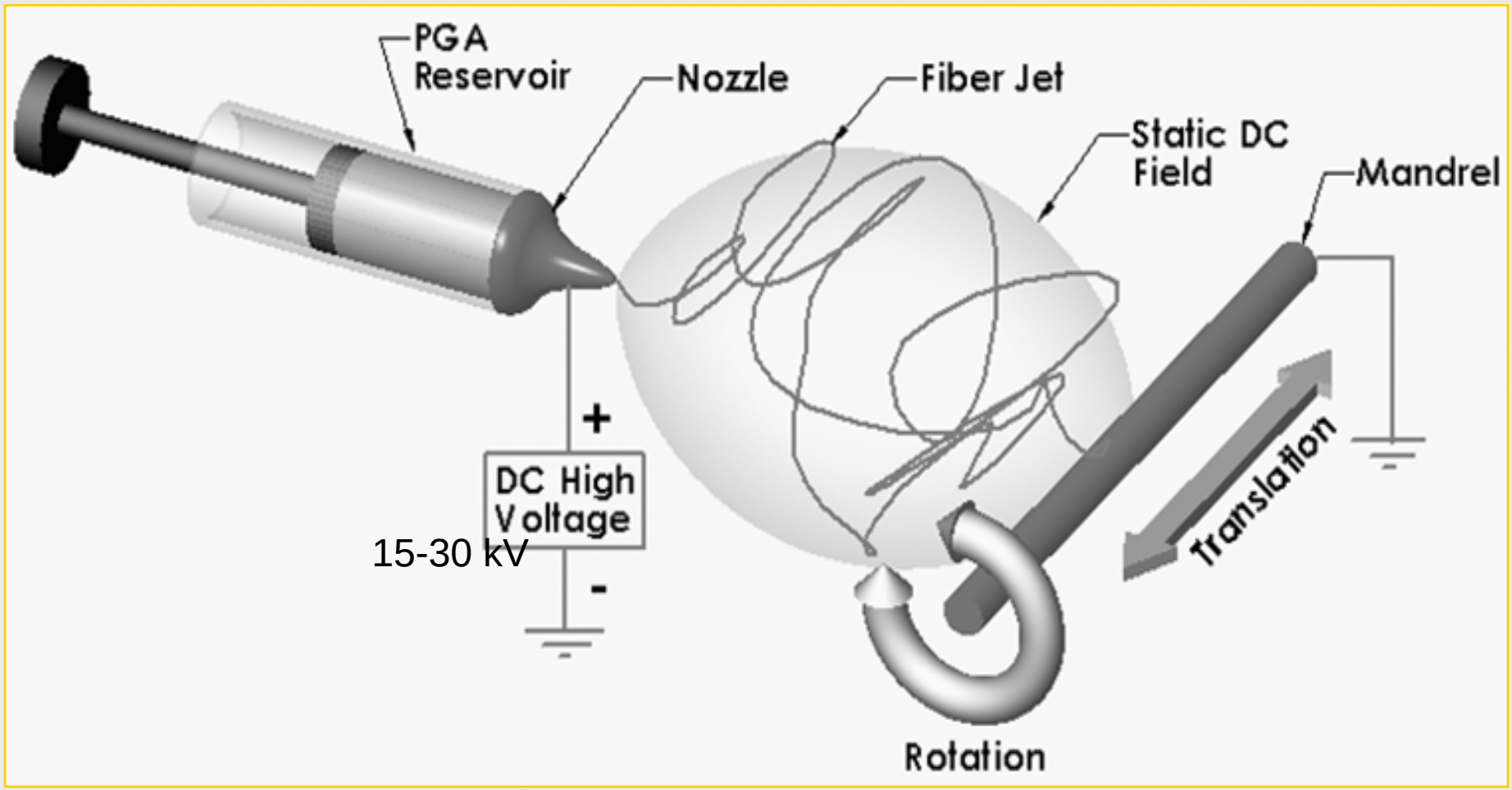
Cell seading with 3.500 RCO-cells/cm², cultivation: 7 days
Red: Vinculin, green: Actin, blue: cell nuclei

T Kunzler et al., Biomaterials, 28:5000-5006 (2007)

Once upon a time.....

Michael DeBakey produces the first artificial artery with the sewing machine of his wife.

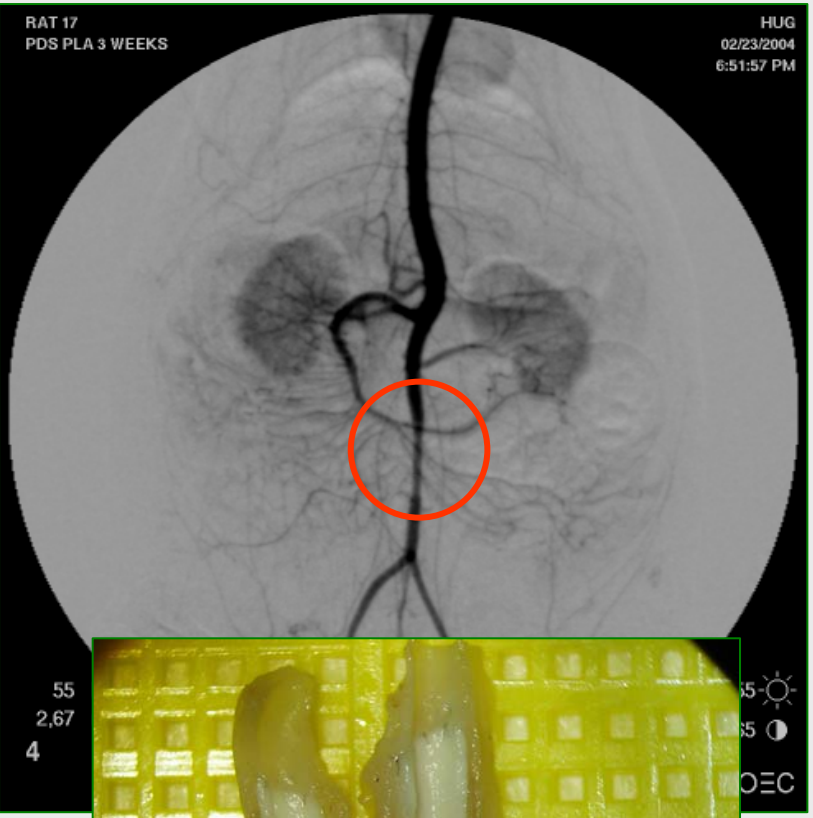
New Vascular Prostheses
with the help of the "BARBAPAPA" Technique

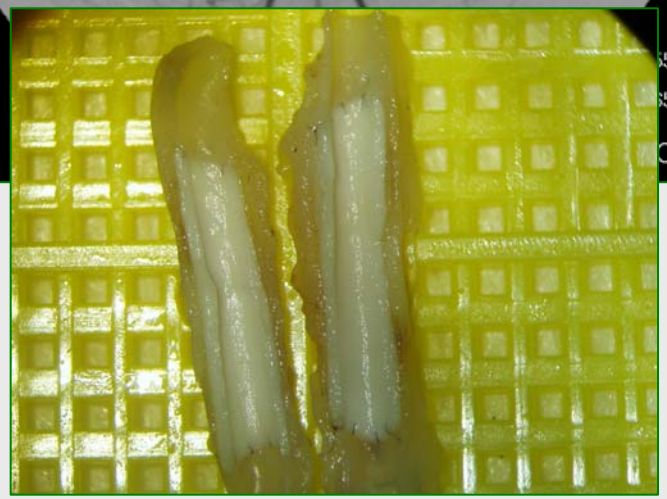


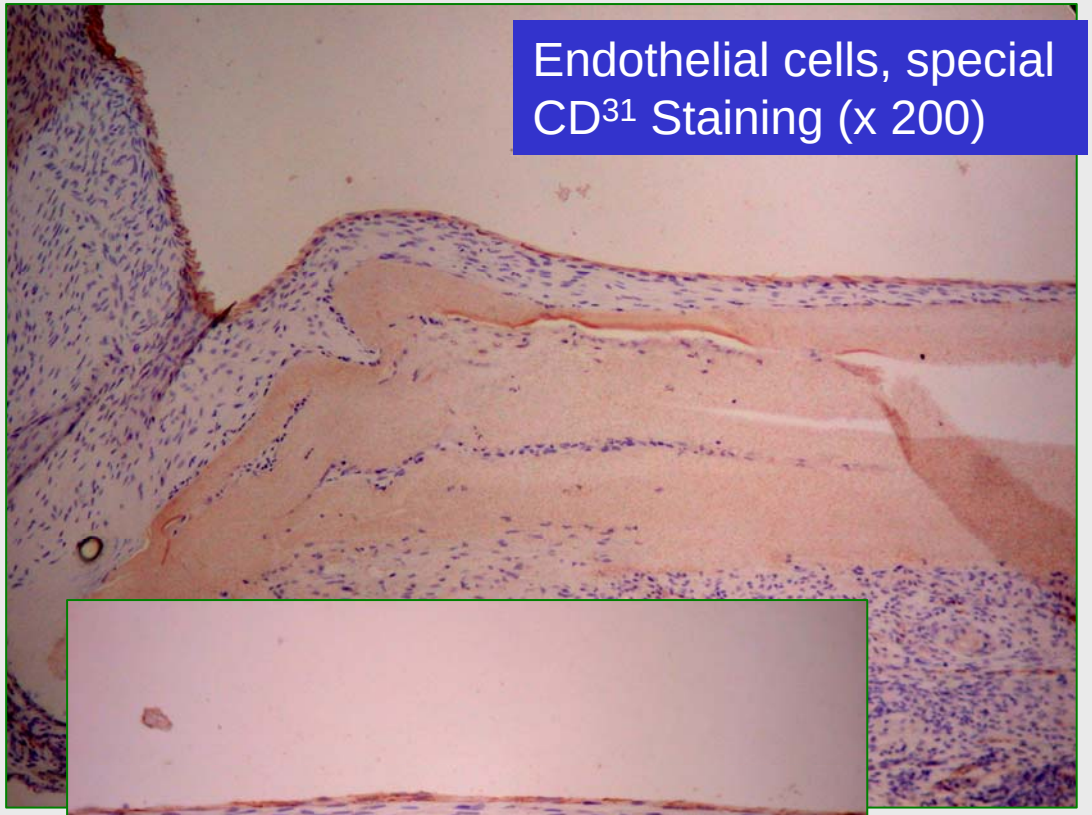
JA Mathews et al.,
Biomacromolecules, 3:232-238 (2002)

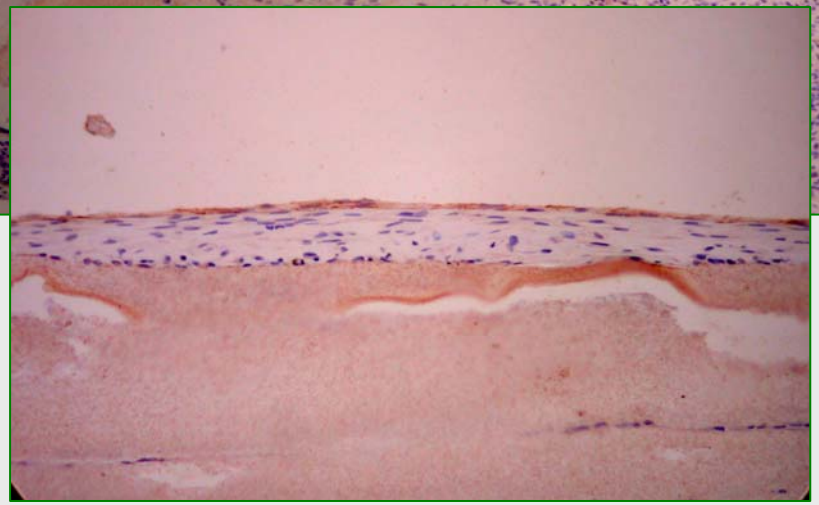
PDS GRAFTS Mixed with Slowly Degradable PLA

n=3, 3 weeks after implantation (Rat model)









Re.: B Walpoth,
HUG Genf 2007

Applications

Polymers with Shape-Memory Effects

Applictions

Particles and magnetic fields

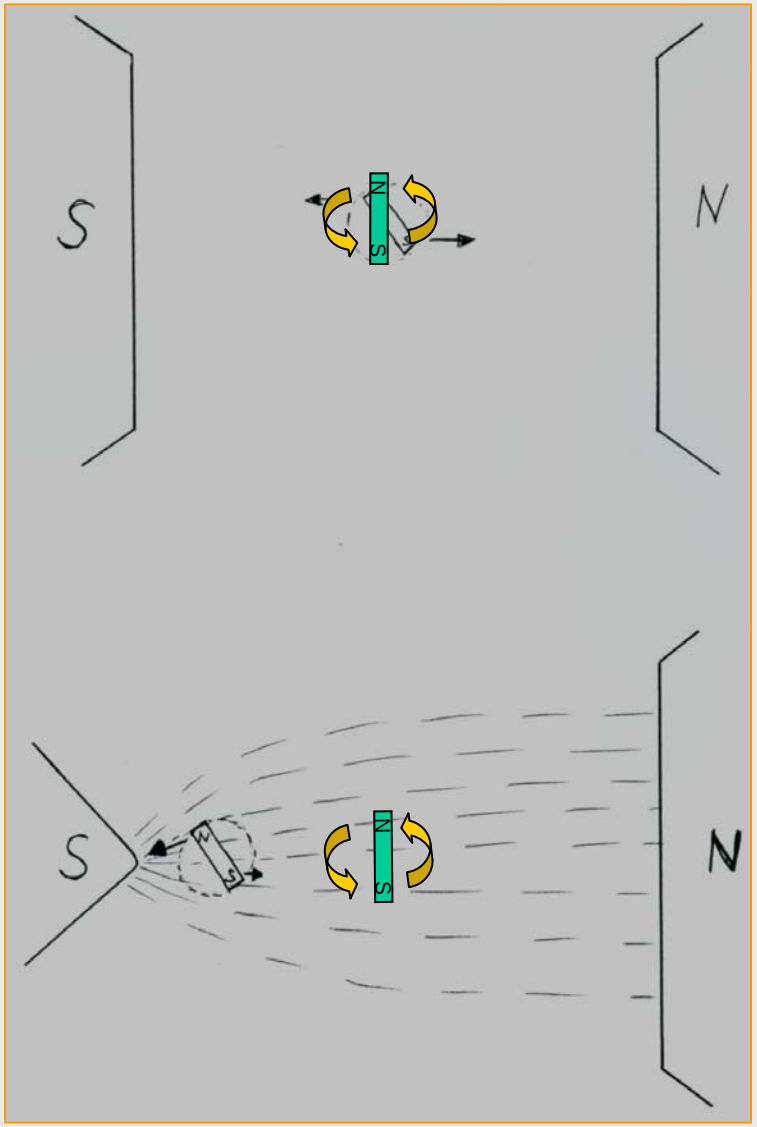
Homogeneous Field

Twist

- **Friction**
- **Heat formation**

Inhomogeneous Field

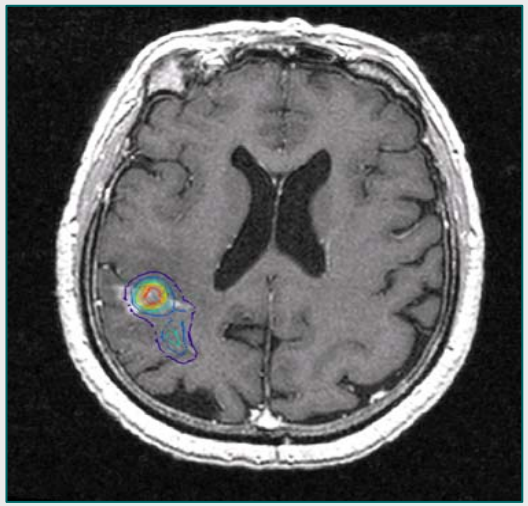
Twist and Movement

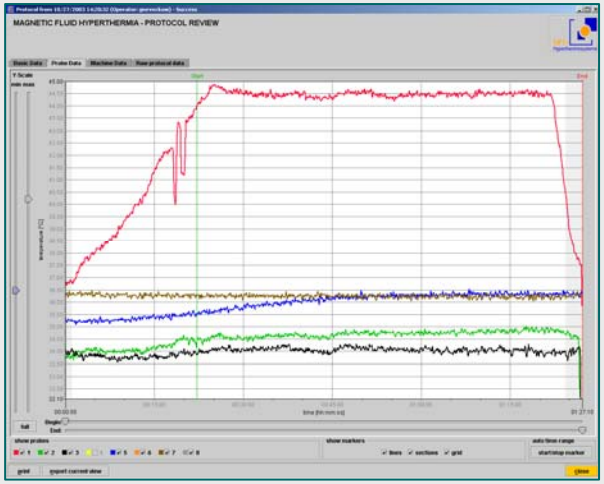



Copyright GKSS 2006

Magnetic Hyperthermy System

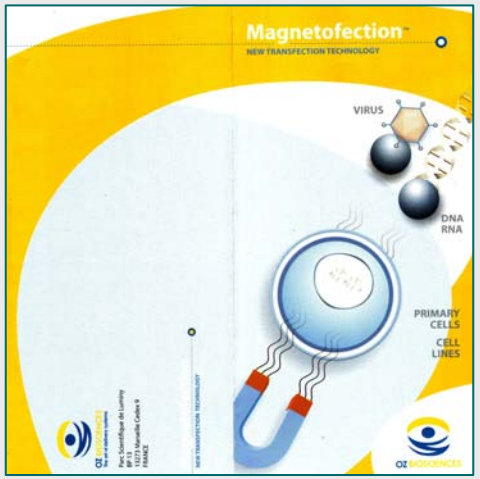
Clinical trials since 2003



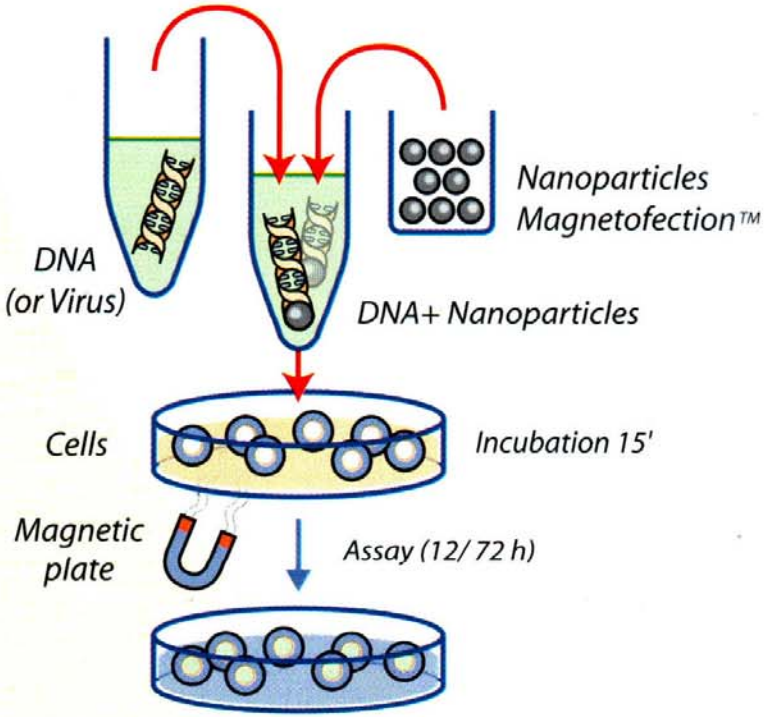


A. Jordan, Charité, Berlin

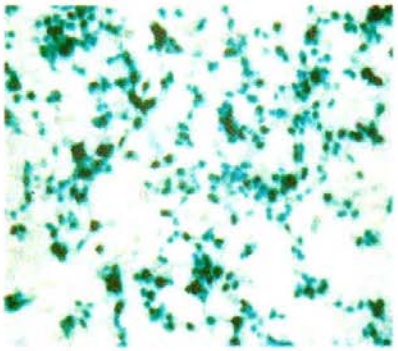
Magnetofection for the cellular uptake of DNA, Genes or Viruses



Simple and rapid protocol: mix, incubate, test.



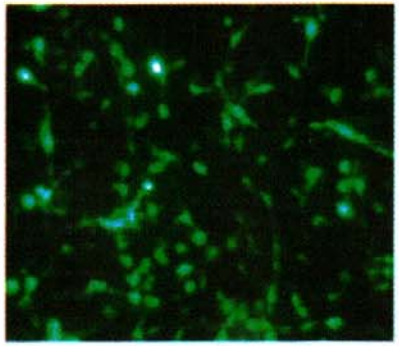
Ideal method to truly transfect primary cells



PRIMARY CELLS

Keratinocytes
Chondrocytes (photo)
Fibrochondrocytes
Peripheral Blood Lymphocytes
Artery Smooth Muscle Cells
Aortic Endothelial Cells
Nasal & Pulmonary Epithelium
HUVEC-C

Extremely efficient technique to transfect cell lines



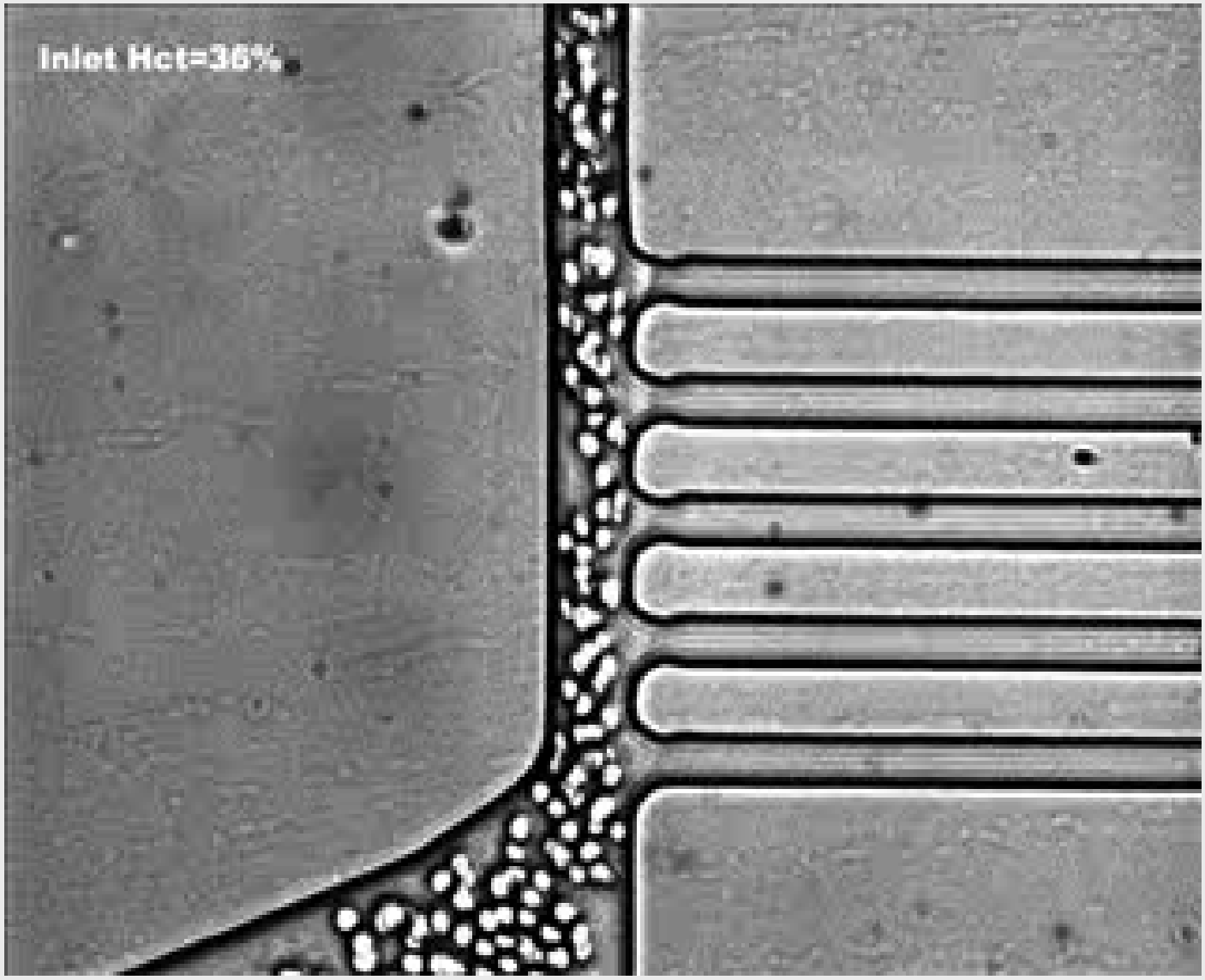
CELL LINES

293	COS 7	L929
16HBE14	CT26	MCF-7
181RDB	CV-1	MDCK
A549	HeLa	NIH-3T3
B16-F0	HepG2	PC-12
CHO-K1	HUVEC	SAOS
COS-1	K562	U937...

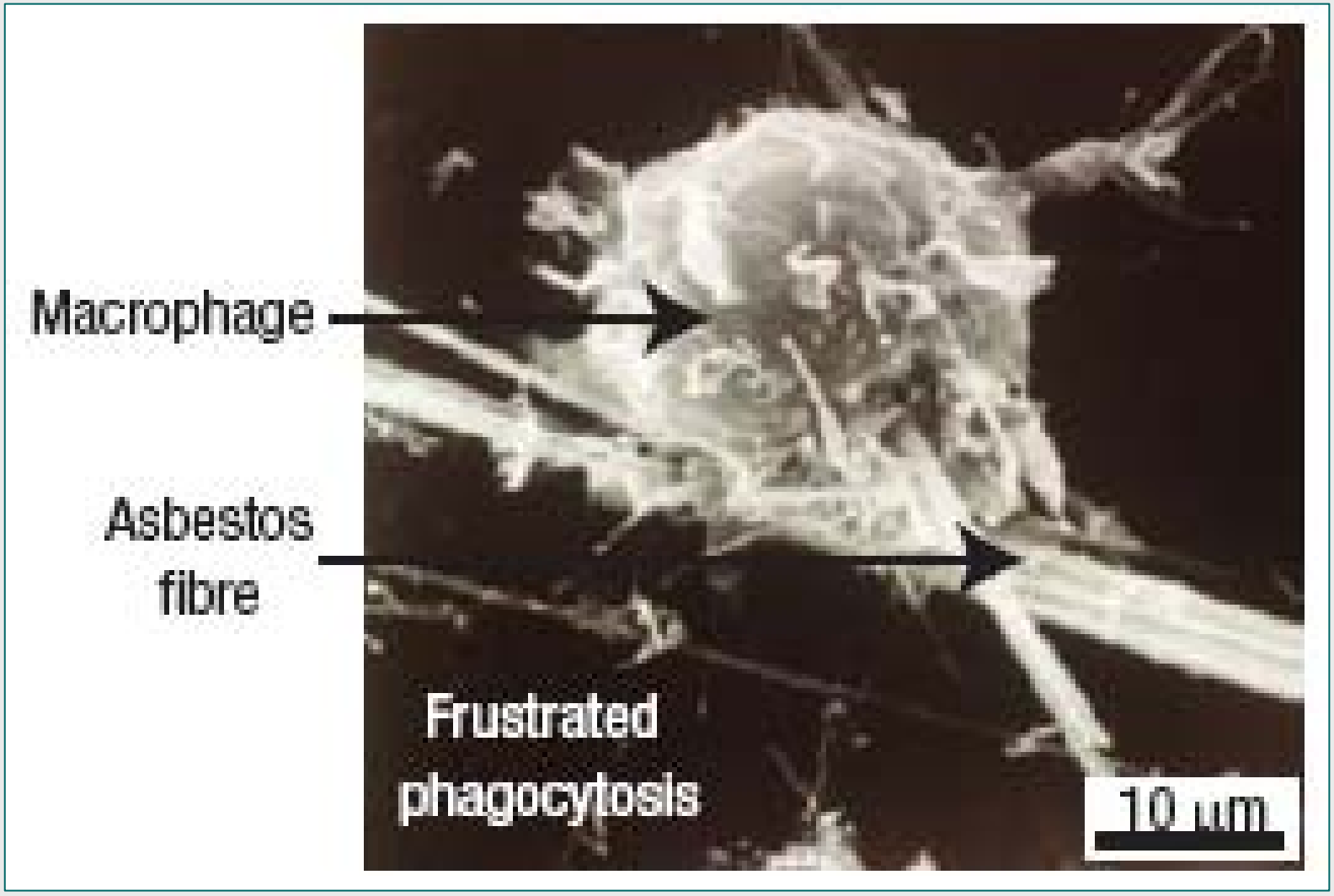
Re: OZ BioSciences, Marseille 2008

Separation of Plasma and Bloodcells

Microfluidic chamber for the analysis of pathological Peptids/Proteins



The Asbestos Case
Lessons learnt from frustrated phagocytosis



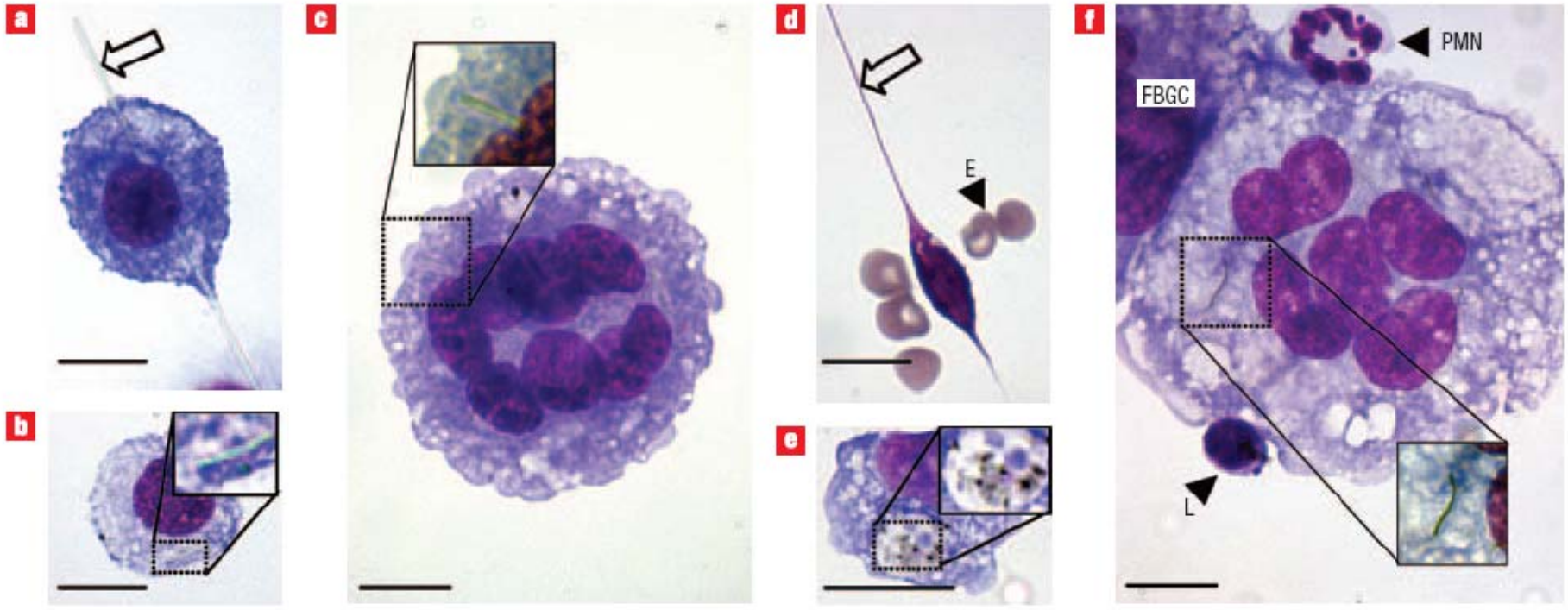
Re: A Kane, A Hurt
Nature Nanotech, 3:378-379 (2008)

Rat Lung Cell Attempts
to ingest a carbon nanotube

Re: R Service
Science,321:1036-37 (2008)

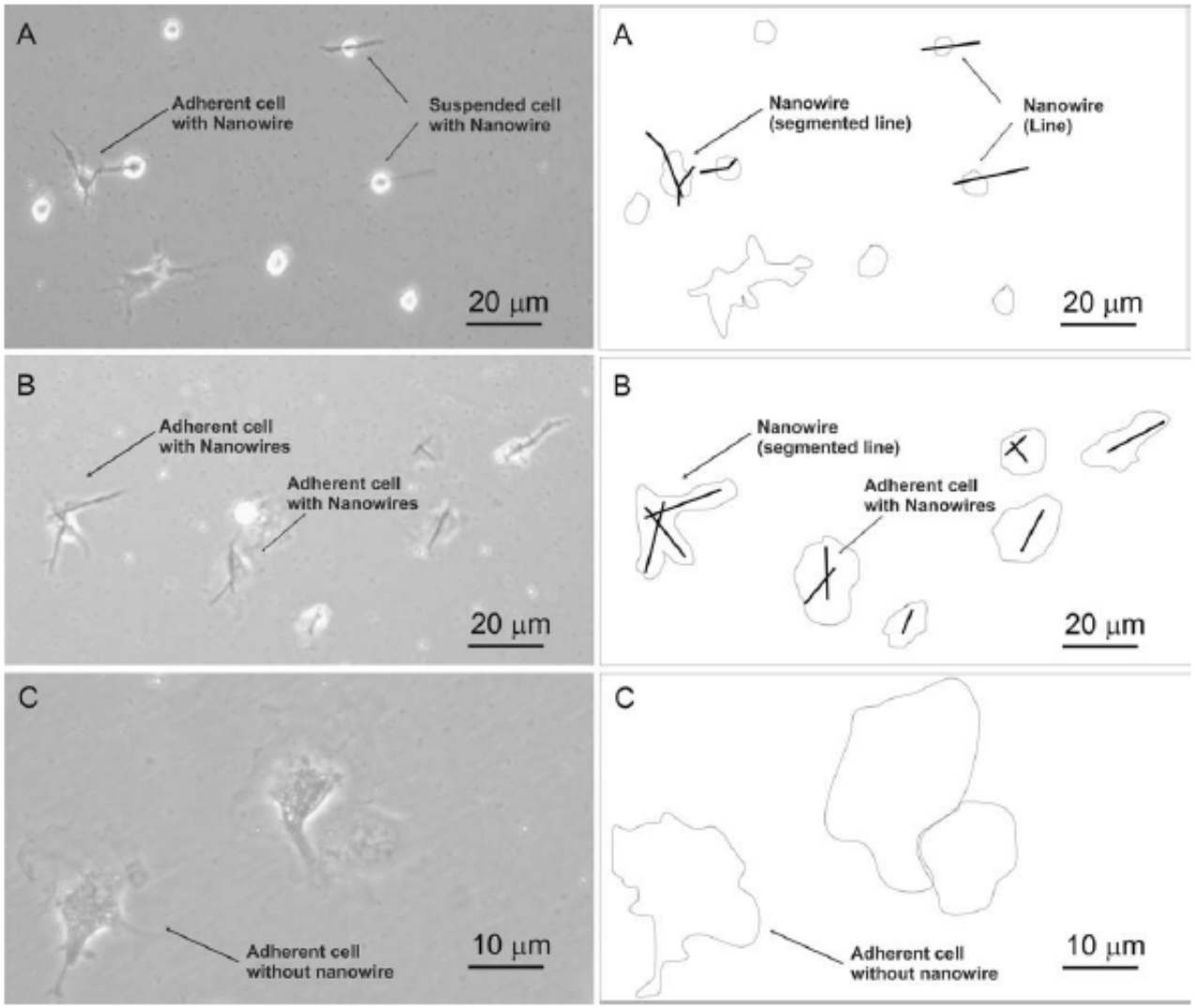
Lessons learnt from Asbestos?

Frustrated Phagocytosis of Carbon Nanotubes



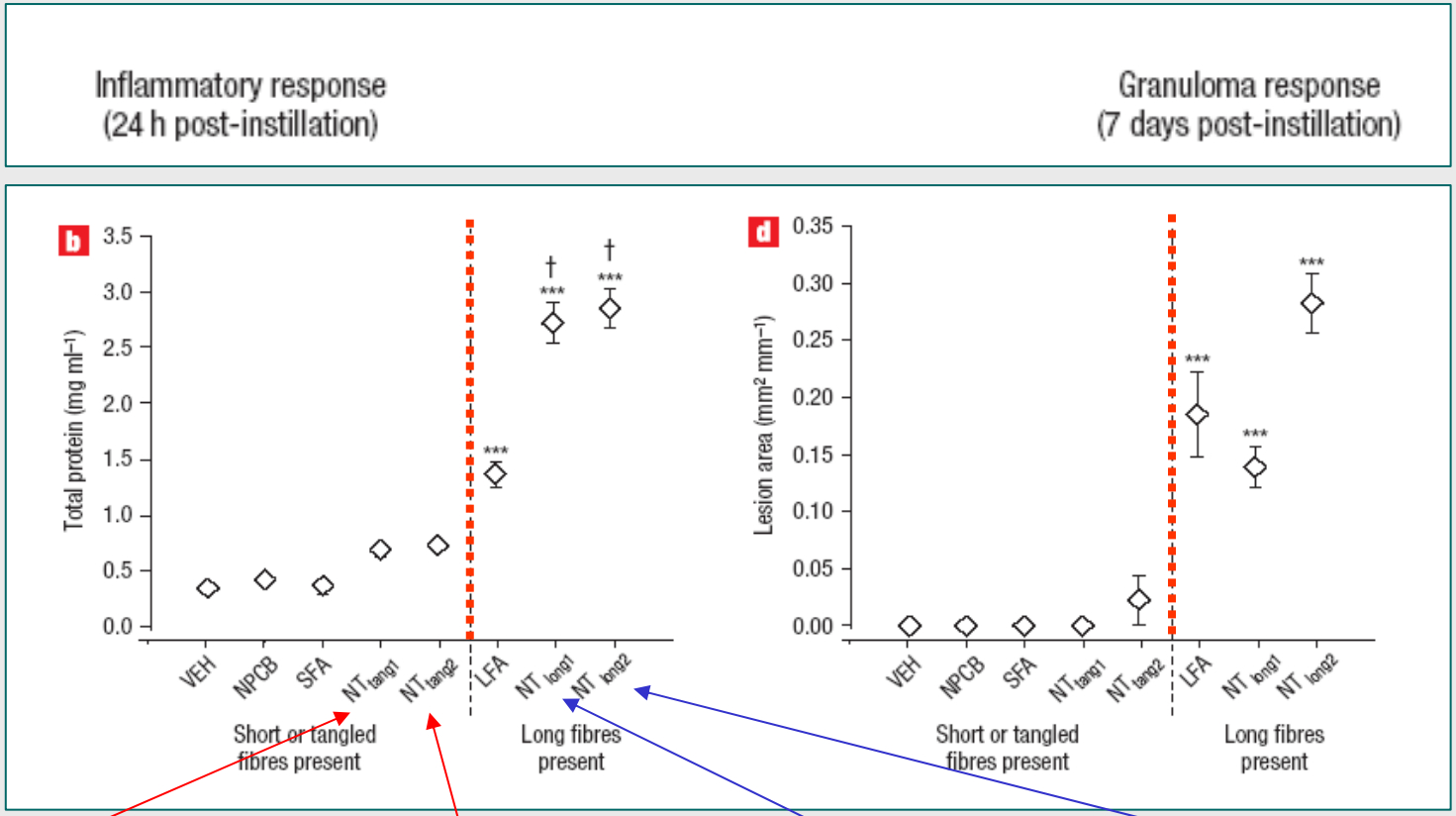
Re: CA Poland et al
Nature Nanotech, 3: 423-428 (2008)

Internalisation of Nanofibres by living cells



Re: A Prina-Mello et al., J Nanobiotechnol, 4:9 (2006)

Fibre-length and -diameter of Nanotubes: Determinants for inflammatory processes



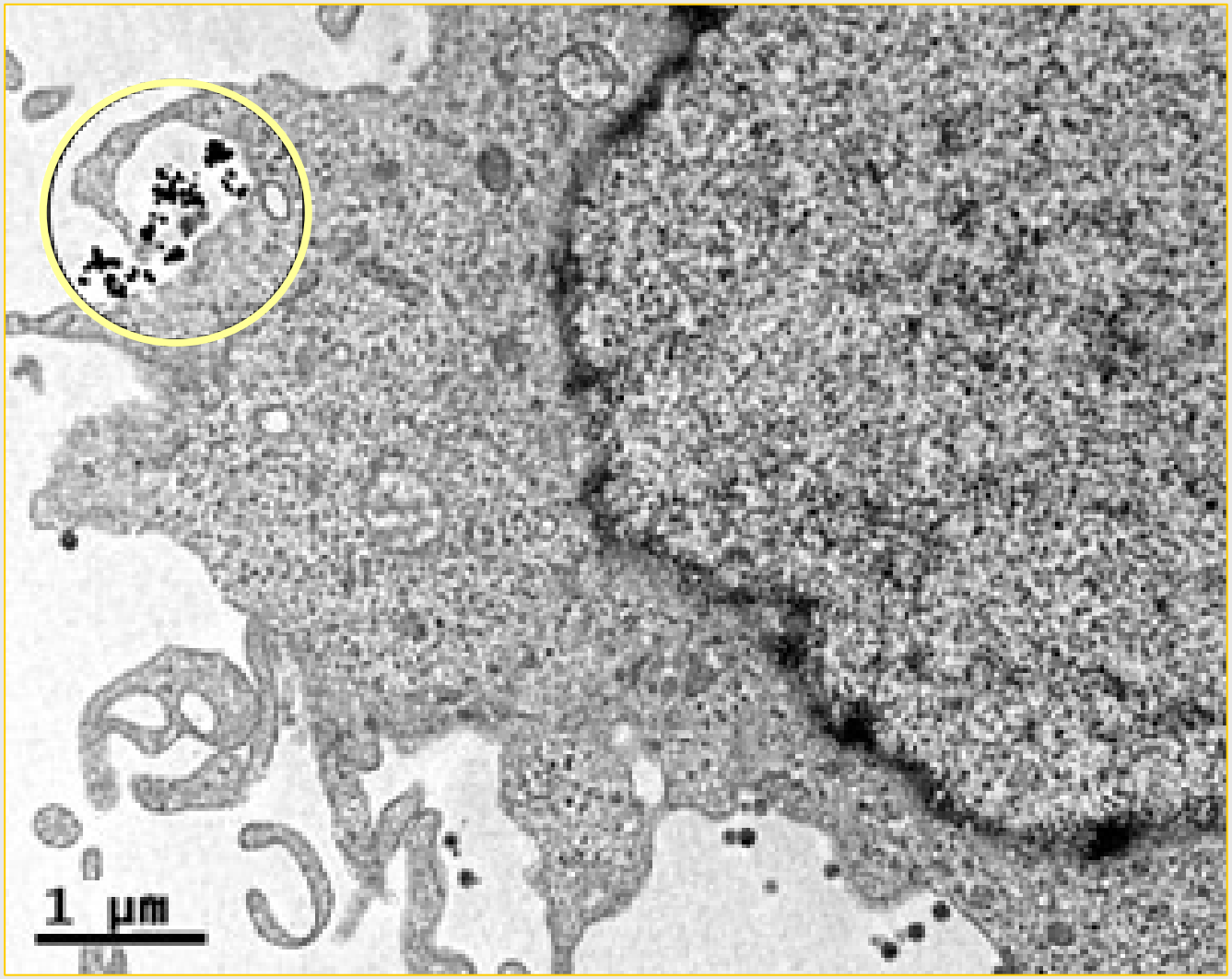
	NTtang1	NTtang2	NTlong1	NTlong2
Diameter as supplied by the manufacturer (nm, mean ± s.e.m.)	15 ± 5	15 ± 5	40–50	20–100
Diameter as determined by authors (nm, mean ± s.e.m.)	14.84 ± 0.50	10.40 ± 0.32	84.89 ± 1.9	165.02 ± 4.68
Length as supplied by the manufacturer (μm)	1–5	5–20	Mean 13	Max 56

* Carbon nanotubes inserted in the abdominal cavity of rats
** NT nanotubes

Re: CA Poland et al
Nature Nanotech, 3: 423-428 (2008)

Phagocytosis of TiO_2 - Nanoparticles
- Formation of free Oxygen radicals -

TiO_2-Particles
∅: 30 nm

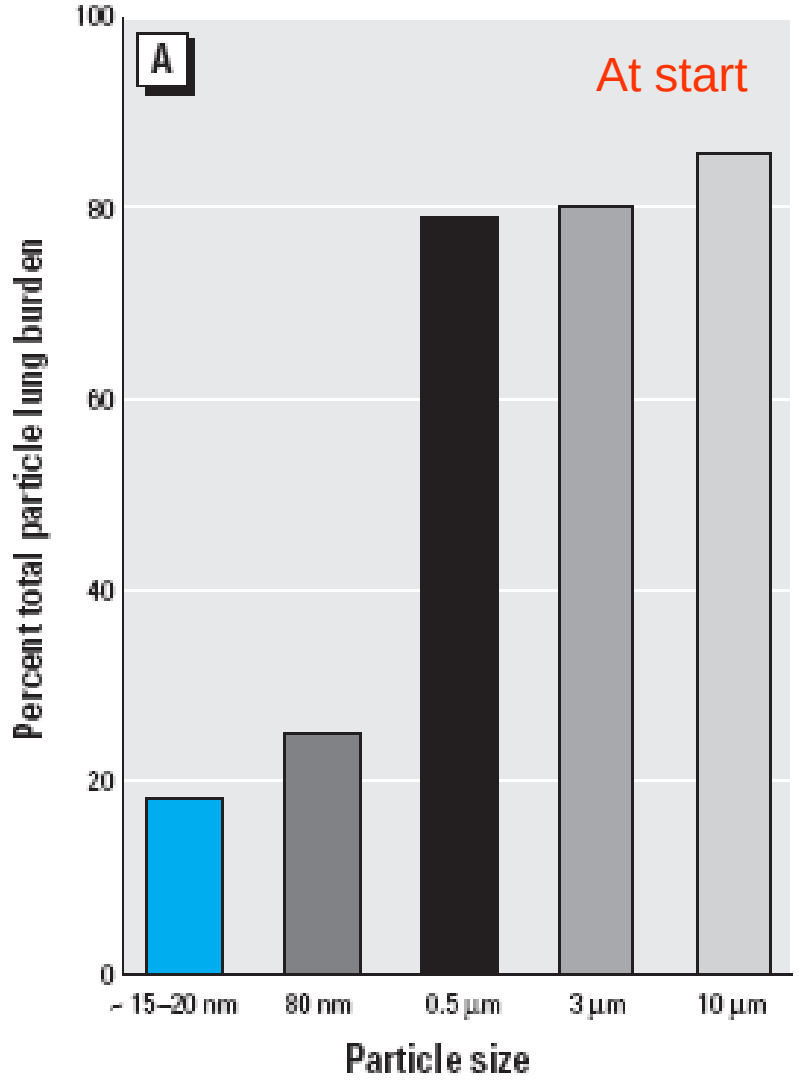


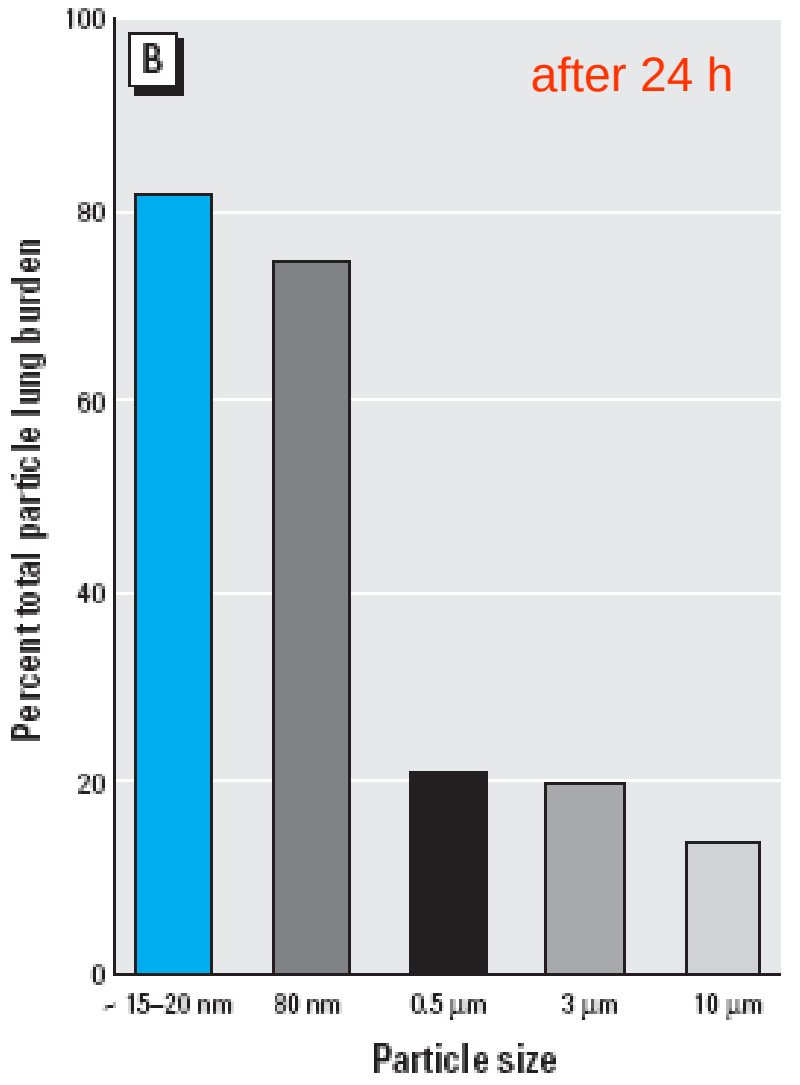
Gliacell

Re: FAZ-Sonntagszeitung
25.06.2006

Lung-Retention of Micro- and Nanoparticles

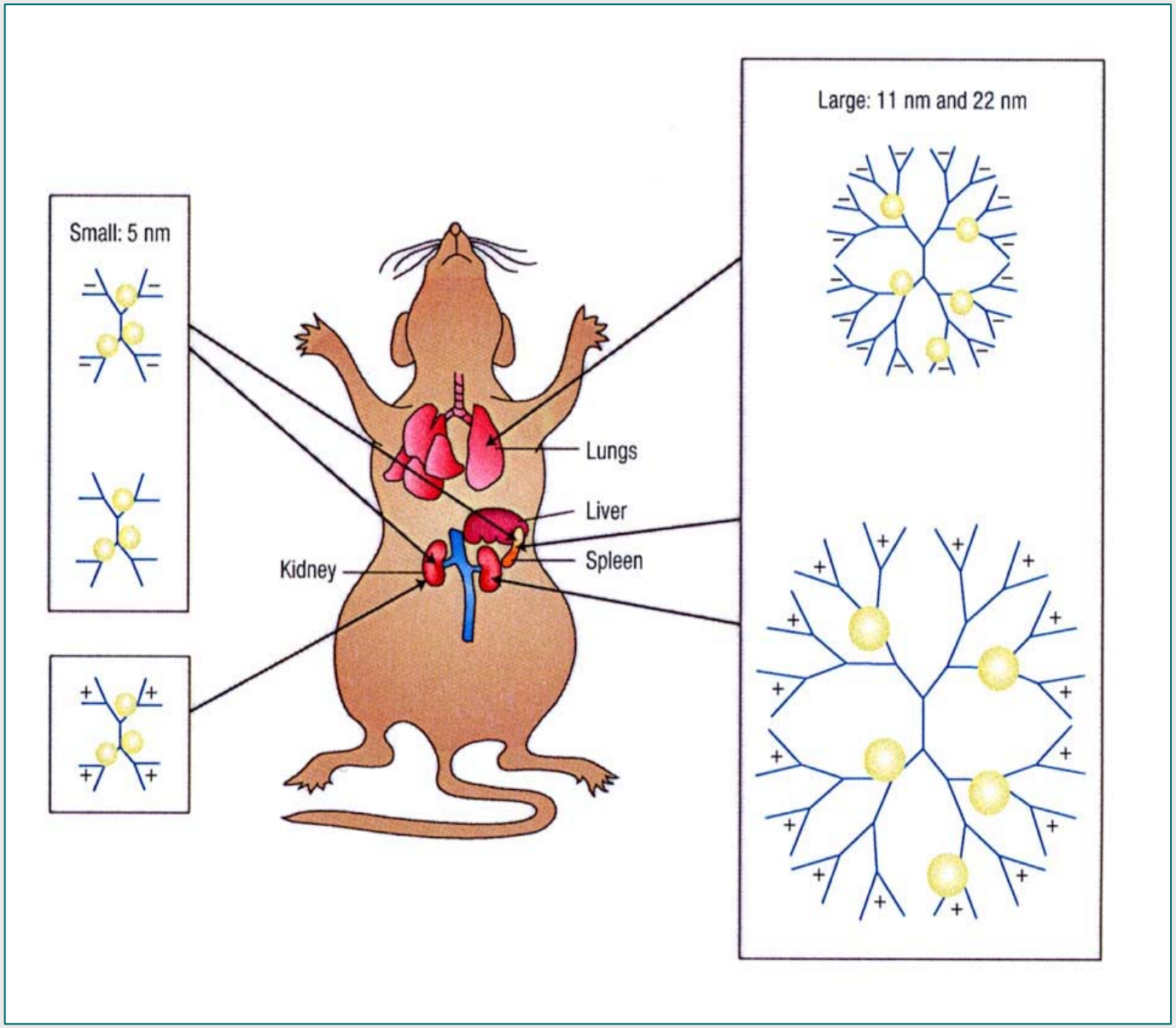
- after in vivo inhalation by alveolar Macrophages -





G Oberdörster et al.,
Environm Health Perspect, 115: 823-839 (2005)

Gold-Dendrimer Particles and Their Biodistribution

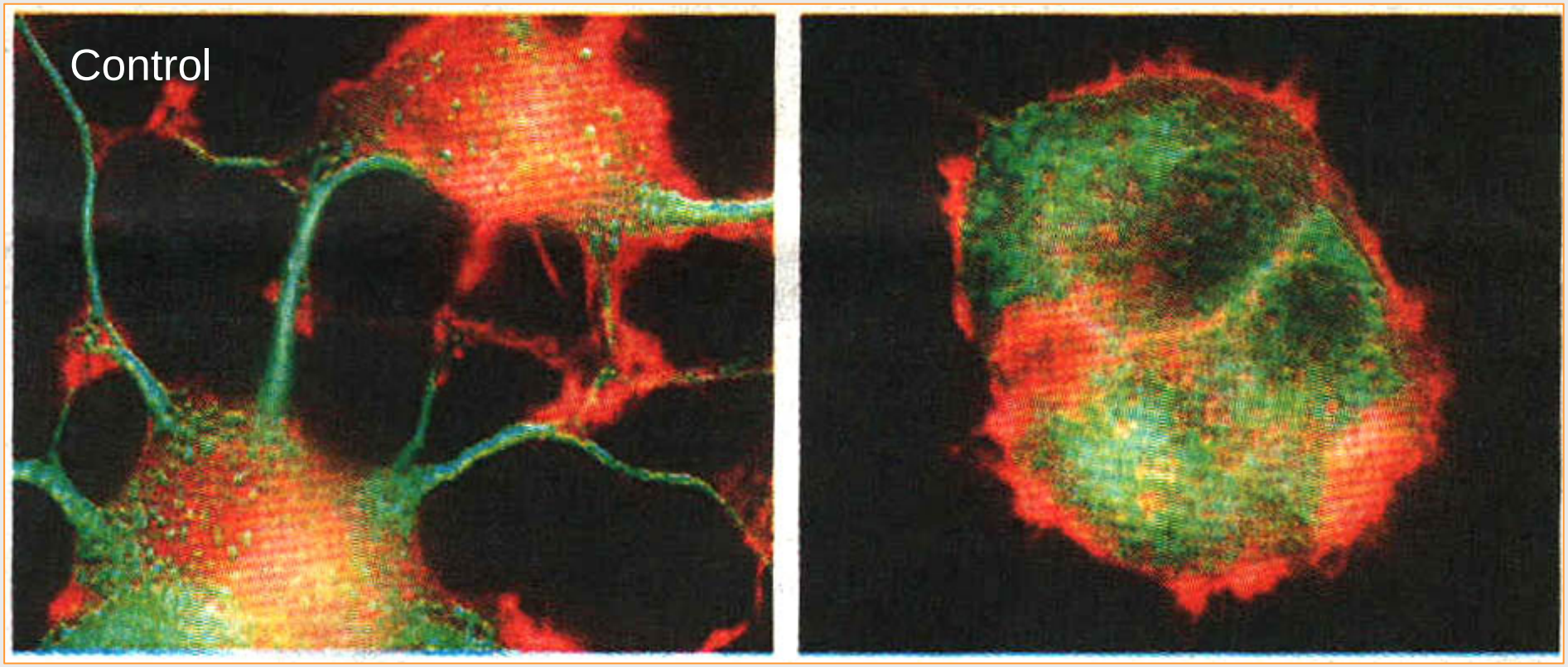


Negatively charged dendrimers

Positively charged dendrimers

Re: R Minchin
Nature Nanotech, 3:12-13 (2008)

Blockage of Axon-Generation in Nervecells by Magnetic Nanoparticles - *in vitro* -



- **Coating of anionic Fe-Nanoparticles with di-mercapto-succinic acid**
- **No cytotoxicity of the individual compounds**

TR Pisanic et al., Biomaterials, 28:2572-2581 (2007)

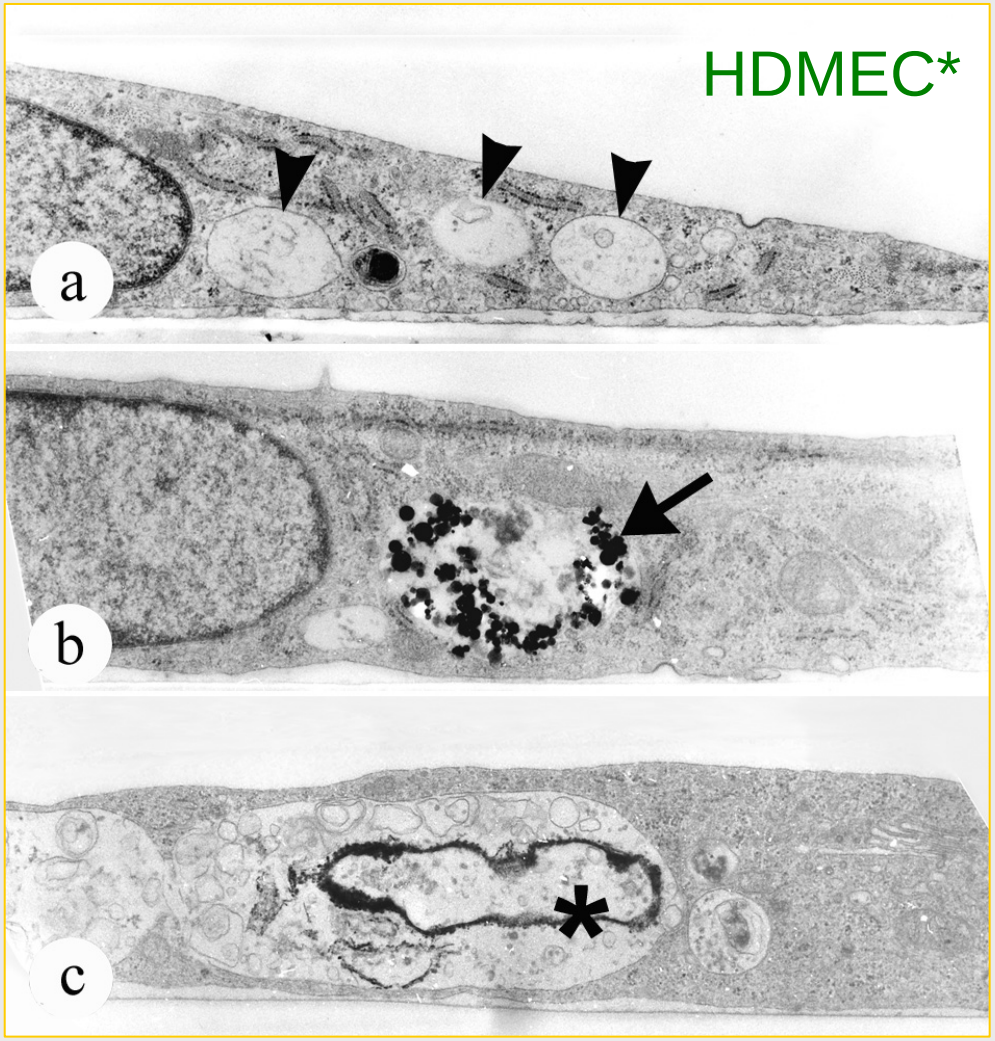
Nanoparticles and Endothelial Cells

TEM (48 h Nanoparticle Exposition)

Untreated Control

TiO_2 Particles

Co Particles

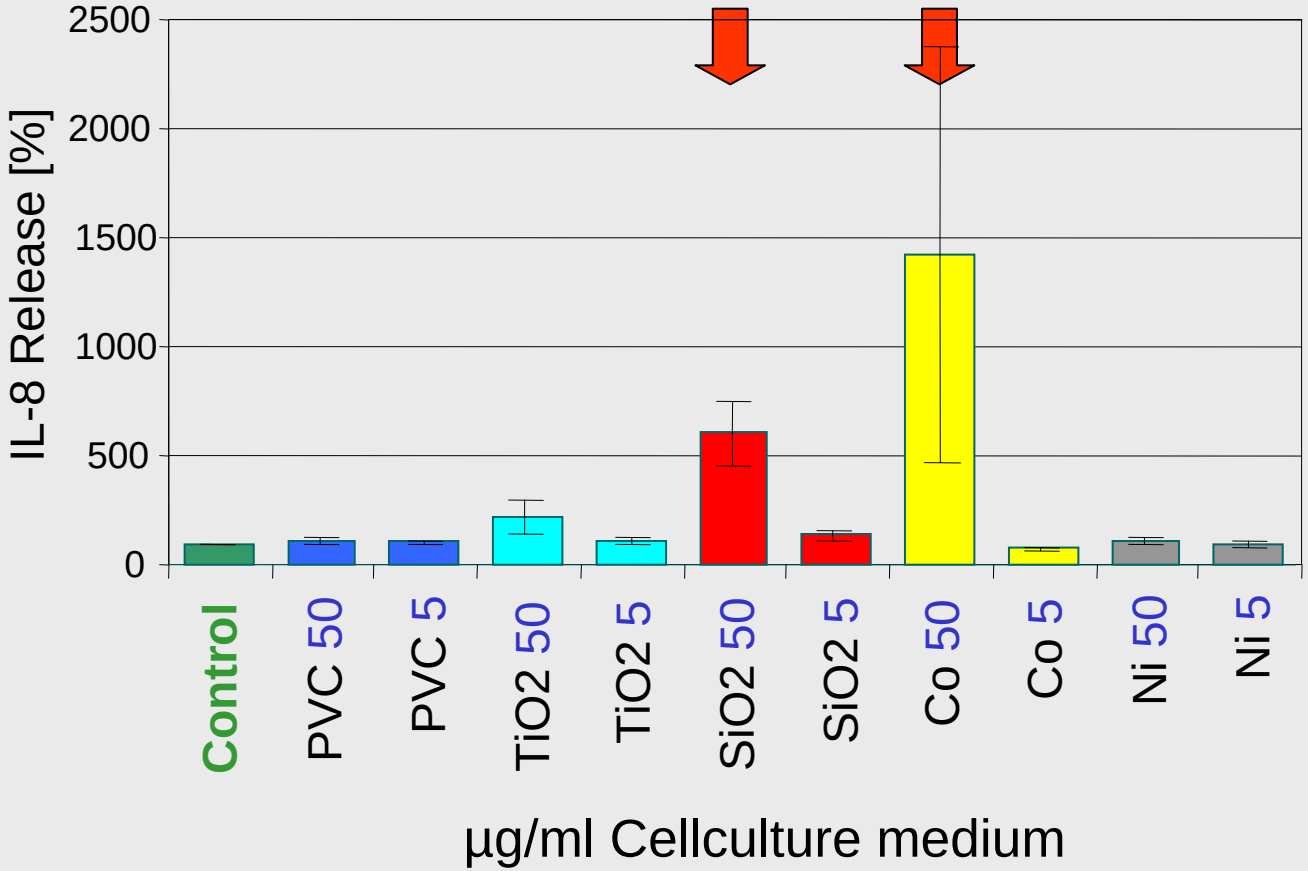


*Human dermal microvascular endothelial cells

Re: Peters et al., JMS MIM 15:319-323 (2004)

HDMEC and Nanoparticles

Pro-inflammatory Cytokine (IL-8 Release)



Nanoparticles
Polymers/Types
PVC (130 nm)
TiO_2 (20-160nm)
SiO_2 (4 – 40 nm)
Co (50 -200nm)
Ni (40- 420nm)

Re: Peters et al.,
JMS MIM (2004) 15: 319-323

Nanoproducts in Medical Application

Risks at the Cellular Level

- **Nano-compounds in medical devices**
  General observations
- **Possible mechanisms of cellular interaction**
- **Observed effects of nano-compounds at the cellular level**
- **Conclusion**

BUSINESS

Nanotech's big issue

Lack of regulation and risk assessment could hamper the nanotechnology sector. **Virginia Gewin** reports.

Nanotechnology is at last set to start delivering on its promise as a growing number of products hit the market place. But even as companies put the finishing touches to their ideas, there is growing unease that the sector is ill-prepared for the rigours of the public arena.

New technologies carry with them an attendant risk, and nanotechnology is no exception. At the start of this year, 'nano' was an advertising buzz word (see *Nature* **440**, 262; 2006) — now, that gloss is already beginning to fade.

In March, a number of German consumers experienced respiratory problems after using the glass sealant Magic Nano, and the tal Protection Agency (EPA) or the Food and Drug Administration (FDA).

BASF has opted to publicize its nanotech research on the web.

BASF

savvy executives saying that some companies had halted their nanotech plans over confusion on how to judge potential hazards. For example, one interviewee said his firm had stopped developing a product when it became clear that it would cost too much to ensure the product would be risk-free for its entire life.

Such concerns can dissuade investors. Steve Jurvetson, managing director of Draper Fisher Jurvetson, a venture-capital firm in Menlo Park, California, says his group simply doesn't invest in areas with an unspecified regulatory regime because it's not worth the risk. Like many investors tempted by the sector, he sticks to supporting nano-sized improvements to existing products rather than novel nanoparticles that, as yet, have little direct application.

Perceived risk is a formidable issue that must be addressed, says Michael Holman, an analyst at Lux. The Magic Nano incident raised the business community's awareness that the public needs to be better

TECHNICAL SPECIFICATION

ISO/TS 27687

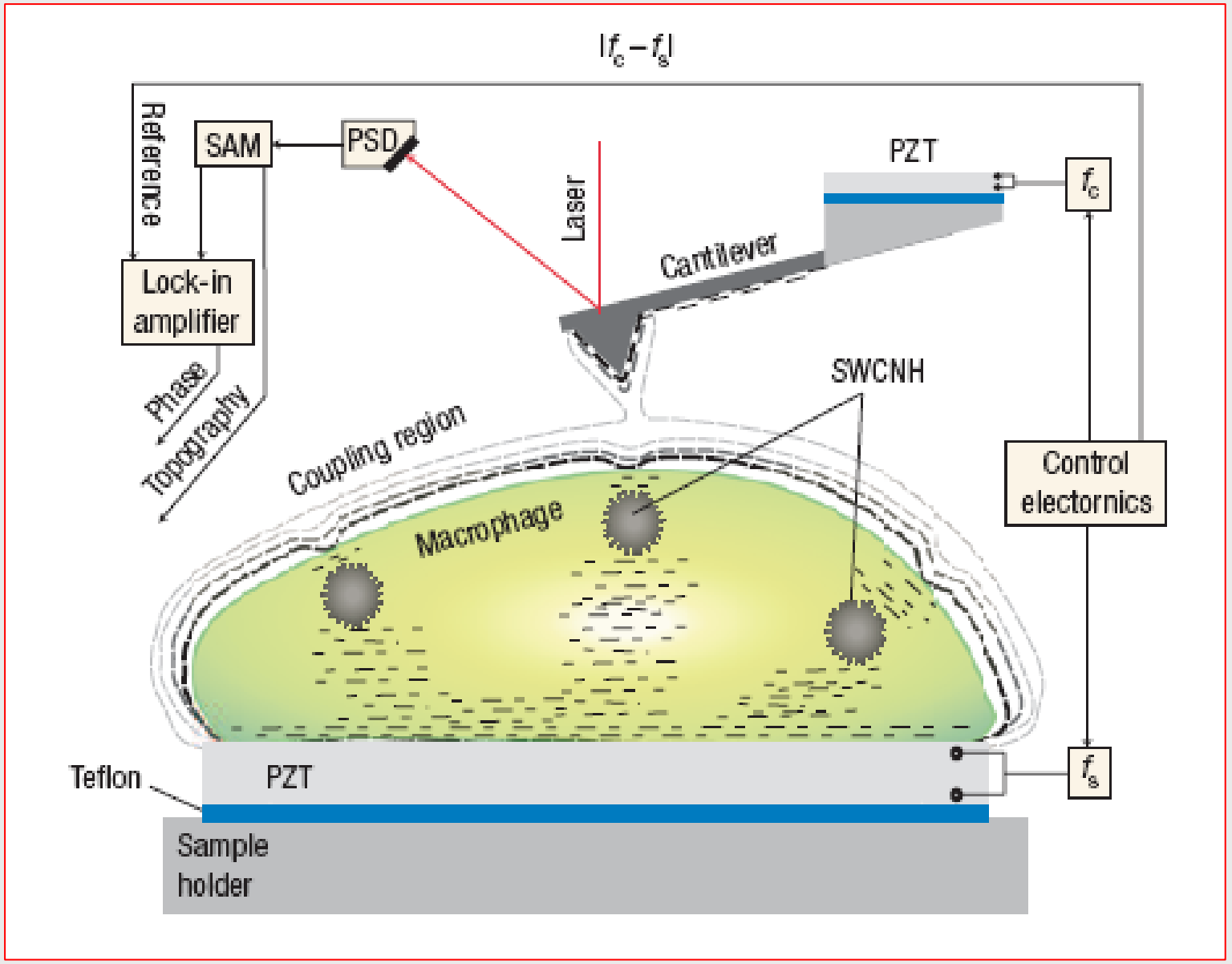
Reference number
ISO/TS 27687:2008(E)

First edition
2008-08-15

Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate

Nanotechnologies — Terminologie et définitions relatives aux nano-objets — Nanoparticule, nanofibre et nanoplat

Detection of Nanoparticles in Biological Cells



Re: L Tetard et al., Nature Nanotechn, 3:505-505 (2008)

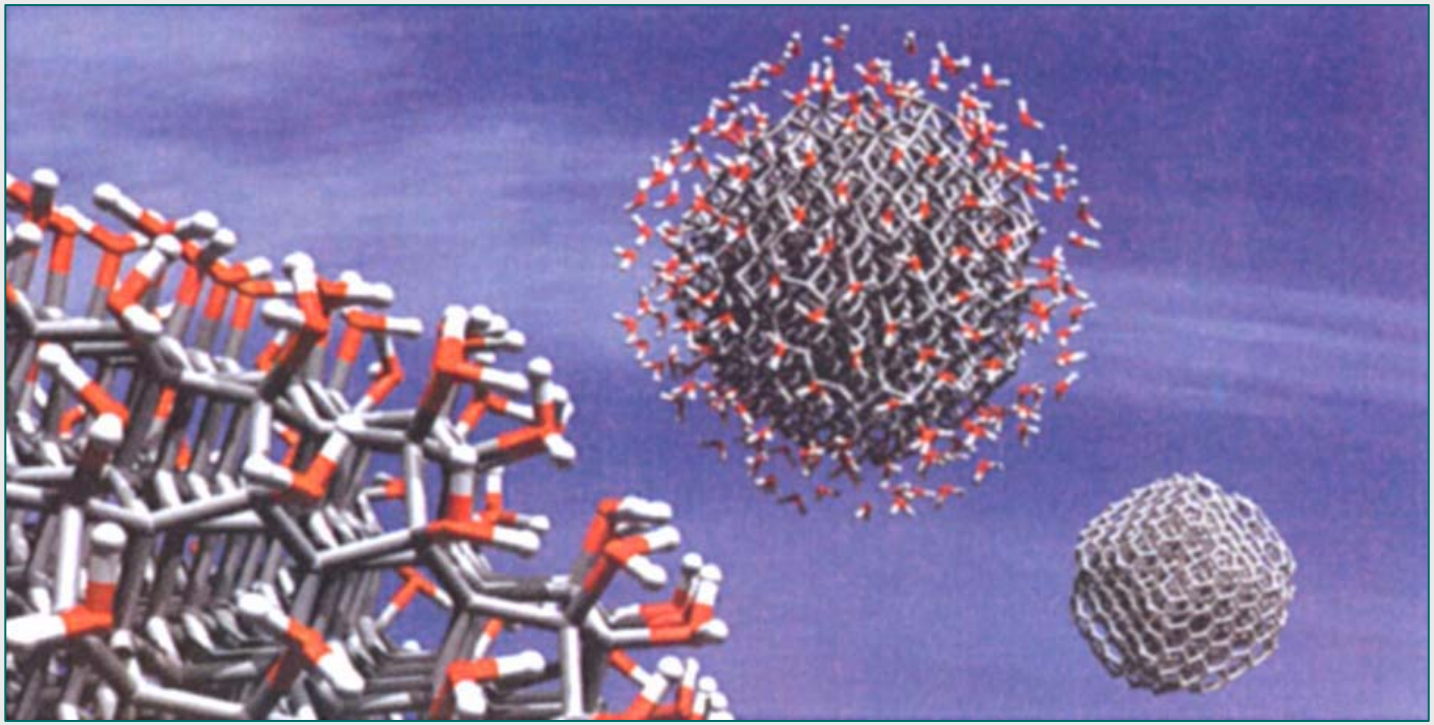
Possible Pathological Risks of Nanoparticles

Experimental NM effects	Possible pathophysiological outcomes
ROS generation*	Protein, DNA and membrane injury,* oxidative stress†
Oxidative stress*	Phase II enzyme induction, inflammation,† mitochondrial perturbation*
Mitochondrial perturbation*	Inner membrane damage,* permeability transition (PT) pore opening,* energy failure,* apoptosis,* apo-necrosis, cytotoxicity
Inflammation*	Tissue infiltration with inflammatory cells,† fibrosis,† granulomas,† atherogenesis,† acute phase protein expression (e.g., C-reactive protein)
Uptake by reticulo-endothelial system*	Asymptomatic sequestration and storage in liver,* spleen, lymph nodes,† possible organ enlargement and dysfunction
Protein denaturation, degradation*	Loss of enzyme activity,* auto-antigenicity
Nuclear uptake*	DNA damage, nucleoprotein clumping,* autoantigens
Uptake in neuronal tissue*	Brain and peripheral nervous system injury
Perturbation of phagocytic function,* "particle overload," mediator release*	Chronic inflammation,† fibrosis,† granulomas,† interference in clearance of infectious agents†
Endothelial dysfunction, effects on blood clotting*	Atherogenesis,* thrombosis,* stroke, myocardial infarction
Generation of neoantigens, breakdown in immune tolerance	Autoimmunity, adjuvant effects
Altered cell cycle regulation	Proliferation, cell cycle arrest, senescence
DNA damage	Mutagenesis, metaplasia, carcinogenesis

A Nel et al.
Science, 311:622-627 (2006)

Questions still open

- Standardisation of test procedures and assays for risk analysis?
- Dose – Response principles?
  - **Individual particles / Agglomerates?**
  - **Mass or particlenumber?**
  - **Analysis of nanoparticles in tissue?**
  - **Limits for cell activation?**
  - ***In vitro* / *in vivo* differences**
  - **Biokinetiks? Bioburden?**
  -
- **Criteria for approval of med-products: case-related or general approach?**



A Riskfactor is not a disease!

R Glassock & C Winearls
Nephron Clin Pract, 110:c39-c47 (2008)

Exogeneous Toxins with fatal Consequences 2001 - 2005

III

CMJ 2001

CROATIAN MEDICAL JOURNAL
CMJ
42(6):606-610,2001
FORUM

Sudden Deaths of Croatian Hemodialysis Patients in October 2001

Vladimir Gašparović, Rajko Ostojić, Ira Gjenero-Marga

Department of Medicine, Zagreb University Hospital Center; [1]C
[2]Department of Medicine, Sisters of Mercy University Hospital, 2

In 2001, there were 2,719 patients with chronic renal failure dialy
10.3%, similar to that in other countries. On October 12, 2001, th
mation that four patients unexpectedly died in the dialysis center i
week, a total of 23 dialysis patients died in Croatia, of whom 5 du
hemodialysis. Those events prompted us to assess the epidemiolo
We used phone contacts and reports of regional centers to collect
was characterized by dyspnea, hypotension, and cardiac arrest; re
all possible risk elements associated with hemodialysis revealed th
were different in all cases, and that the only common denominat
tured by *Baxter*, USA, and distributed by *Pliva*, Croatia.

Key words: *Croatia; fluorocarbons; membranes, artificial; renal dialysi*

NDT 2002

Nephrol Dial Transplant (2002) 17: 545–548

Performance liquid test as a cause for sudden deaths of dialysis patients: perfluorohydrocarbon, a previously unrecognized hazard for dialysis patients

Bernard Canaud on behalf of the European Experts Panel*

Department of Nephrology, Lapeyronie University Hospital, Montpellier, France

Keywords: haemodialysis hazards; life-threatening complications; perfluorocarbon toxicity

Haemodialysis hazards

Over the last two decades, routinely performed haemodialysis (HD) has continued to evolve as a safe procedure of renal replacement therapy (RRT). Despite the apparent overall safety of the HD procedure, the nephrology community must not forget that HD can be the cause of serious reactions leading eventually to life-threatening complications [1,2]. The

JASN 2005

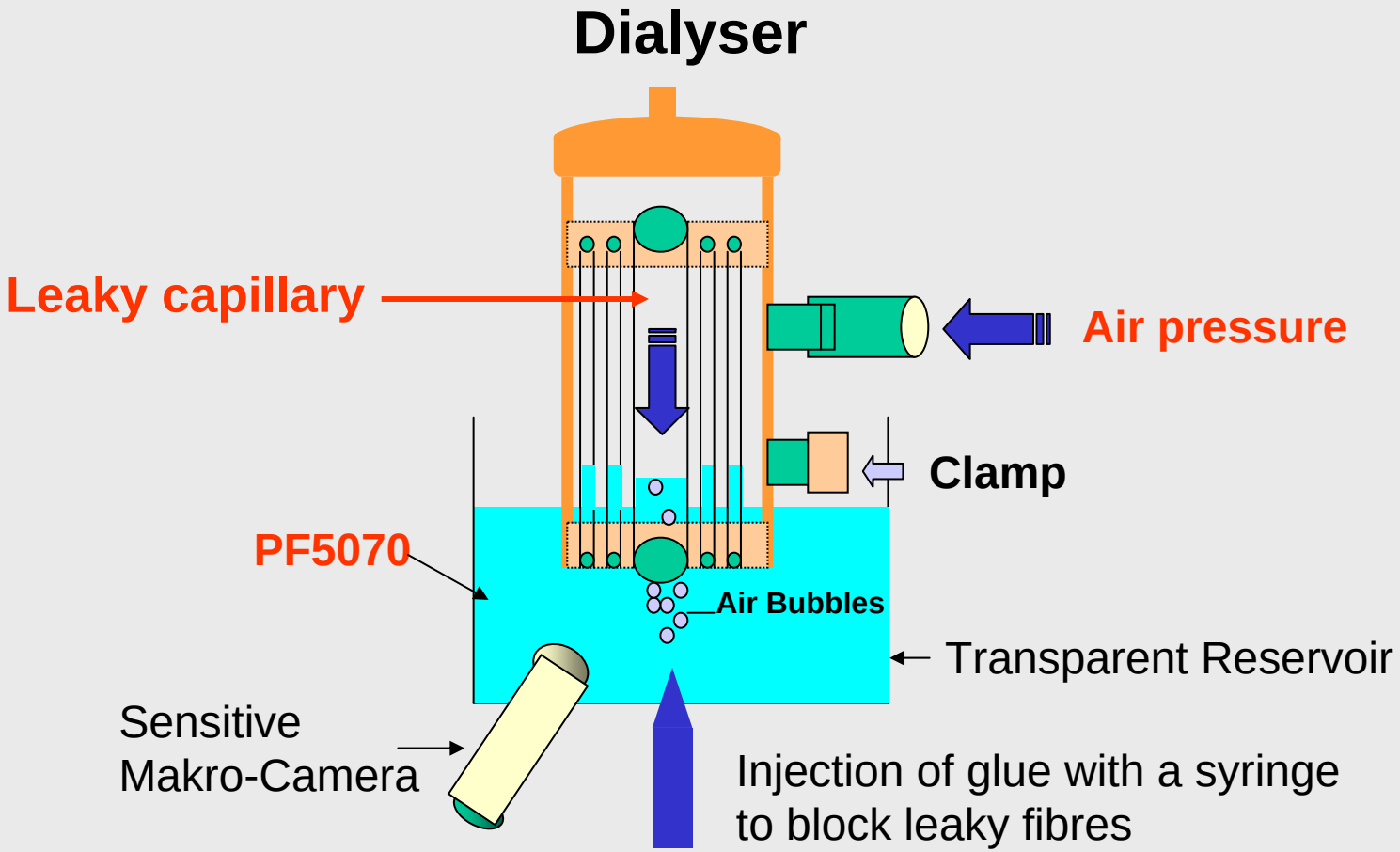
Pathochemical Toxicity of Perfluorocarbon-5070, a Liquid Test Performance Fluid Previously Used in Dialyzer Manufacturing, Confirmed in Animal Experiment

Bernard Canaud,* Pedro Aljama,† Christian Tielemans,‡ Vladimir Gasparovic,§ Alberto Gutierrez,‖ Francesco Locatelli¶

Nephrology, Lapeyronie University Hospital, Montpellier, France; †Nephrology, Reina Sofia University Hospital, Cordoba, Spain; ‡Erasme Hospital, Brussels, Belgium; §Department of Internal Medicine, Emergency & Intensive Care, Rebro, Zagreb, Croatia; ‖Department of Clinical Science, Division of Renal Medicine, Huddinge University Hospital, Huddinge, Sweden; ¶Department of Nephrology, Alessandro Manzoni Hospital, Lecco, Italy

In the light of clustered deaths in late 2001 associated with hemodialysis (HD), this article analyzes the pathochemical toxicity of the perfluorocarbon-5070 (PF-5070), a liquid used as test performance fluid for detecting capillary leaks during dialyzer manufacturing. Residual PF-5070 in some Althane dialyzers of the involved brands was infused in the injured patients during hemodialysis. The clinical presentation was in contrast with other previously described severe reactions to HD. Foam material was discovered in the right ventricle and caval vein of the patients who underwent postmortem examination. Deaths were attributed to gas embolism without the external causes identified. To explore the pathochemical toxicity of the inert liquid PF-5070, an animal model was developed. In a rabbit model, single slug intravenous injections as bolus of increasing doses of PF-5070 were performed. In a first set of experiments, three groups of three rabbits were administered increasing doses of PF-5070 at 4, 40, or 160 μl/kg. After intravenous injection, the animals were observed for clinical signs of adverse effects and underwent autopsy after death. Doses were normalized to animal body weight to allow comparison with supposed patient

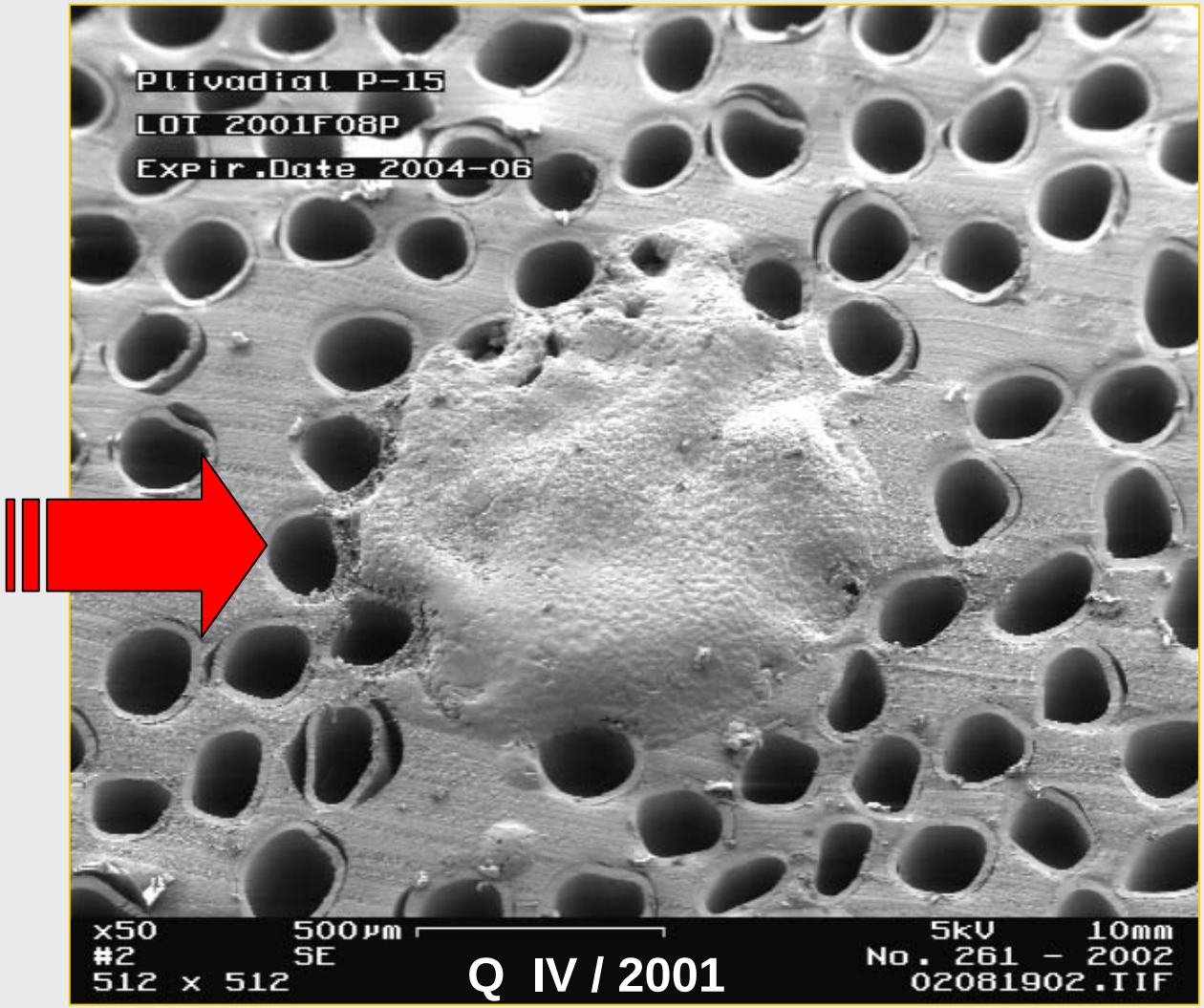
Dialyser - Repair
- with the help of Perfluorocarbon (PF5070) solutions -



Dialyser - Repair in 2001

Coverage and blockage of leaky fibers with PUR

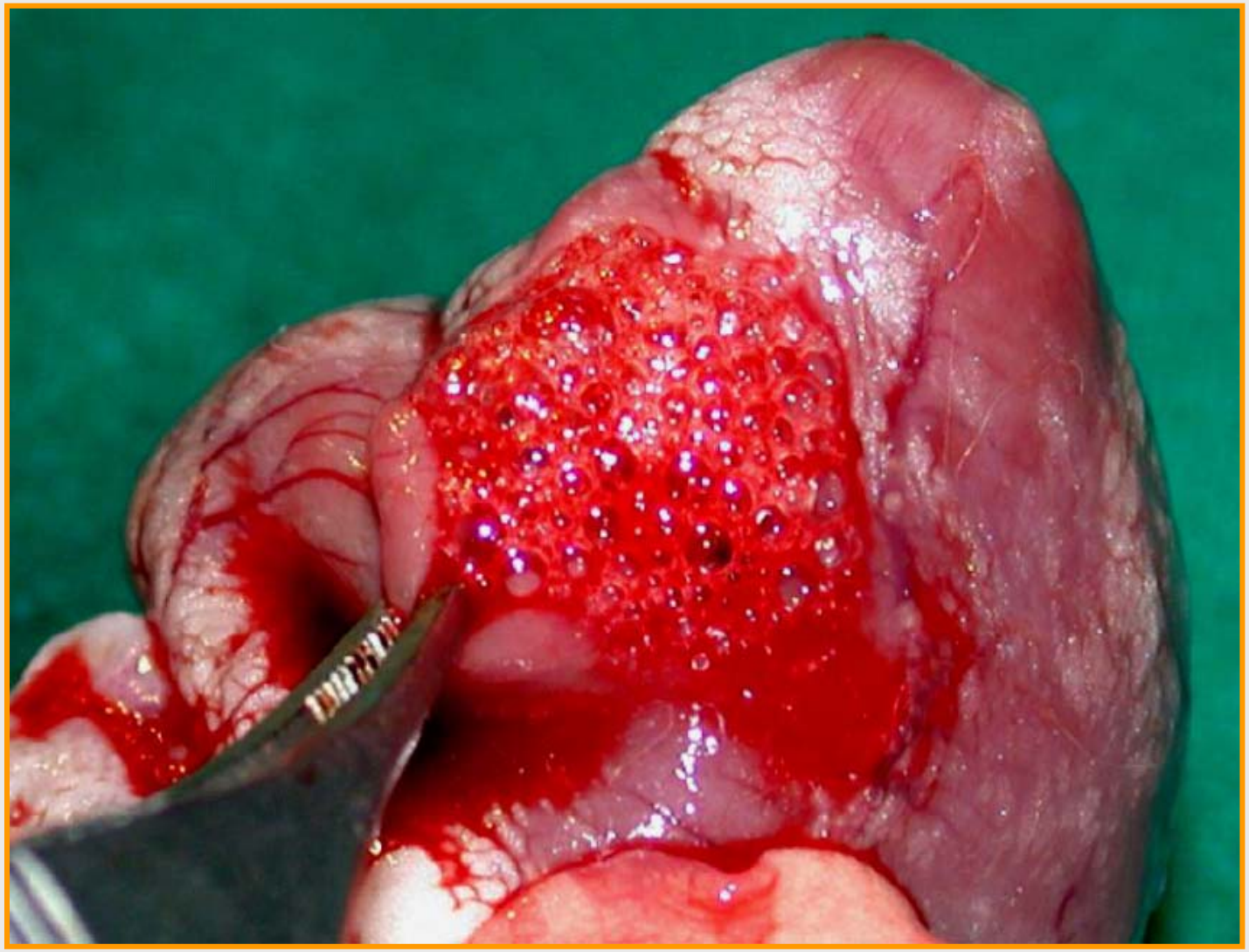
III



Heart of a Rabbit (after Autopsy)

Consequence of an Injection of 160 µl/kg PF5070

III



B Canaud et al.,
J Am Soc Nephrol, 16:1819-1823 (2005)

The New England Journal of Medicine

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VOLUME 346 FEBRUARY 14, 2002 NUMBER 7

PURE RED-CELL APLASIA AND ANTIERYTHROPOIETIN ANTIBODIES IN PATIENTS TREATED WITH RECOMBINANT ERYTHROPOIETIN

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Conclusions Neutralizing antierythropoietin antibodies and pure red-cell aplasia can develop in patients with the anemia of chronic renal failure during treatment with epoetin. (N Engl J Med 2002;346: 469-75.)

The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes

Katia Boven, Scott Stryker, John Knight, Adrian Thomas, Marc van Regenmortel, David M. Kemeny, David Power, Jerome Rossert, and Nicole Casadevall

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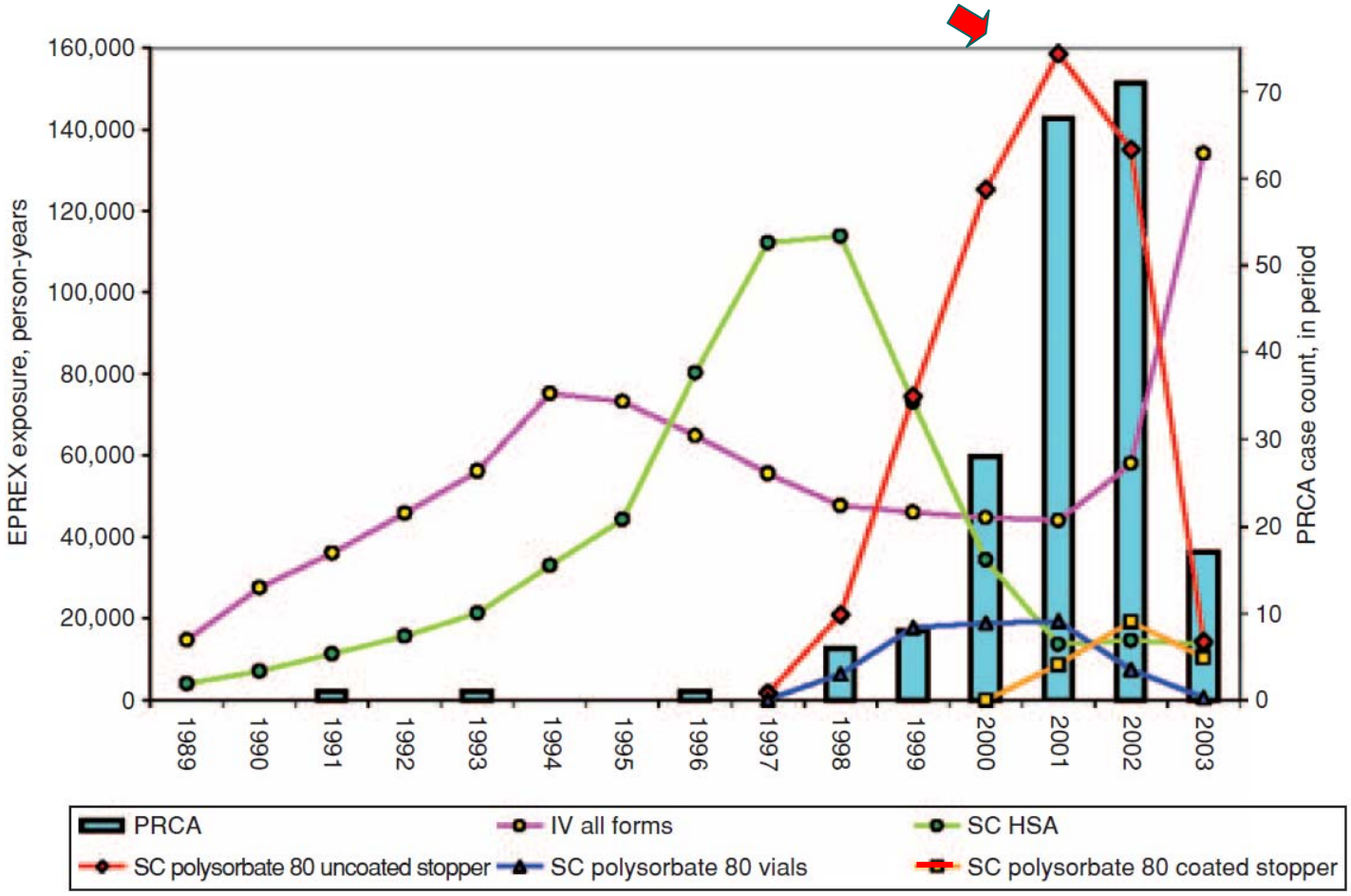

K Boven et al.,
Kidney Int, 67:2346 - 2353 (2005)

Aplasia (PRCA) in Dialysis patients

- Analysis of leachables by „Reverse Phase HPLC“ -

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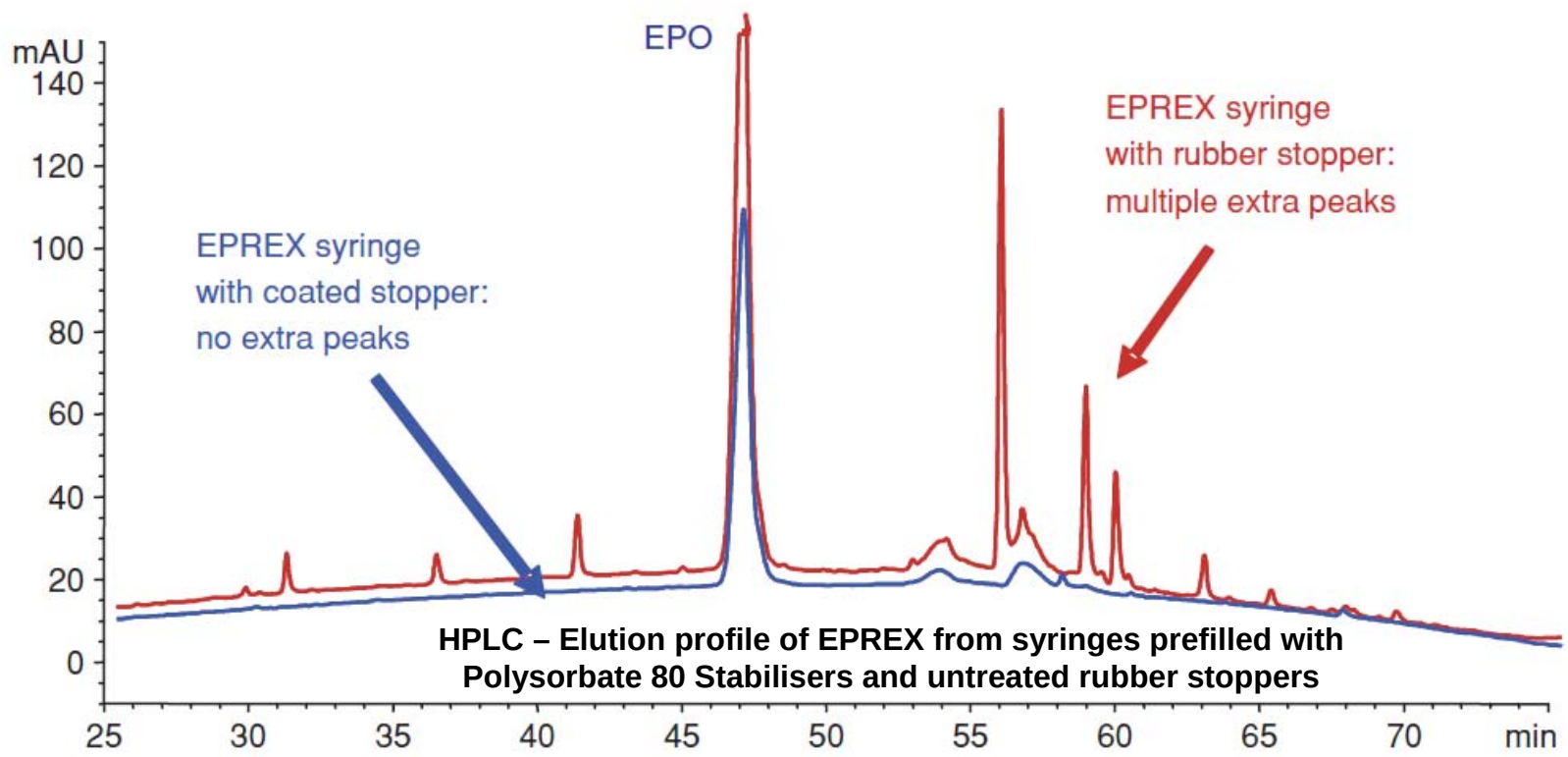

HPLC – Elution profile of EPREX from syringes prefilled with Polysorbate 80 Stabilisers and untreated rubber stoppers

PRCA:

- **acute severe isolated Anaemia**
- **no red precursor cells in bone marrow**
- **Reticulocyte number < $10x10^9$ / L**

K Boven et al.,
Kidney Int, 67:2346-2353 (2005)

Kidney International, Vol. 67 (2005), pp. 2346–2353

The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes

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The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes.

Background. The incidence of pure red cell aplasia (PRCA) in chronic kidney disease patients treated with epoetins increased substantially in 1998, was shown to be antibody mediated, and was associated predominantly with subcutaneous administration of Eprex®. A technical investigation identified organic compounds leached from uncoated rubber stoppers in prefilled syringes containing polysorbate 80 as the most probable cause of the increased immunogenicity.

Methods. This study investigated whether the incidence of PRCA was higher for exposure to the product form contain-

Pure red cell aplasia (PRCA) is a rare disorder that manifests itself as a severe, isolated anemia of sudden onset, characterized by an almost complete absence of red cell precursors in the bone marrow and a reticulocyte count below 10×10^9/L [1]. Many potential causes for PRCA have been reported, but most concern only isolated case reports, and about 50% of cases have no known cause [2]. Over the decade following its introduction in 1989, three cases of PRCA were associated with recombinant human erythropoietin (epoetin) treatment for

Conclusion. The epidemiologic data, together with the chemical and immunologic data, support the hypothesis that leachates from uncoated rubber syringe stoppers caused the increased incidence of PRCA associated with Eprex. Currently, all Eprex prefilled syringes contain fluoro-resin coated stoppers, which has contributed to decreased incidence of PRCA with continued surveillance.

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