Safer-by-Design Principles for Nanofibers at the Human-Nanotechnology Interface

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Acknowledgments

Rona Silva & Kent Pinkerton
University of California – Davis, CA

French Lab-Ex

Serenade
Safe(r) Ecodesign Research and Education applied to NAnomaterial DEvelopment.

EU–US ERA-NET

NanoWIr²es SIINN

National Institutes of Health
RC2 ESO18812

Safe by Design

Several definitions

Georgios Katalagarianakis, European Commission, Belgium

Monday 7th Nov

- Materials chosen for safety and performance
- Jobs always and everywhere done in a prescribed manner
- Develop skills along the complete manufacturing chain
- Operations should be sustainable
- Control and feedback mechanisms
- Learning from failures
- Acceptable risk insured against affordable premiums

Safe by Design

Several definitions

Georgios Katalagarianakis, European Commission, Belgium

Monday 7th Nov

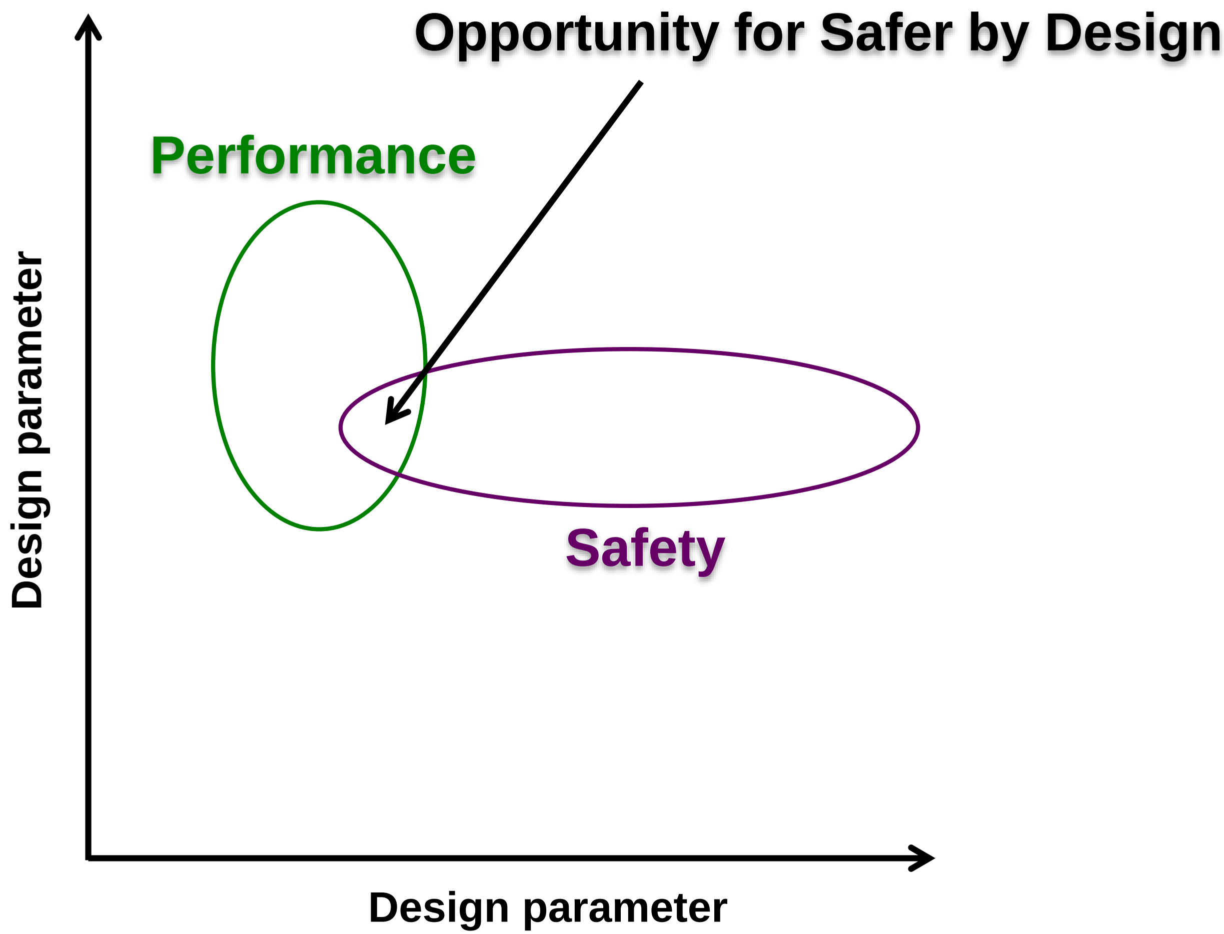
Consumer health

- Materials chosen for safety and performance
- Jobs always and everywhere done in a prescribed manner
- Develop skills along the complete manufacturing chain
- Operations should be sustainable
- Control and feedback mechanisms
- Learning from failures
- Acceptable risk insured against affordable premiums

Occupational health

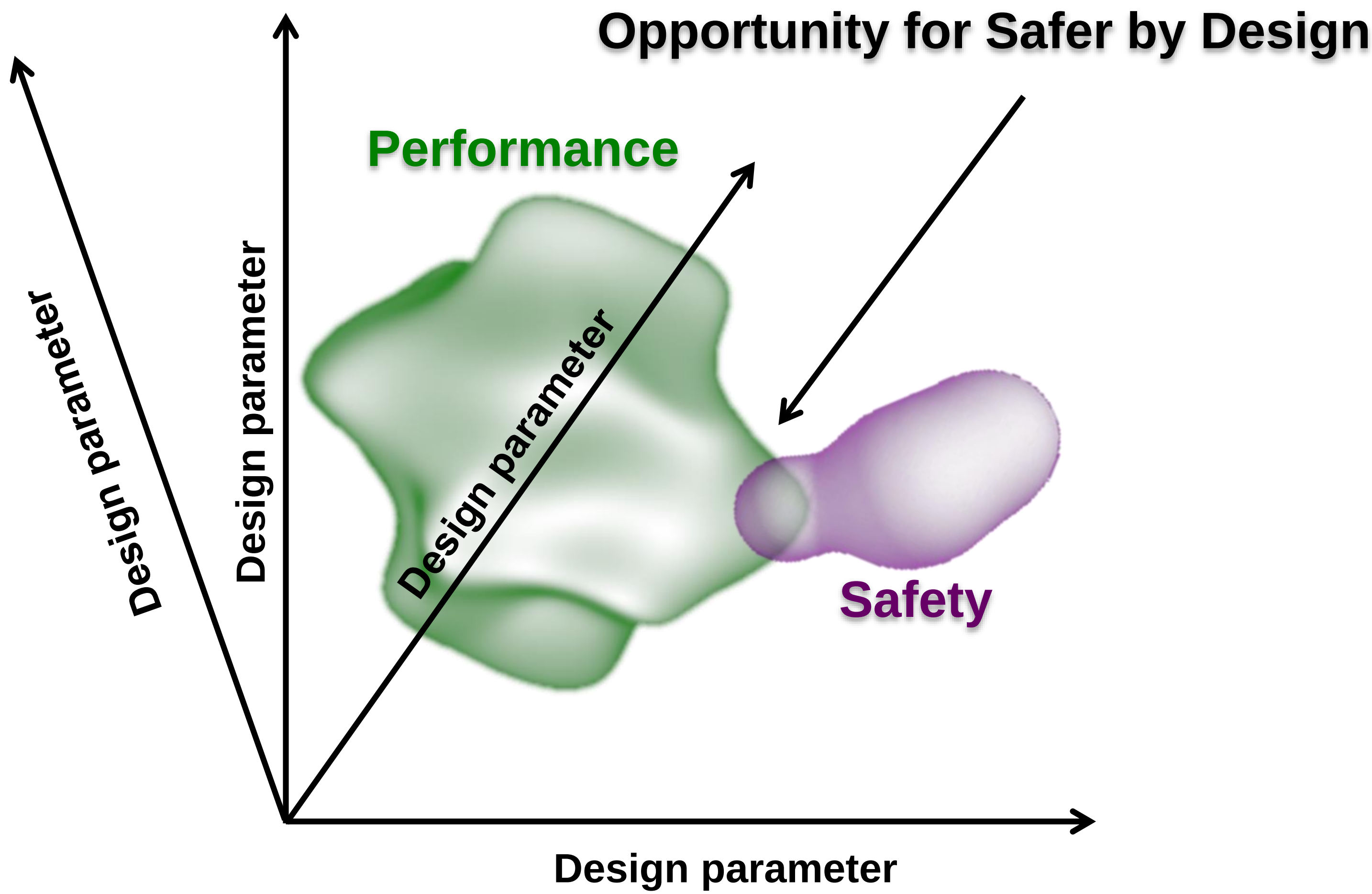
Safer by Design

The “Holy Grail”



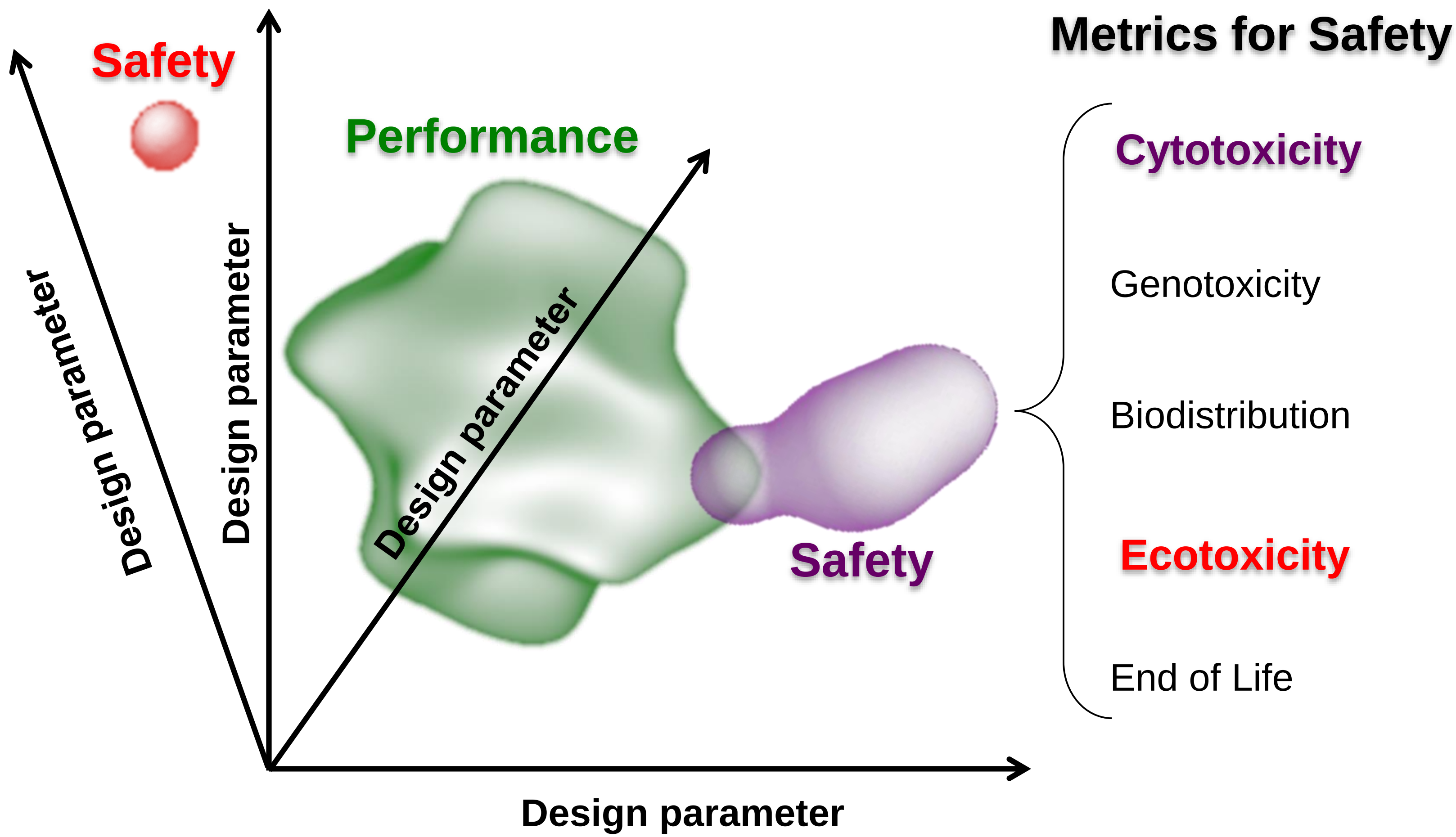
Safer by Design

The Challenge



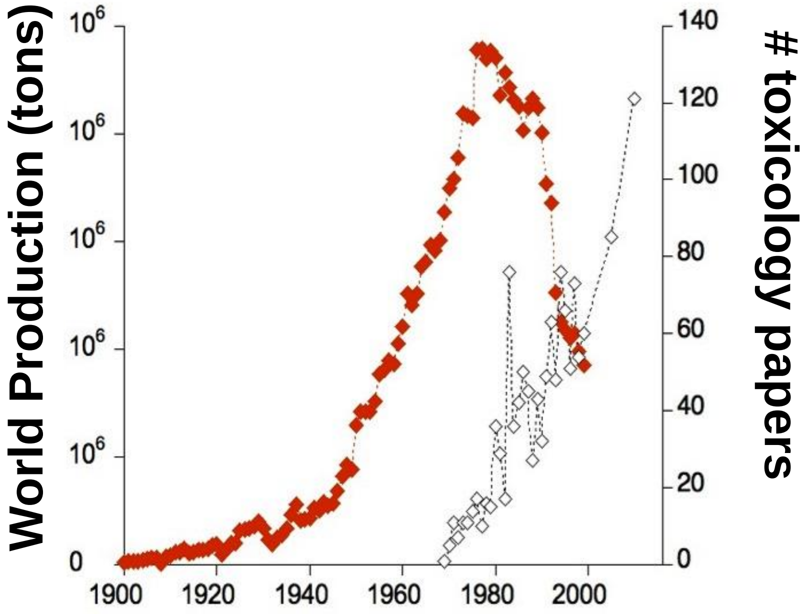
Safer by Design

The Challenge

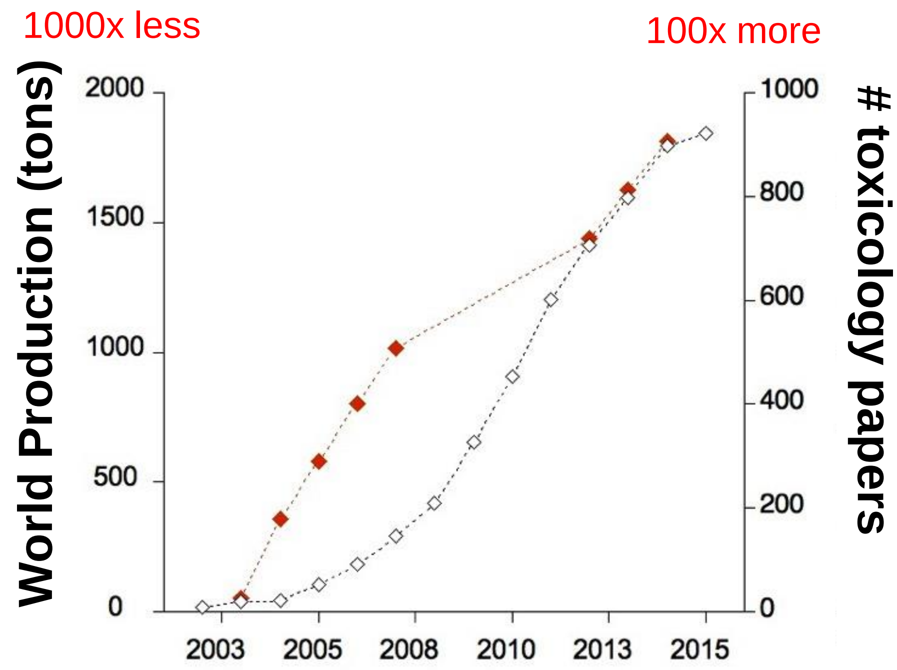


Can we Learn from Previous Examples?

Asbestos



Nanotechnologies



Graphs courtesy of Melanie Auffan, CEREGE

Safer by Design

A focus on nanomaterial SbD

Session 3.2: Safer by design nanomaterial and process // Auditorium Platine

Chair: Benjamin GILBERT, University of Berkeley, USA

SUB-SESSION 1: EU PROJECTS DEFINING SAFER BY DESIGN

PS3.2-1
2:45 - 3:00

Safe-by-design implementation approach in production systems: Case study, protective composite coatings based in Nano-particles

Rita Alberto, Marina Peixoto, Maria Farinha, Ana Cabral, Ricardo Rato, Marco Estrela, Eduardo Silva, (ISQ - Instituto de Soldadura e Qualidade, Portugal)

PS3.2-2
3:00 - 3:15

Banding approach for engineered nanomaterial risk assessment and control

Alain Pardon, Nausikaa Van Hoornick, Dimiter Prodanov, (IMEC, Belgium)

PS3.2-3
3:15 - 3:30

Implementation of the Nanoreg Safe-by-Design approach for different nanomaterial applications

Christian Micheletti, Marco Roman, Erik Tedesco, Iolanda Olivato, Federico Benetti, (ECAMRICERT-ECSIN, Italy)

PS3.2-4
3:30 - 3:45

NanoStreeM: Strategies for safety assessment in advanced integrated circuits manufacturing

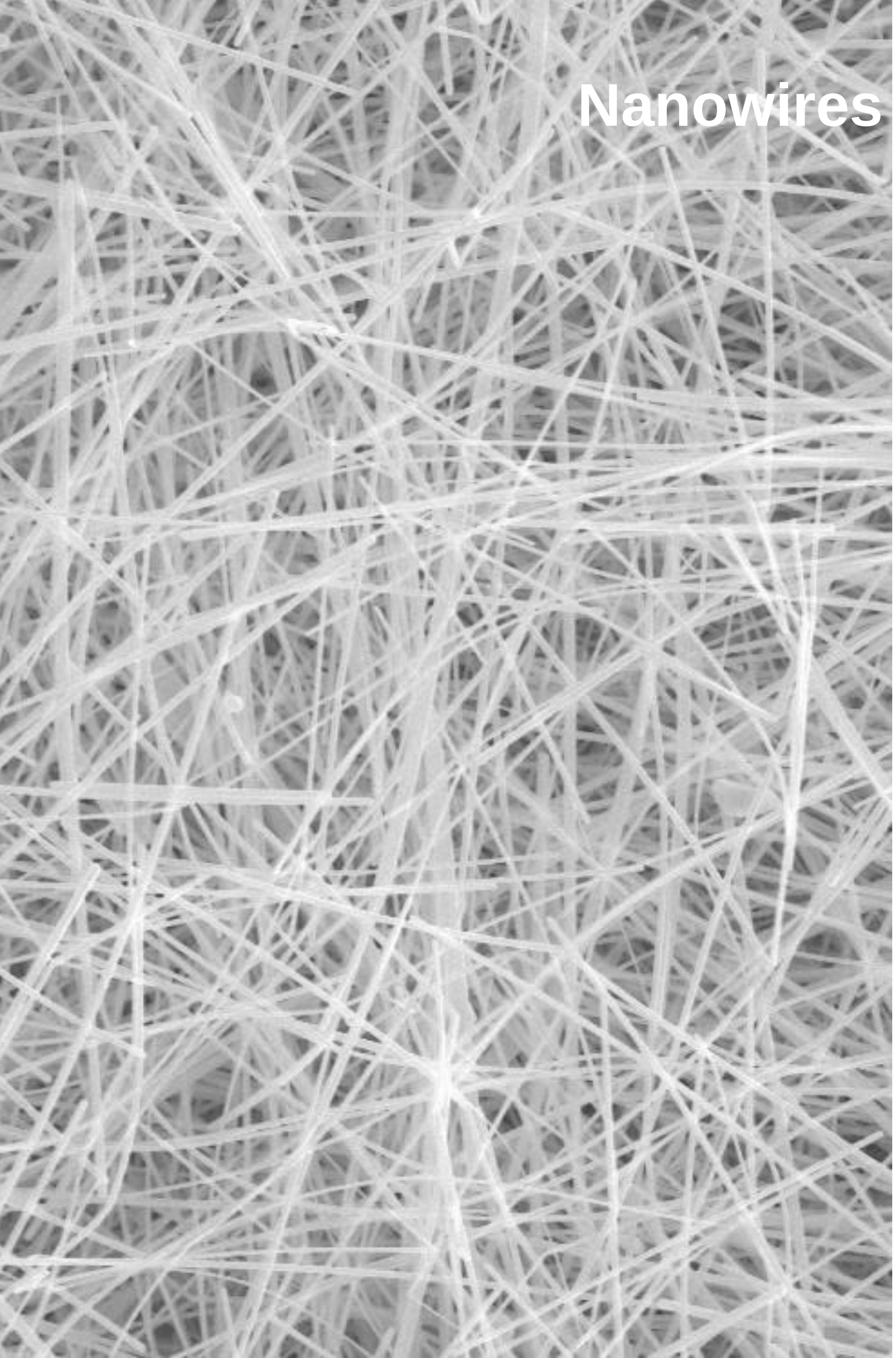
Dimiter Prodanov, (IMEC, Belgium)

PS3.2-5
3:45 - 4:00

Save and Sustainable by Design: A potential Approach for nanoinnovations

Christa Schimpel, Susanne Resch, Celina Vaquero, Andreas Falk, (BioNanoNet Forschungsgesellschaft mbH, Austria)

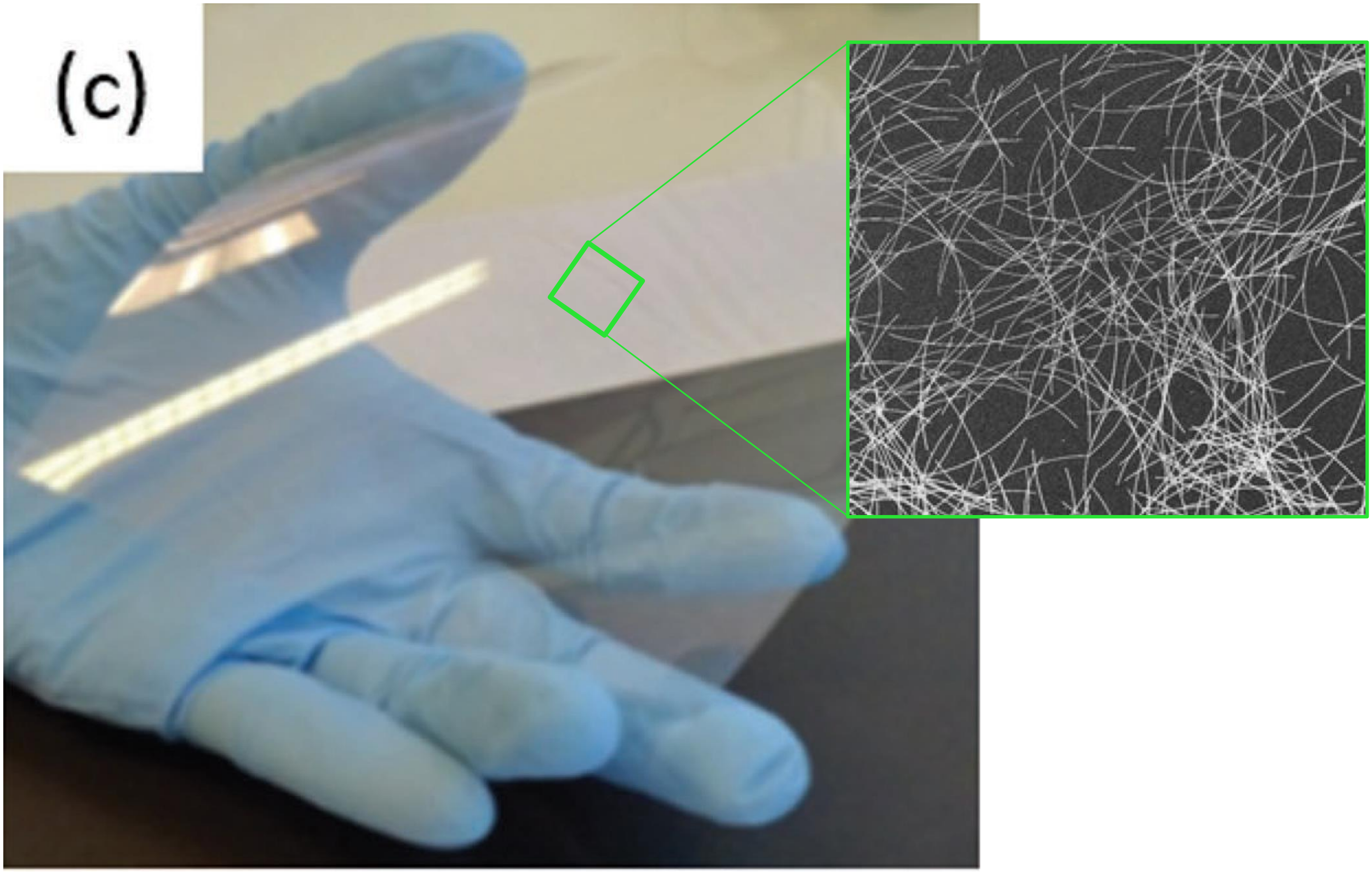
Nanowires and Nanofibers



Nb supercapacitor yarn
Mervakili ACS Nano 2015

Silver Nanowire Applications

Flexible electrically-conductive, optically-transparent networks



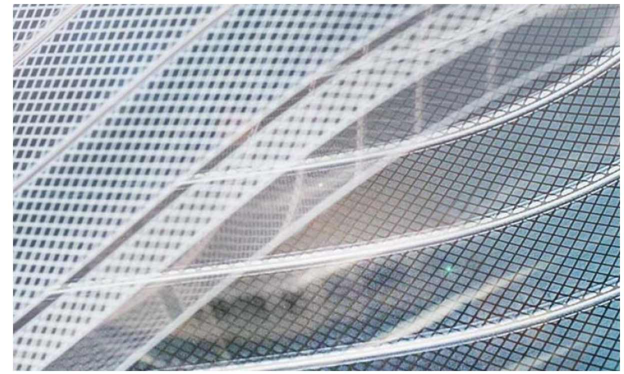
Sannicolo Small (2016)

Silver Nanowire Companies

cambrios ADVANCED MATERIALS

INNOVA DYNAMICS

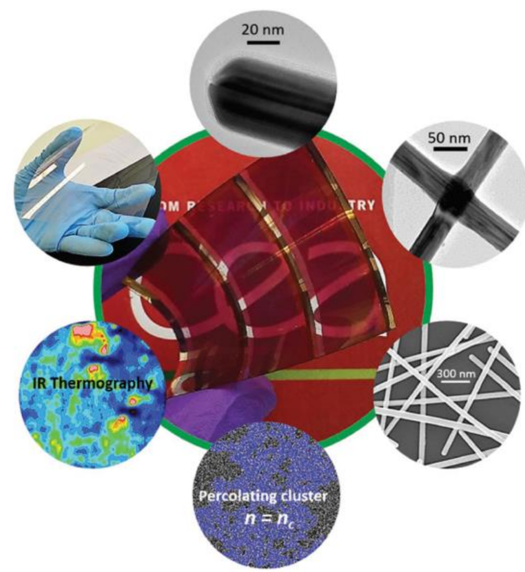
ARMOR



Curved or flexible touchscreens and displays

Windows and roofs with photovoltaics

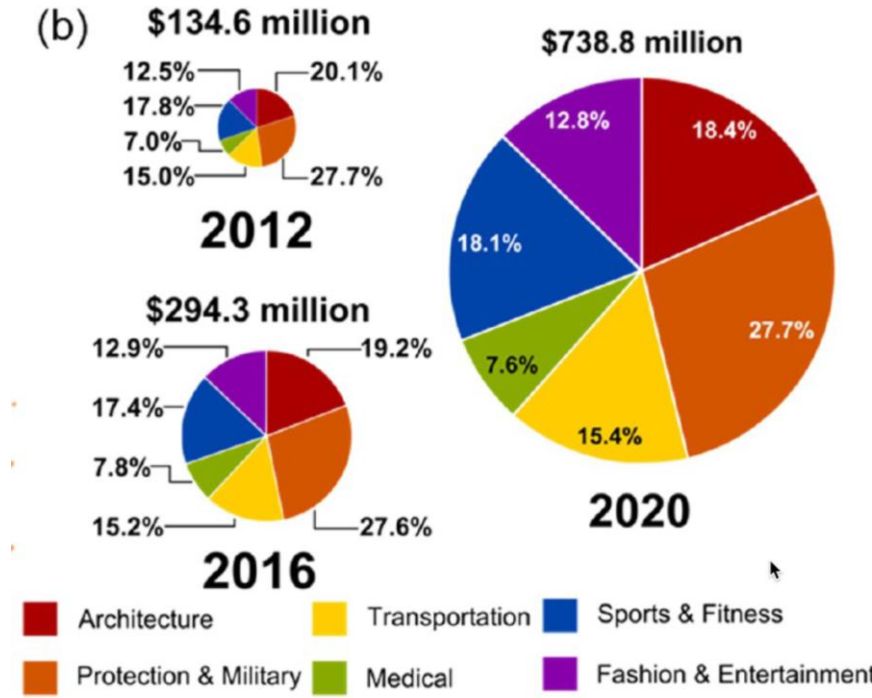
ITO market >$1.5B in 2015
Flexible display market >$10B by 2020



Smart Textile Companies

BeBop Sensors

American smart textiles market revenue 2012–2020



Advanced Functional Fabrics of America

affoa

MIT, Cambridge

Non-profit distributing $85M
8th Manufacturing Innovation Institute

Goals and Approach for SbD Research

Establish Consumer Exposure Scenarios

- Nanowire and nanofiber types
- Exposure pathways

Depend entirely on applications and commercial decisions

Determine Intrinsic Nanomaterial Toxicity

- Prior relevant epidemiological and toxicological data
- New research studies

1st Tier of SbD

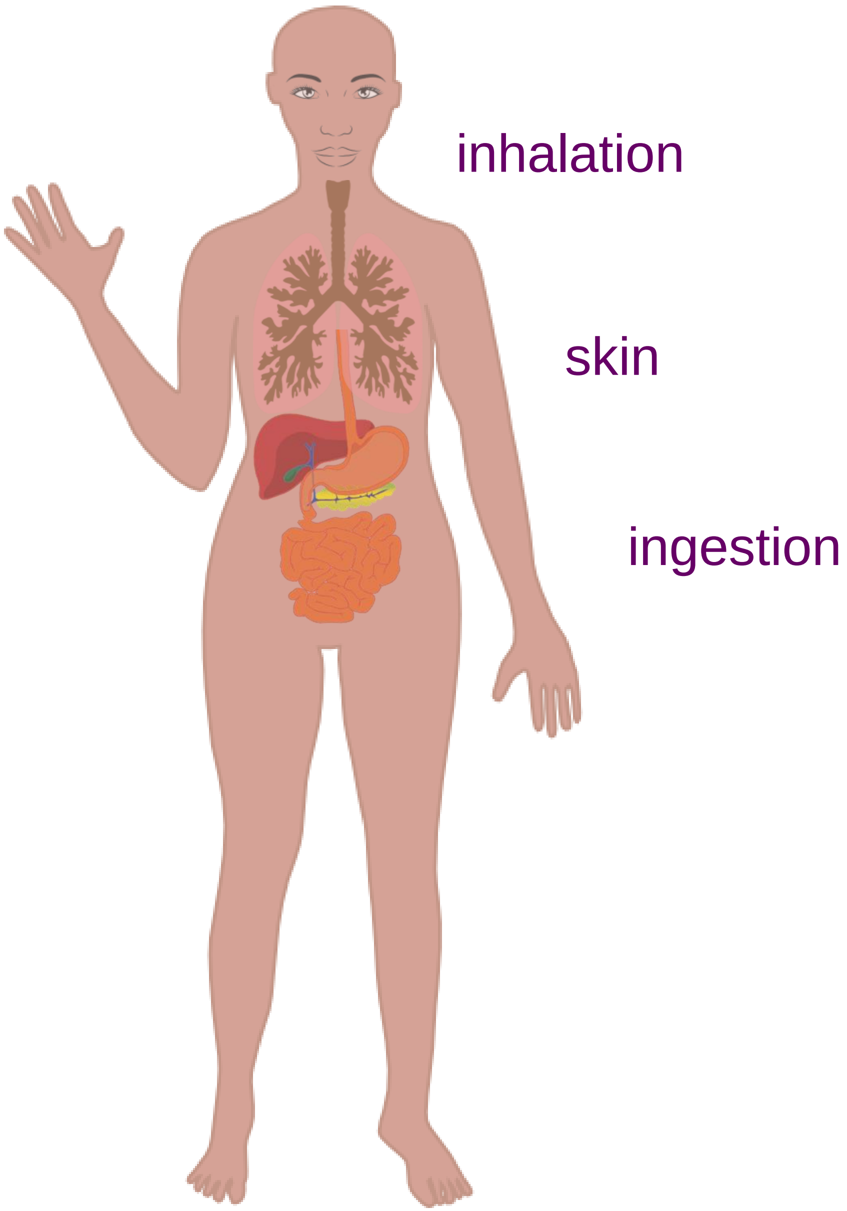
Find Opportunties to Mitigate Nanomaterial Toxicity

- Compromise between technological performance and nanomaterial hazard

Translate to Real Consumer Devices

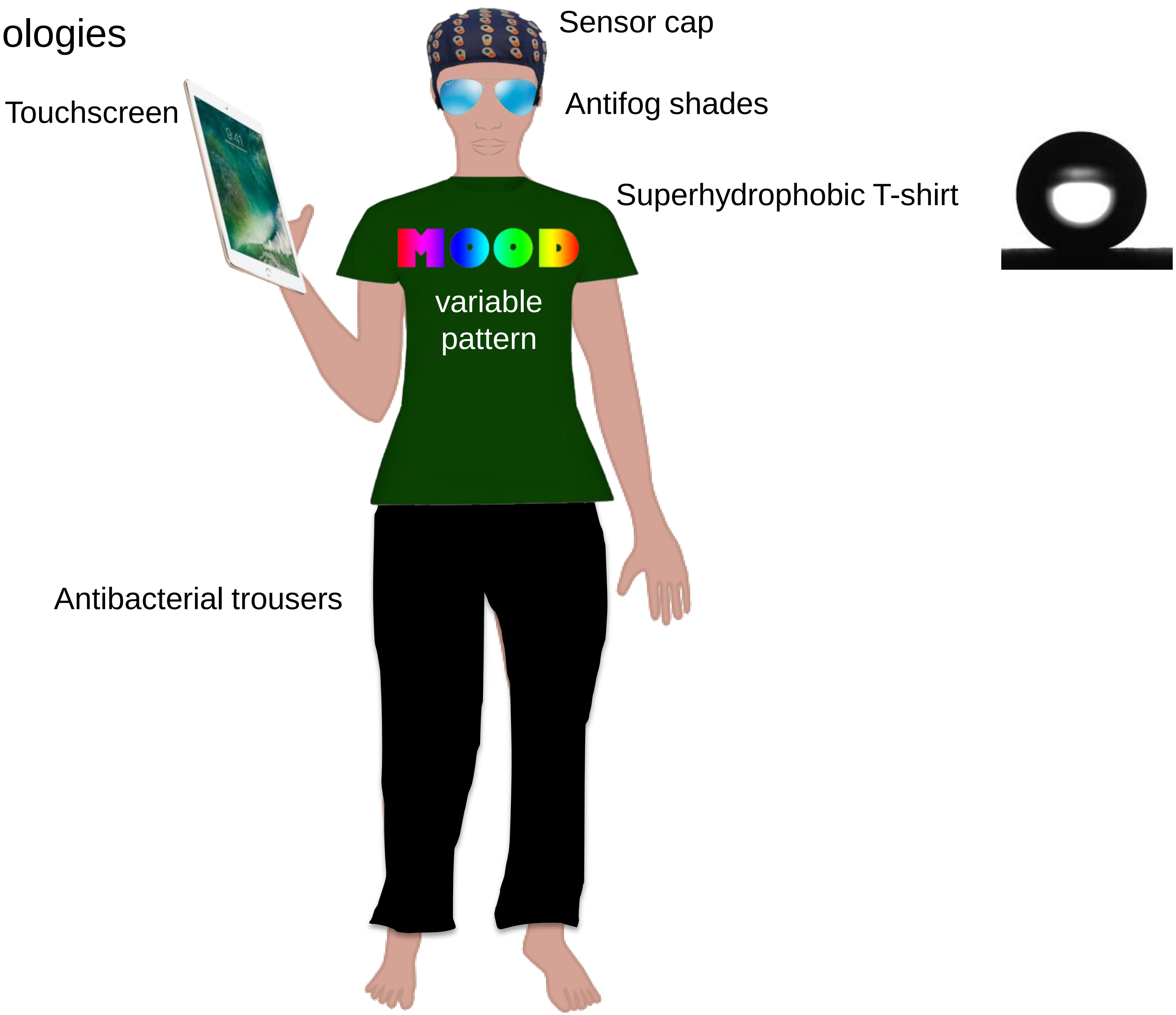
- Test that assumptions and strategies are still valid
- Develop additional tiers of Safer-by-Design safeguards as necessary

Human Exposure Pathways



Consumer Exposure Pathways for Nanowires and Nanofibers

Example technologies



Consumer Exposure Pathways for Nanowires and Nanofibers

Possible release of NM during use and washing

Inhalation exposure

From nano-clothes …

… to nano-lint?

Consumer Exposure Pathways for Nanowires and Nanofibers

Close association of nanomaterials and human skin

Skin exposure

The New Review, The Observer, p 35, Aug 14

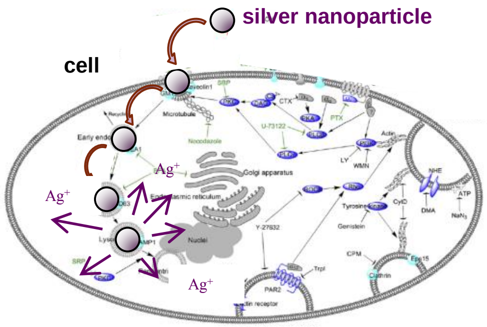
Possibly Predictive Paradigms

✓ Asbestos

Fiber Pathogenicity Paradigm

✓ Silver Nanoparticles

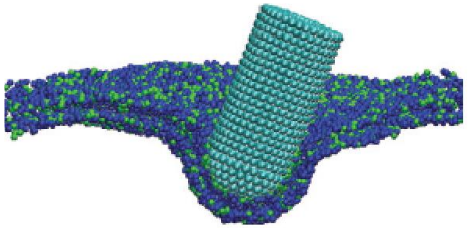
Internalization and dissolution



(✓) Carbon Nanotubes

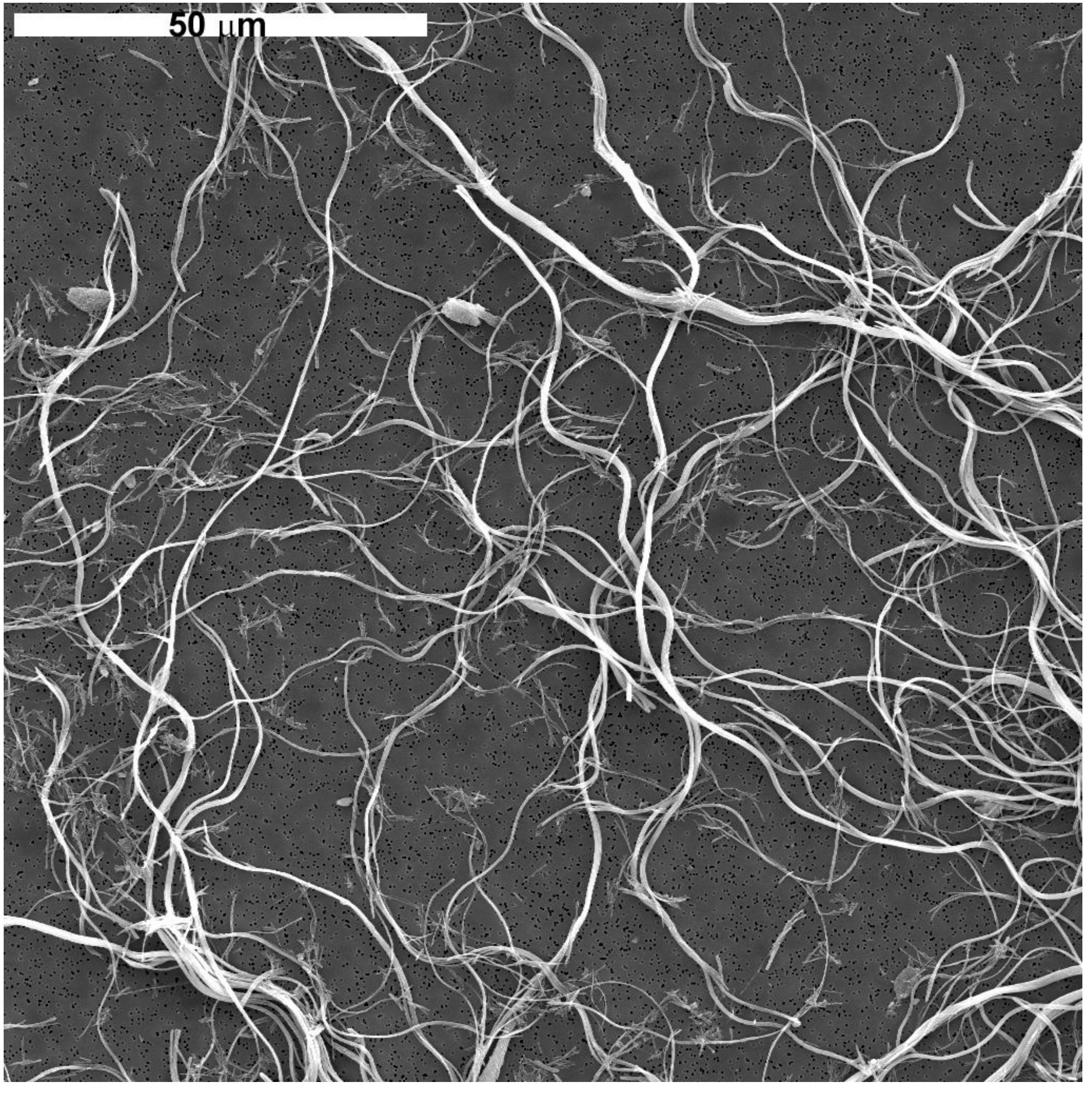
Active internalization via tip entry

Donaldson Part. Fiber Tox. (2010)



Shi Nature Materials (2011)

Asbestos



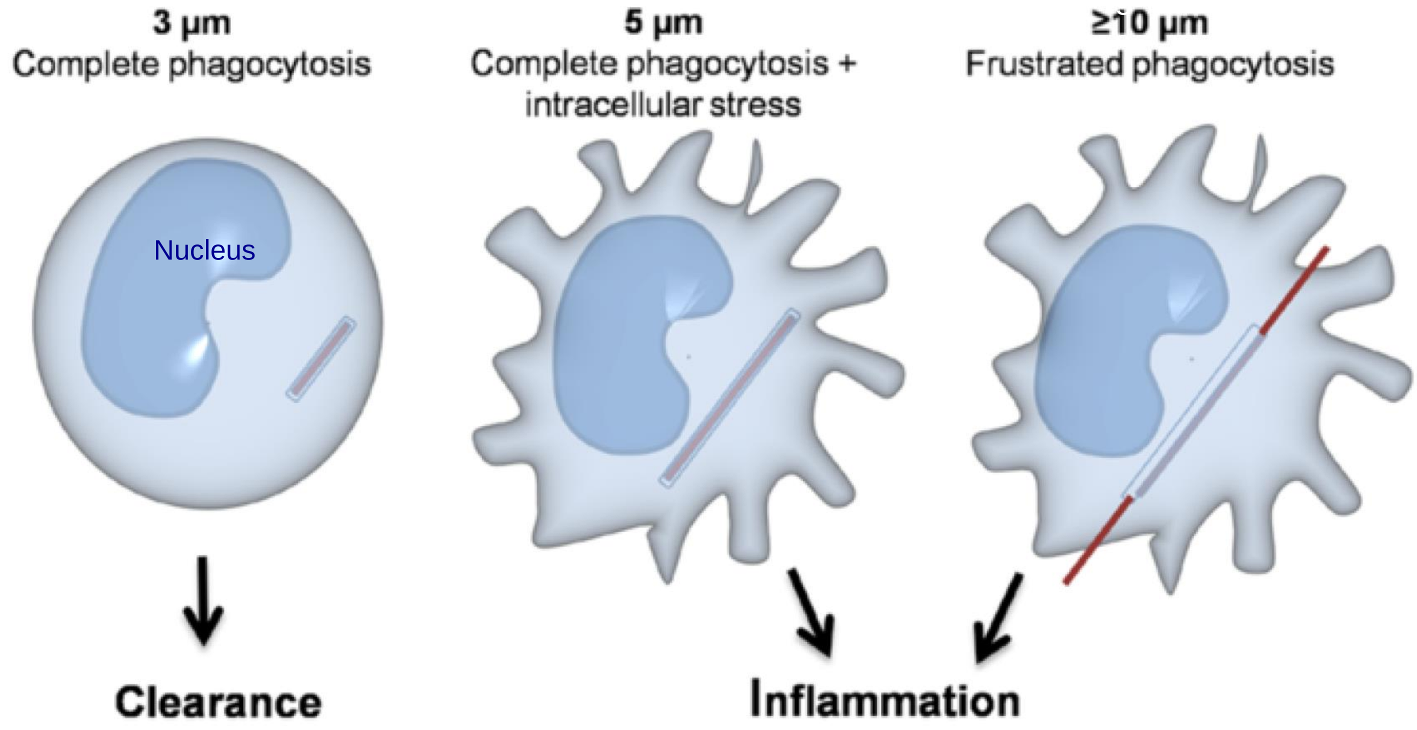
A diverse class of fibrous minerals

USGS Denver

Fiber Pathogenicity Paradigm

High-aspect-ratio fibers are highly inflammatory

- Macrophages cannot engulf and clear fibers
- Frustrated phagocytosis causes granulomas and chronic inflammation



Schinwald & Donaldson *Part. Fibre Tox.* (2012)

Fiber Pathogenicity Paradigm

Crystalline fibers are highly biopersistent

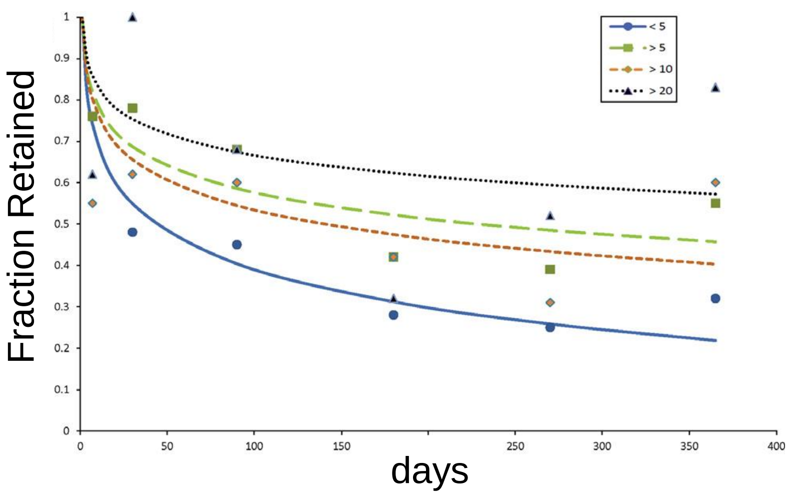
Substituation of asbestos for Synthetic Vitreous Fibers (SVF) is earlier example of (retroactive) Safer by Design

“The combined evidence from epidemiology and toxicology provide little evidence that exposure to SVF increases the risk of mesothelioma.”

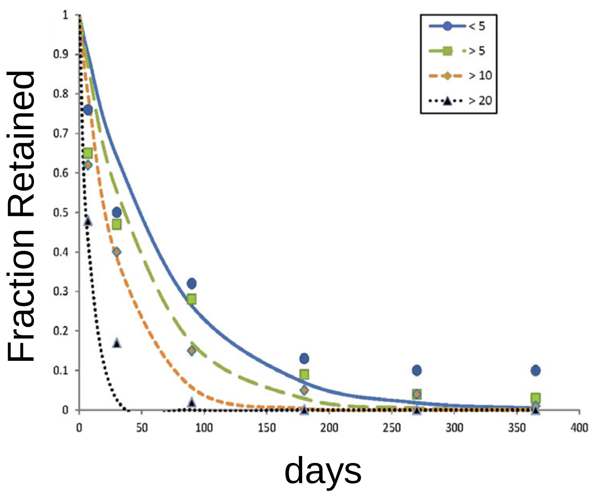
Boffetta *Critical Reviews Tox.* (2014)

Biopersistence of fibers in the rat lung

Crocidolite Asbestos



Fiber Glass Wool



Hesterberg *Reg. Tox. Pharma.* (2012)

Lessons from Prior Toxicity Studies

Long (>10 µm) crystalline fibers are highly biopersistent

Reduction of nanowire length

Soluble (amorphous) fibers much less toxic than insoluble (crystalline) fibers

$Ag(s)$ dissolution requires oxidation and generates $Ag^{+}(aq)$

Ag NW Toxicity Studies

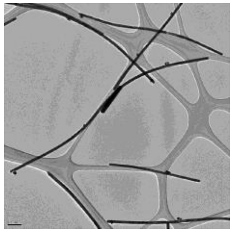
Mani Tagmount
Chris Vulpe

Rat Macrophage Toxicity *in vitro*

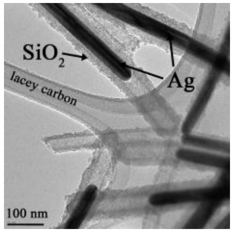
Cell viability

Dose-dependent cell death for all Ag NW studied

PVP-coated



Silica-coated



Markers of inflammation and oxidative stress

- Cell count
- Cell size
- Nuclear size & morphology
- Mitochondrial membrane potential
- Plasma Membrane permeability

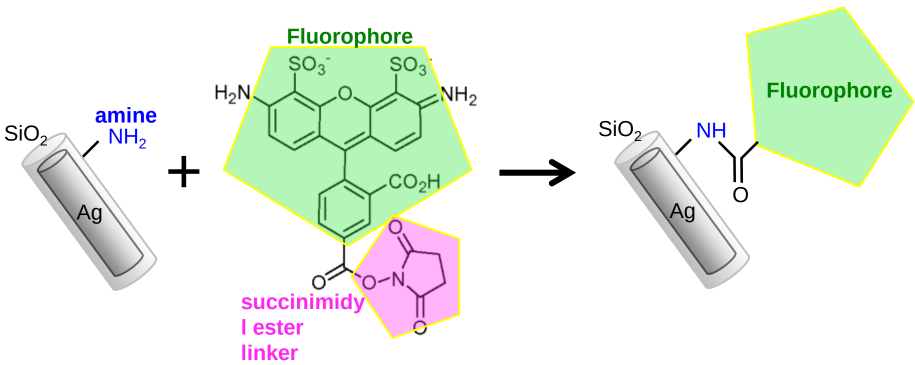
- Cell count
- DNA damage
- Oxidative stress
- Lysosomal mass - pH
- Externalization of phosphatidylserine
- Apoptosis
- IL beta release

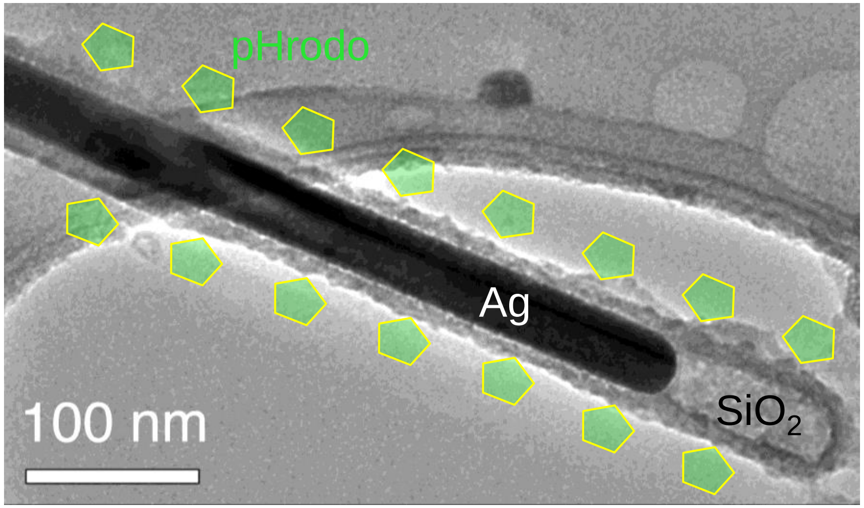
Test for the role of active internalization pathways

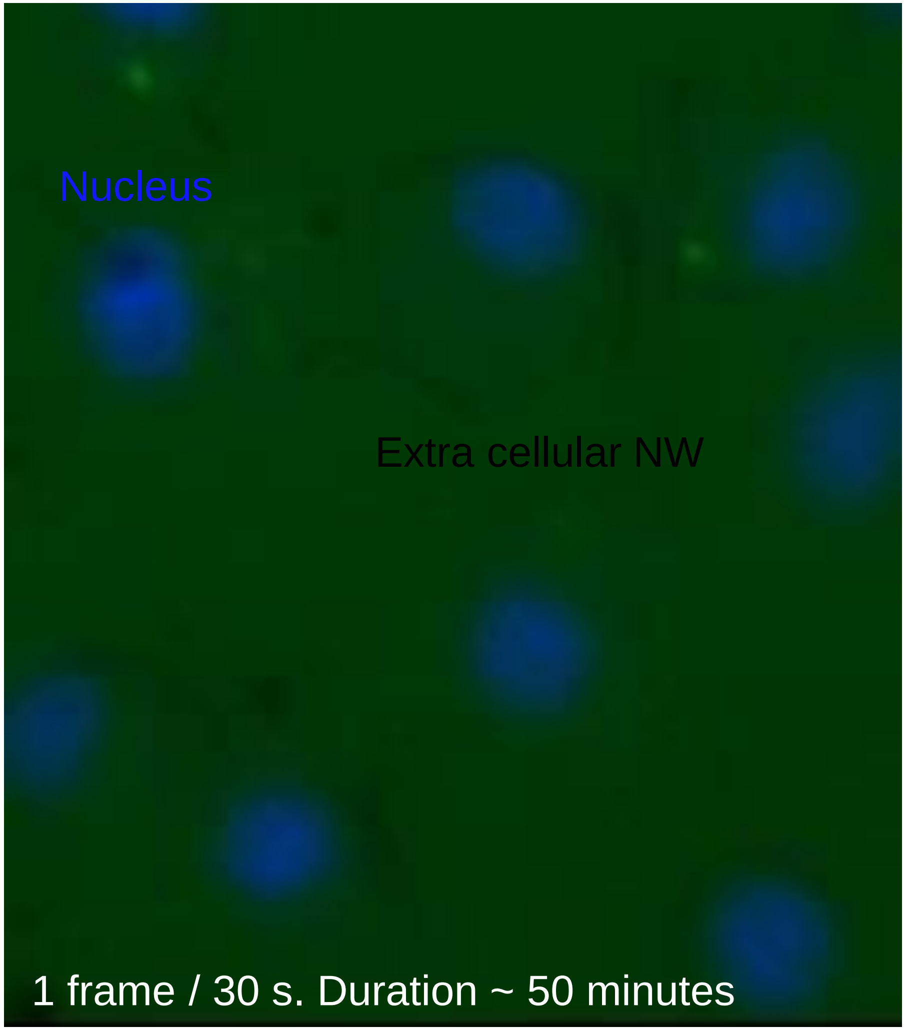
Rat Macrophage Toxicity *in vitro*

Observe internalization using time-lapse fluorescent microscopy

pHrodo is a pH-sensitive dye (**green** at low pH) can be linked to amino groups

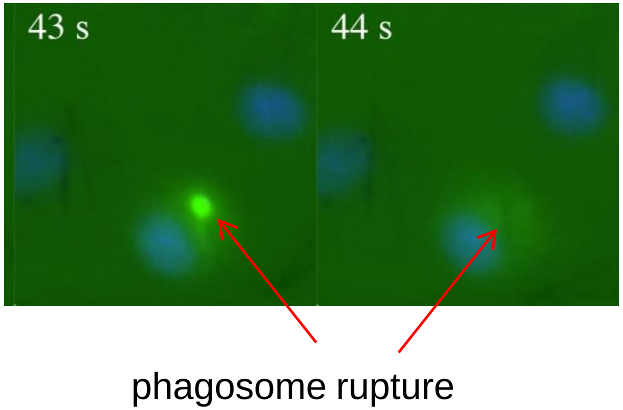


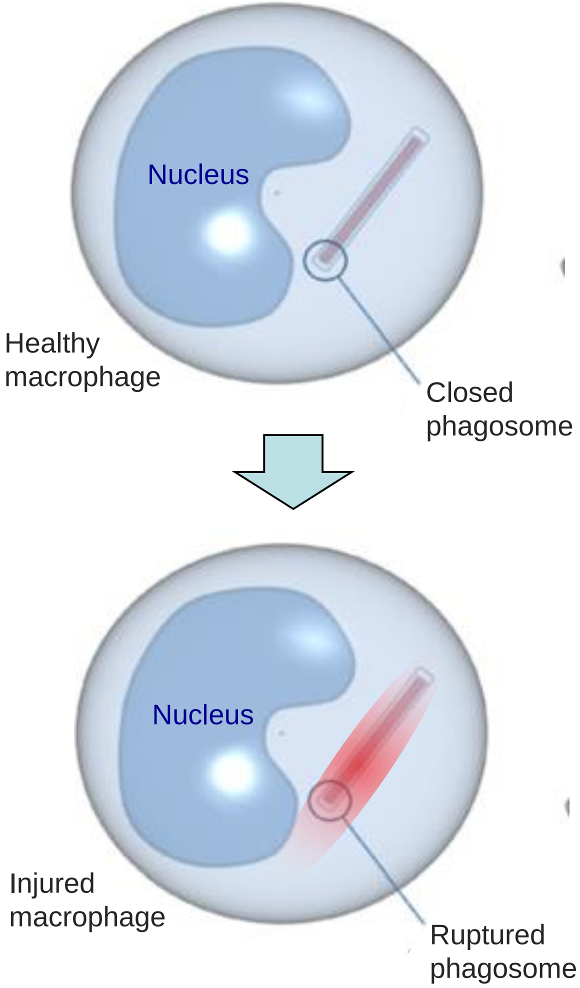




Rat Macrophage Toxicity *in vitro*

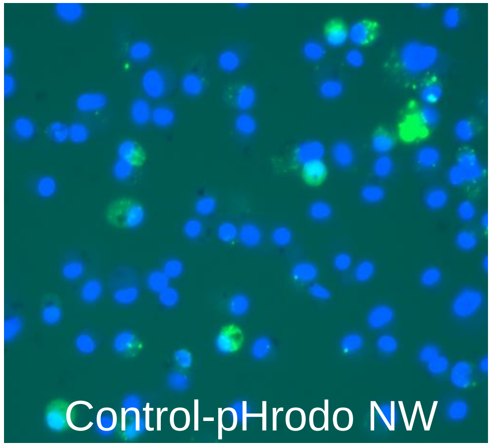
Phagosome rupture following phagocytosis

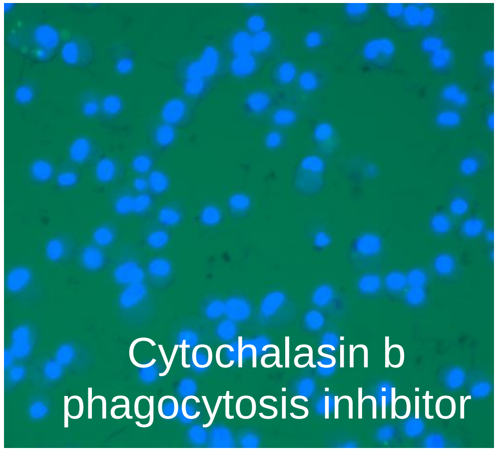


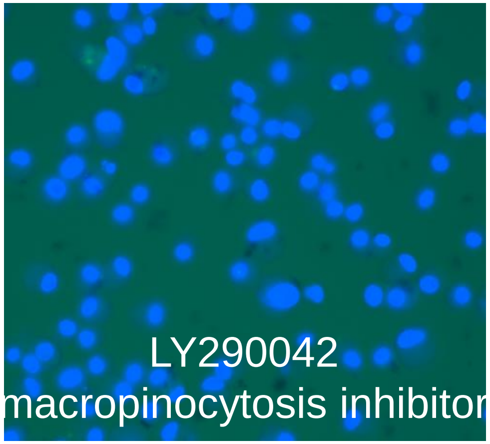


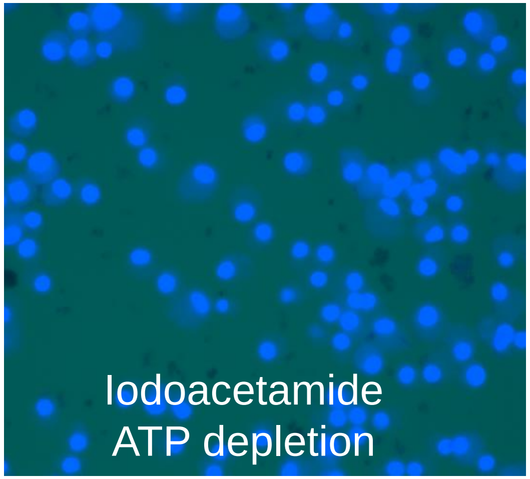
Rat Macrophage Toxicity *in vitro*

Internalization of 2-µm AgNW is reduced by inhibiting ATP-dependent uptake

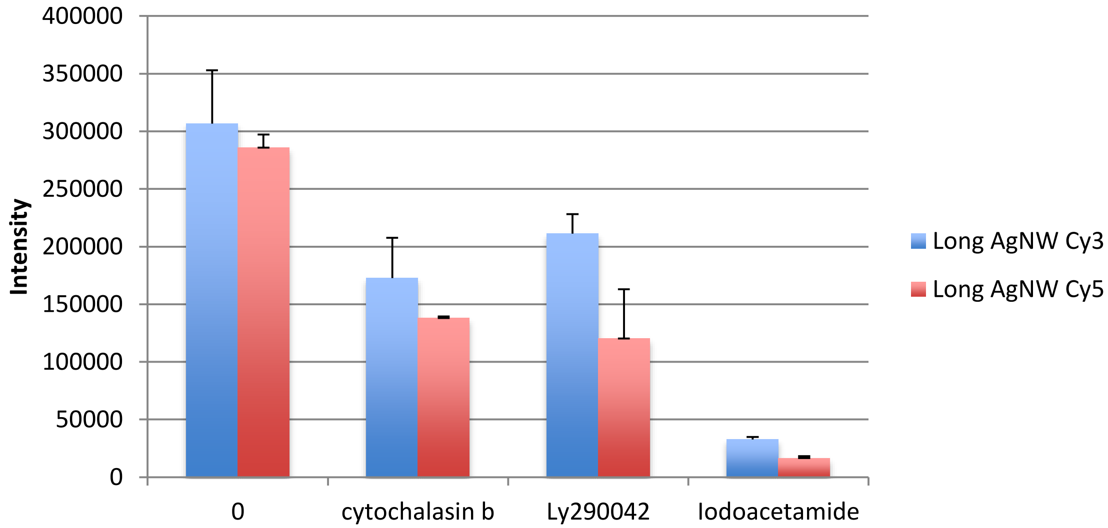








Effect of Cytochalasin b, LY290042 and iodoacetamide on AgNW uptake



Rona Silva
Kent Pinkerton

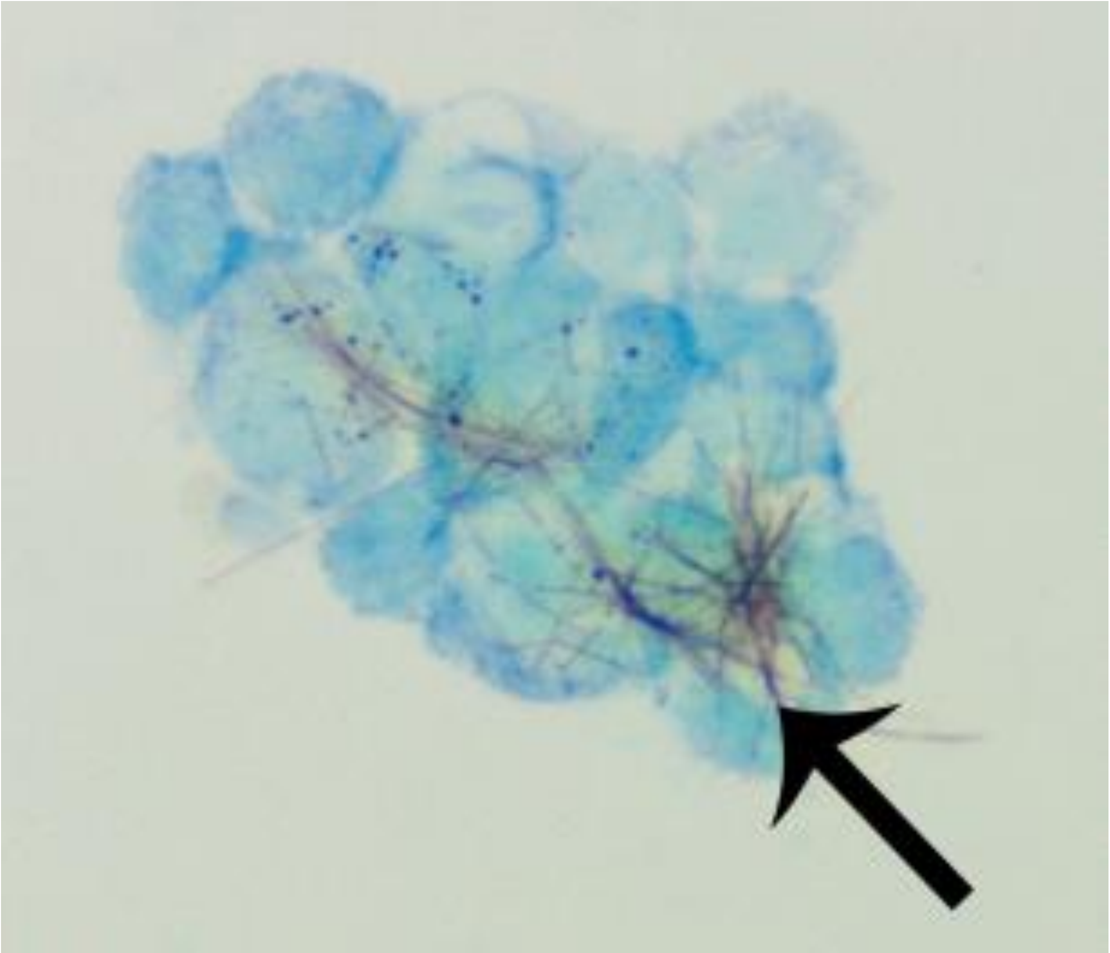
Rat Pulmonary Toxicity *in vivo*

Intra tracheal instillation studies

Sprague-Dawley rats

20-µm Ag NW instillation causes

- Frustrated phagocytosis
- Inflammation
- Granuloma formation



Cells from broncho-alveolar lung fluid at day 1

Silva et al., Part. Fiber Tox. (2014)

Ag NW Toxicity to Immune Cells

Nanowires conform to the fiber pathogenicity paradigm

- Frustrated phagocytosis
- Evidence for internalization through other ATP-dependent pathways

Additional modes of action

- 2-µm Ag NW are also cytotoxic
- Possible role for Ag^{+}(aq) release (ESRF experiment planned early 2017)

Ag NW Toxicity to Non-Immune Skin Cells

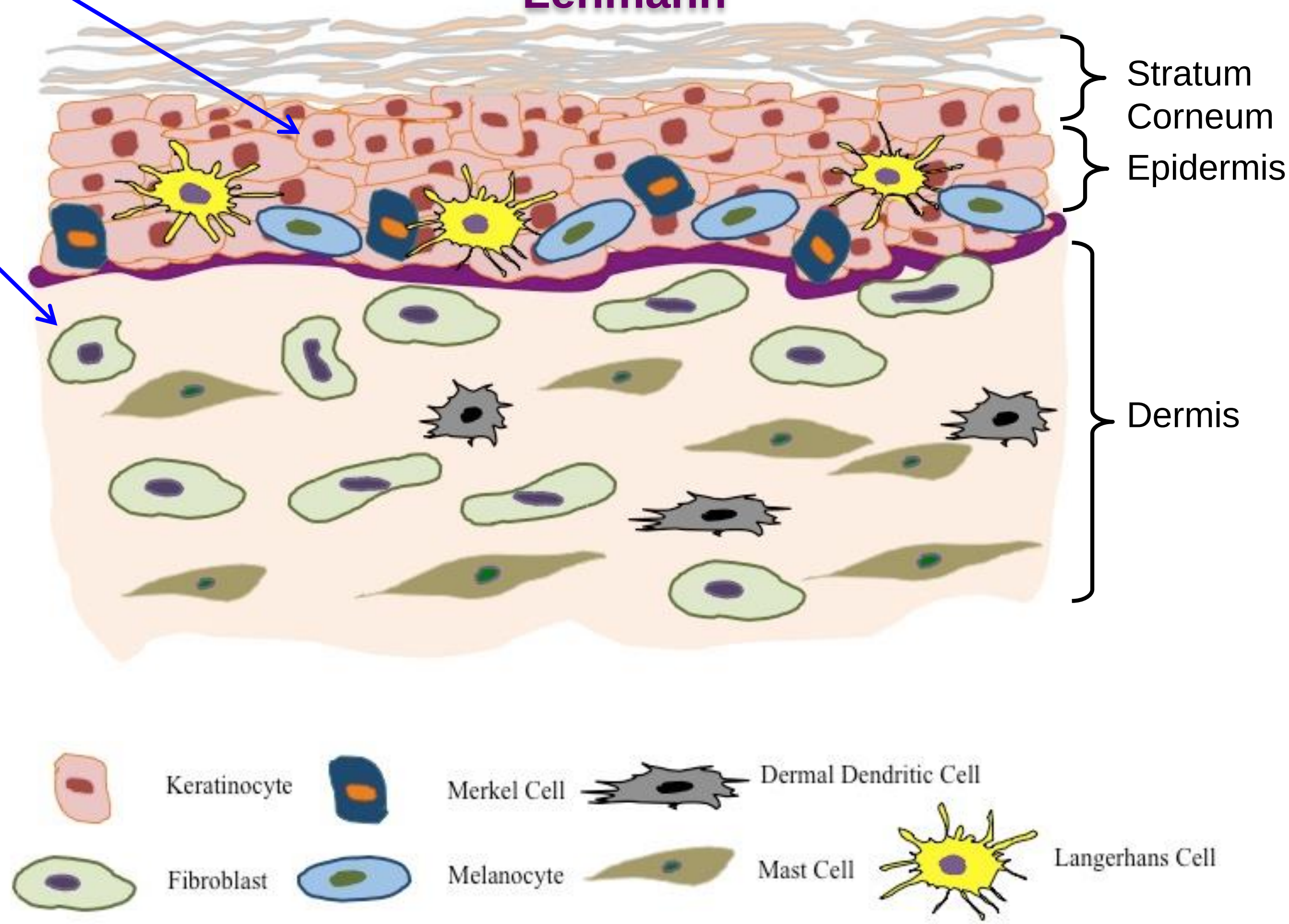
Human skin cells

Keratinocytes – outermost layer of skin

Syliva Lehmann

Fibroblasts

Muriel Viau

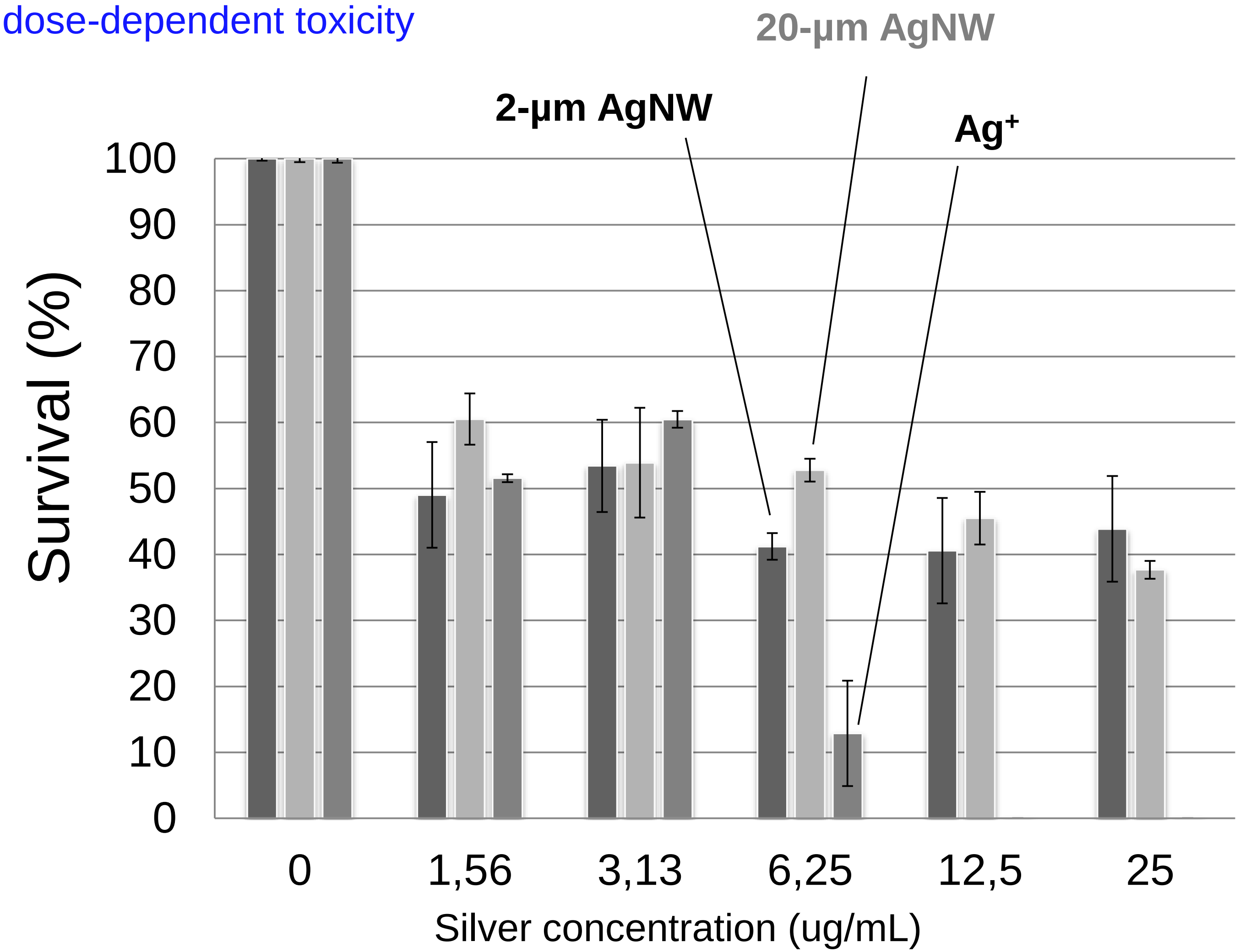


Ag NW Toxicity to Non-Immune Skin Cells

Syliva Lehmann

Human primary keratinocytes

- 24 hour exposure
- Mild dose-dependent toxicity



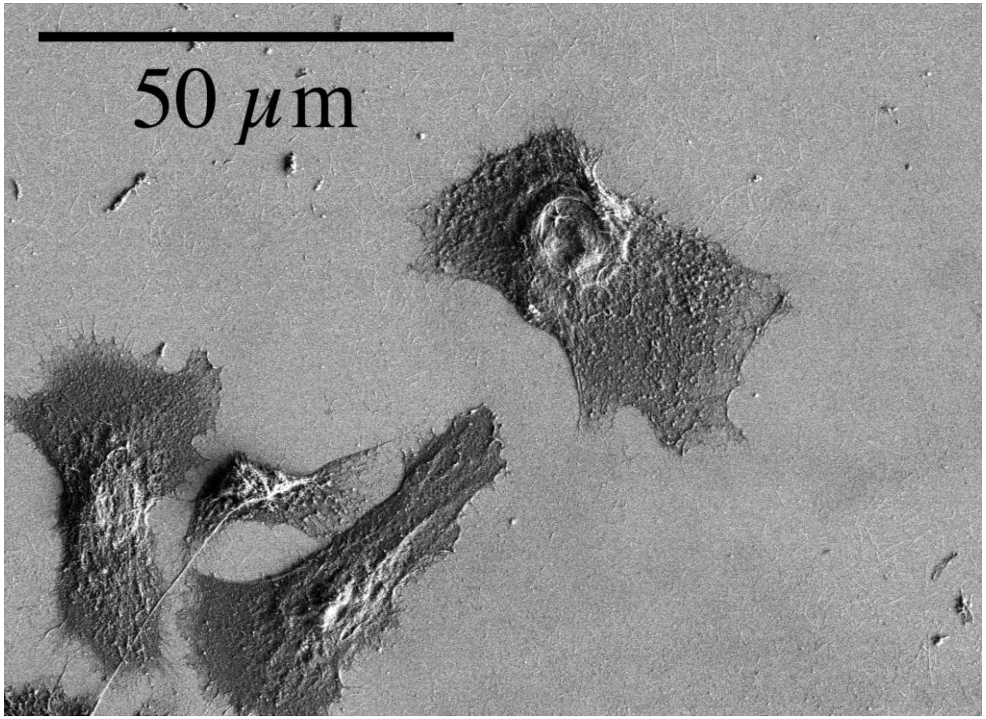
Syliva
Lehmann

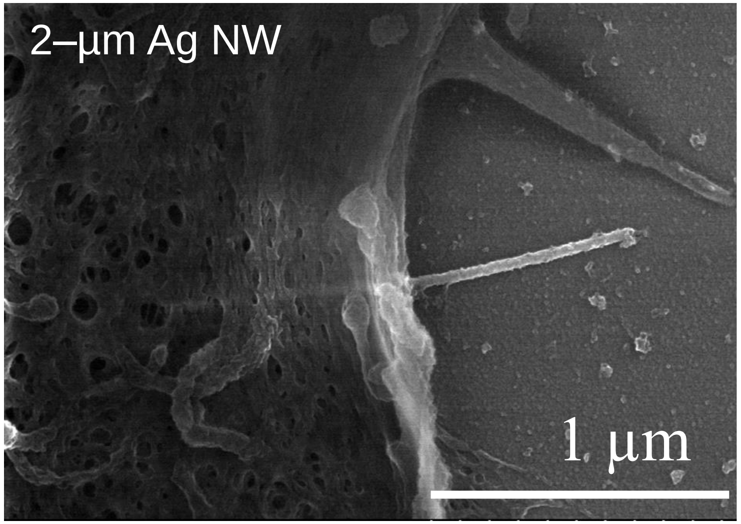
Ag NW Toxicity to Non-Immune Skin Cells

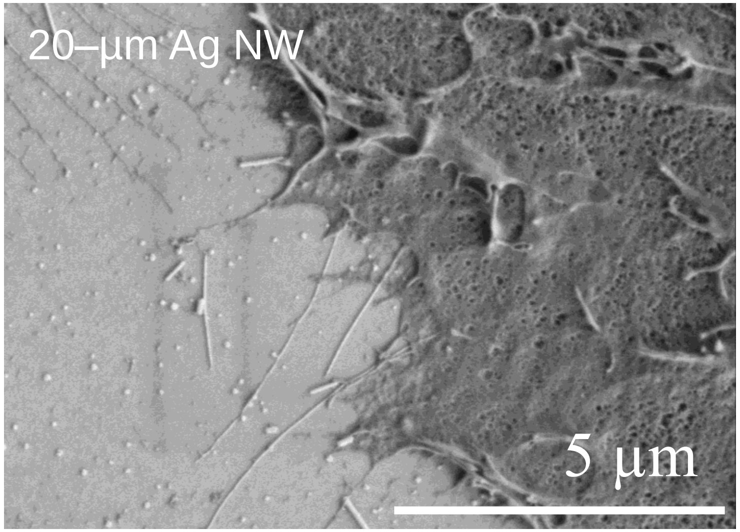
Human primary keratinocytes

2–µm and 20-µm Ag NW are internalized

Scanning electron microscopy





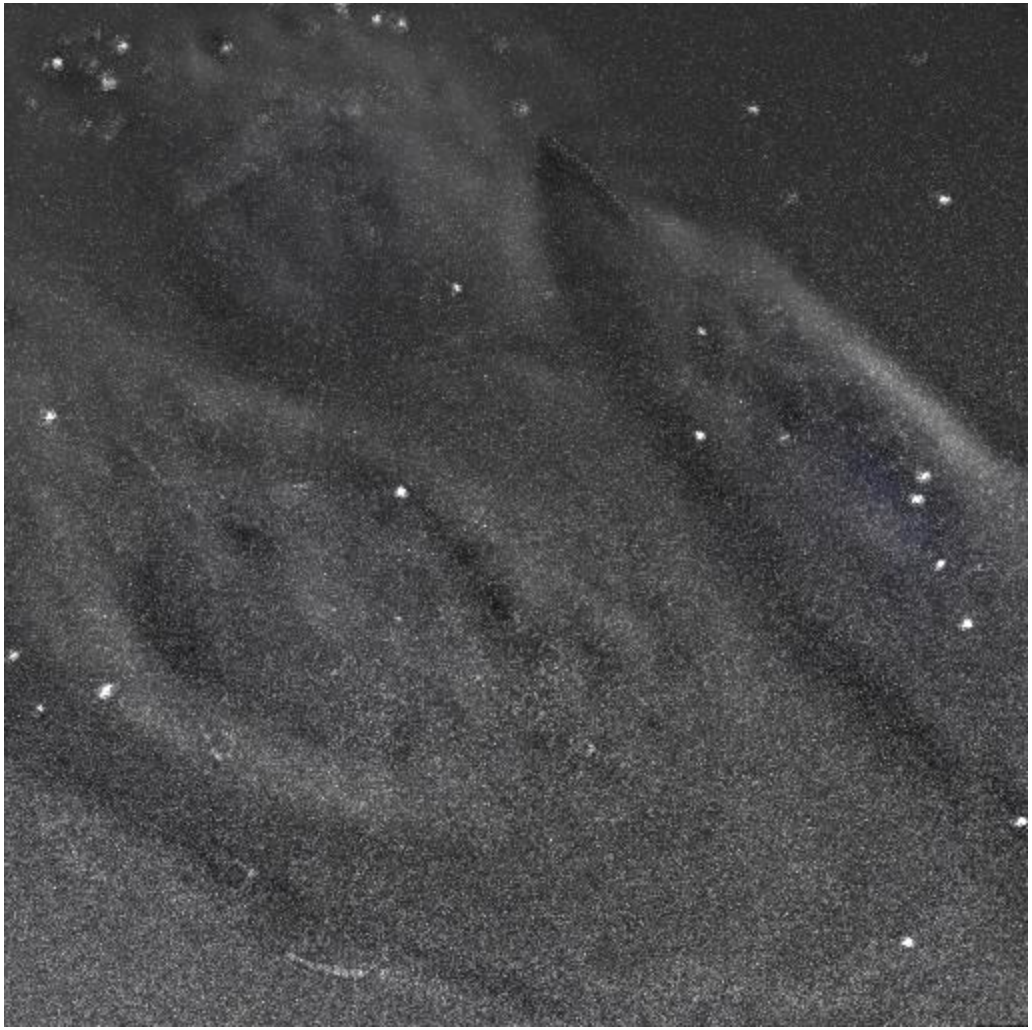


Ag NW Toxicity to Non-Immune Skin Cells

Human primary keratinocytes

- 2–µm and 20-µm Ag NW are internalized
- Located in cytoplasm
- Evidence of Ag NW bending

Confocal optical microscopy
Blue = Hoechst stain for nucleus



Ag NW Toxicity to Non-Immune Skin Cells

Clear evidence of internalization

Moderate toxicity

Tentative interpretation:

Complete or frustrated endocytosis

Intracellular fate does not lead to Ag^{+} generation or other causes of cellular stress

Use SbD Approach for Safer Touchscreens

Control AgNW dimensions to minimize damage due to active internalization

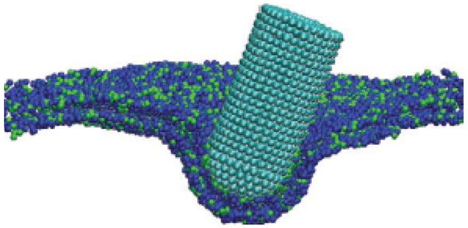
Nanowire length

Below the limit for frustrated phagocytosis/endocytosis

Nanowire diameter

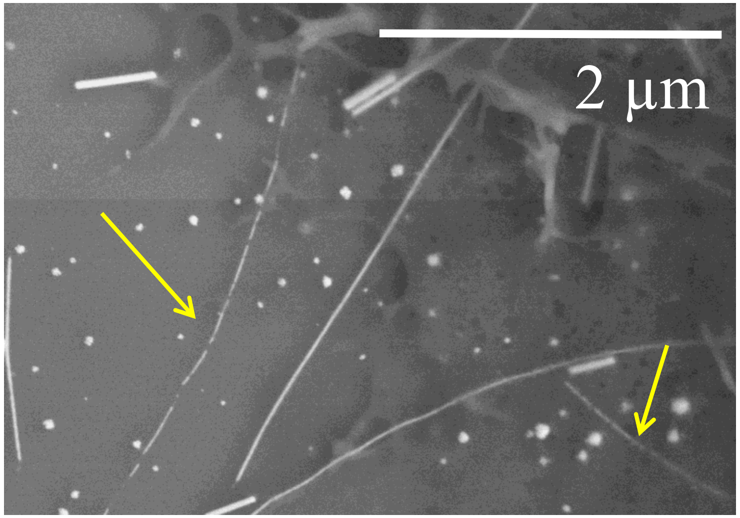
Can diameter control uptake and toxicity?

Alter tip–membrane interaction strength



Shi Nature Materials (2011)

Weaken AgNW



Lehmann et al *in prep*

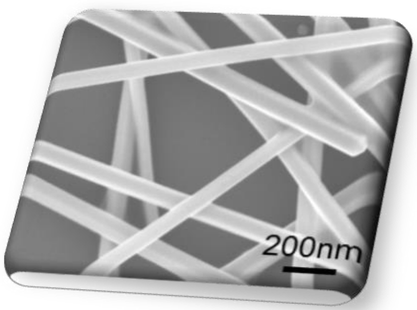
Use SbD Approach for Safer Touchscreens

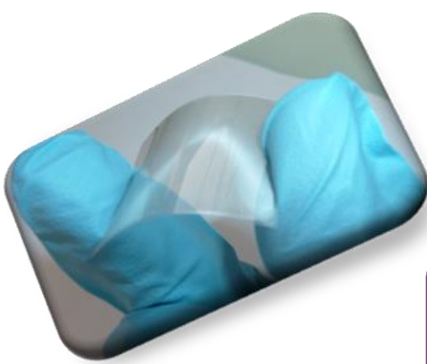
Safer by Design

Ag NW dimensions
Surface coating

Materials Properties

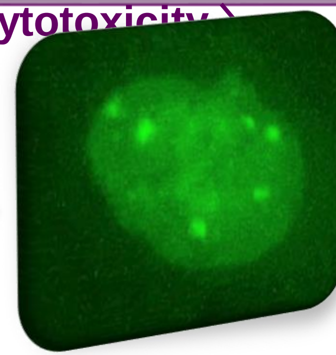
Synthesis





Toxicity

Lower intrinsic cytotoxicity



Prototype

Optimization Goals

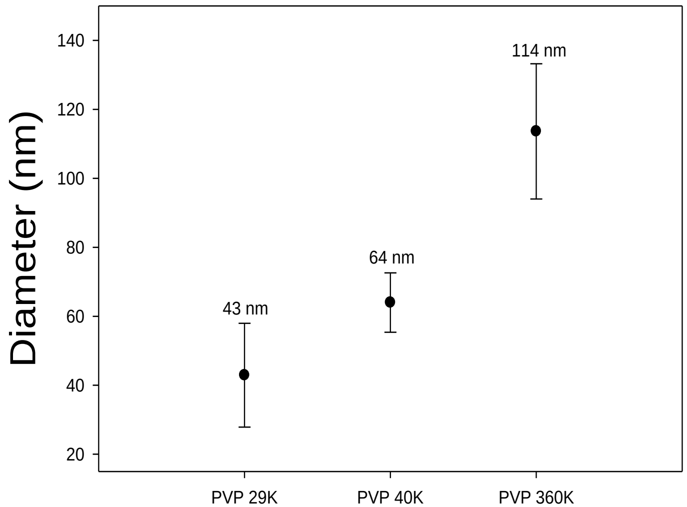
Product Performance

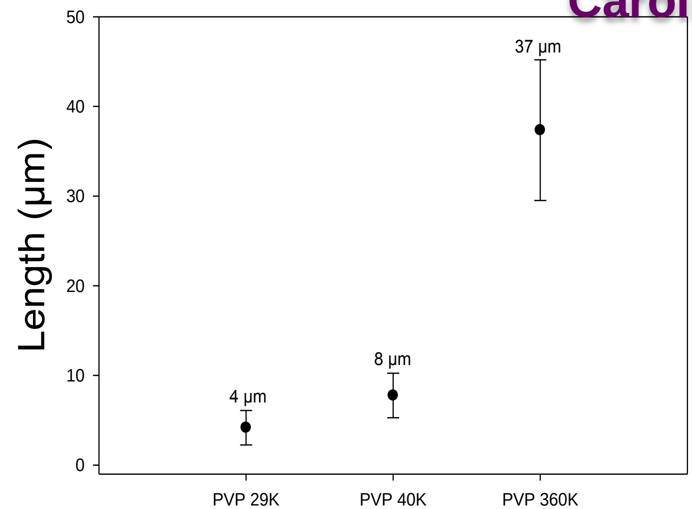
Electrical resistance < 50 Ω/cm^2
Optical transparency 90% T

Djadidi Toybou
TALK 5:45 today
Jean-Pierre Simonato
Caroline Celle

Control of Ag NW Dimensions

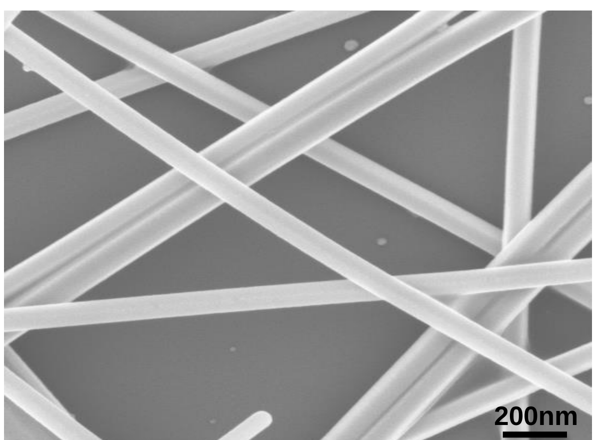
Example: Molecular weight of capping agent



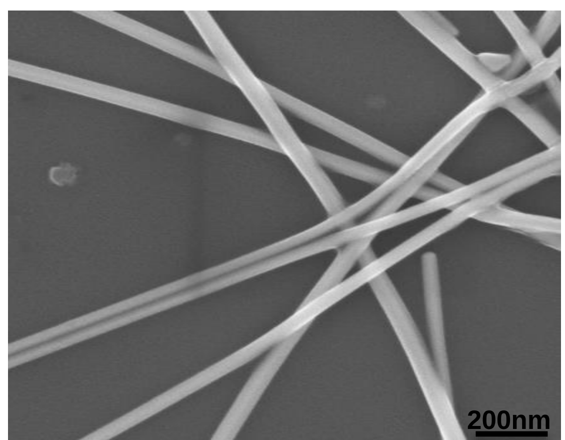


All NW achieve transparent conductor performance targets

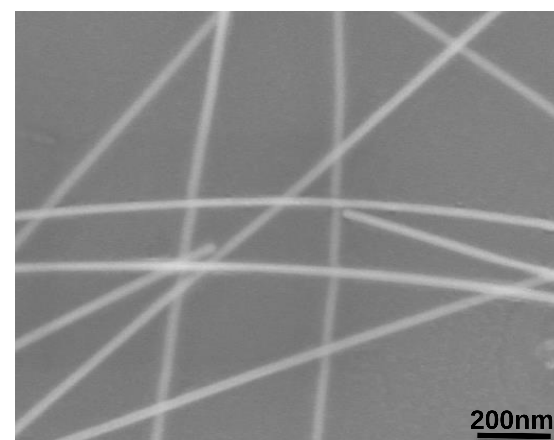
Thick ≈ 90nm



Medium ≈ 60nm



Thin ≈ 30nm



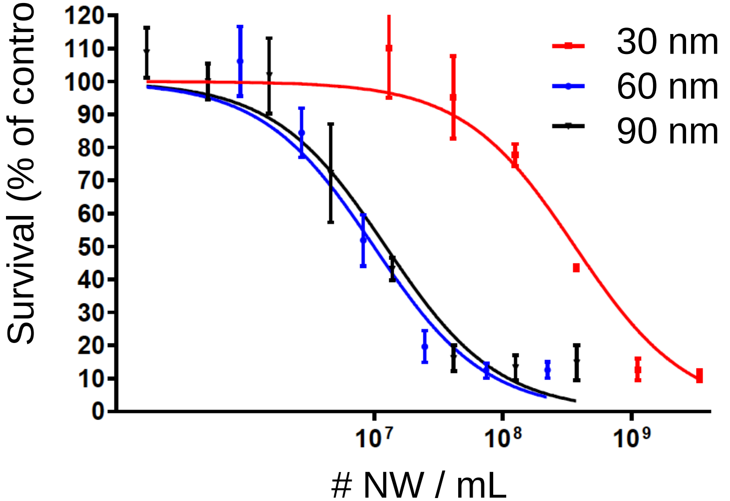
Muriel Viau
POSTER

Ag NW Diameter Affects Cell Viability

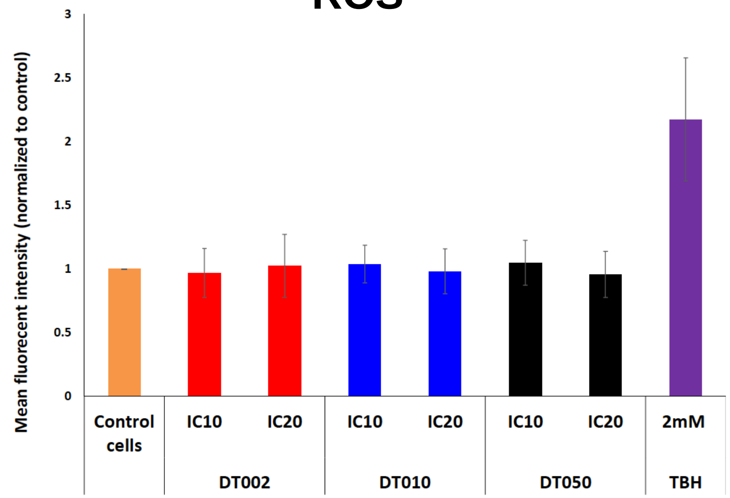
Human primary fibroblasts

Ag-NW: 8–9 µm length and variable diameter

Viability

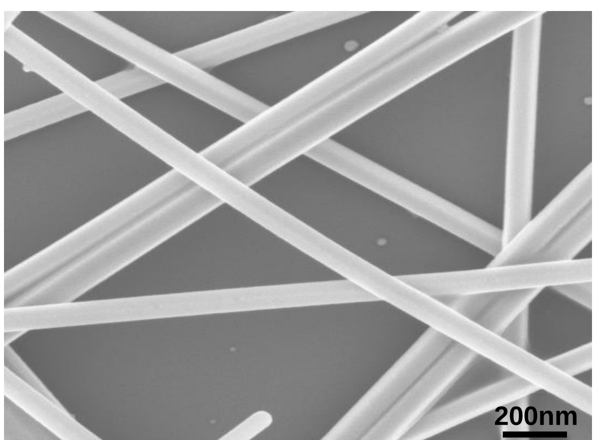


ROS



DNA damage
Apoptosis
…

Thick ≈ 90nm



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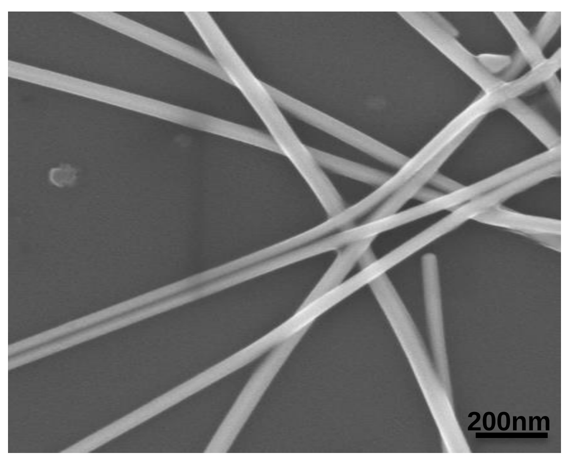
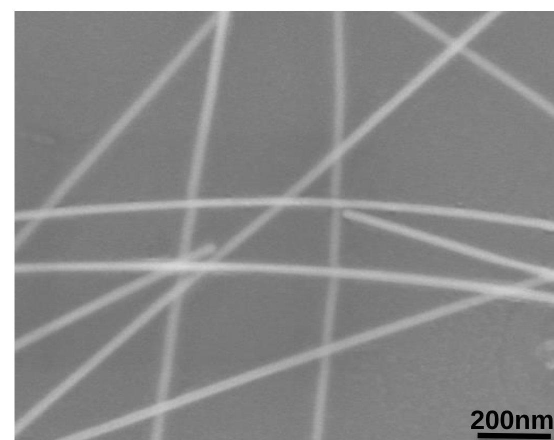


Thin ≈ 30nm



Ag NW Diameter

Human primary fibroblasts

8-µm Ag NW show higher toxicity to primary fibroblast cells than 20-µm Ag NW to primary keratinocytes

Cell viability improves with smaller diameter (30 nm)

Possible mechanisms …

- Less internalization
- NW fracturing and less membrane damage
- To be determined!

A new possible strategy for SbD nanowire products

Mouse macrophage cells

Next project!

Collaboration with Dr. Thierry Rabilloud, CEA

Could Nanowire/fiber Exposure Cause Skin Disease?

The major asbestos related diseases for occupational and non-occupational exposure are all respiratory

- Lung cancer
- Mesothelioma
- Asbestosis
- Pleural plaques

All associated with inhalation route

One suggested link between asbestos and malignant melanoma

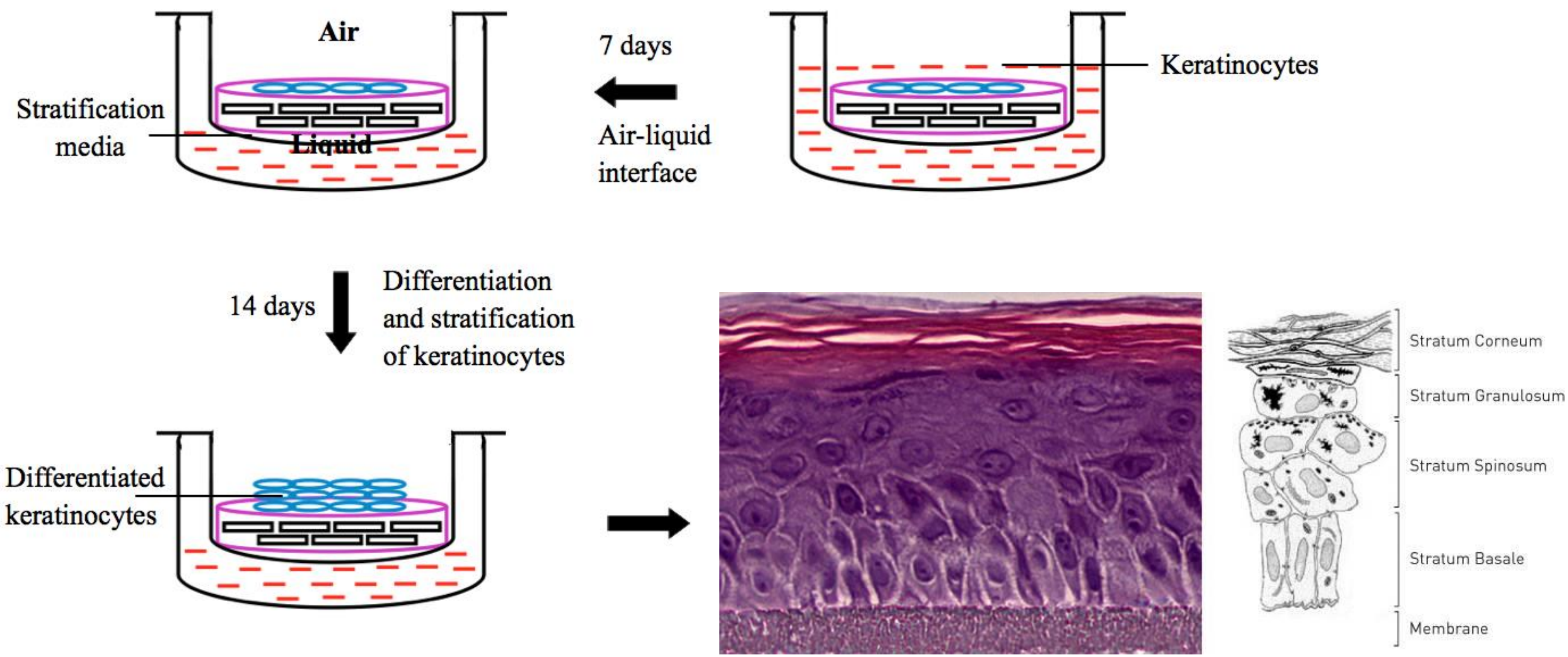
Hilt et al. Am. J. Indust. Medicine (1985)

Syliva
Lehmann

3D Model for Skin Exposure

3D human reconstructed epidermis (epiCS)

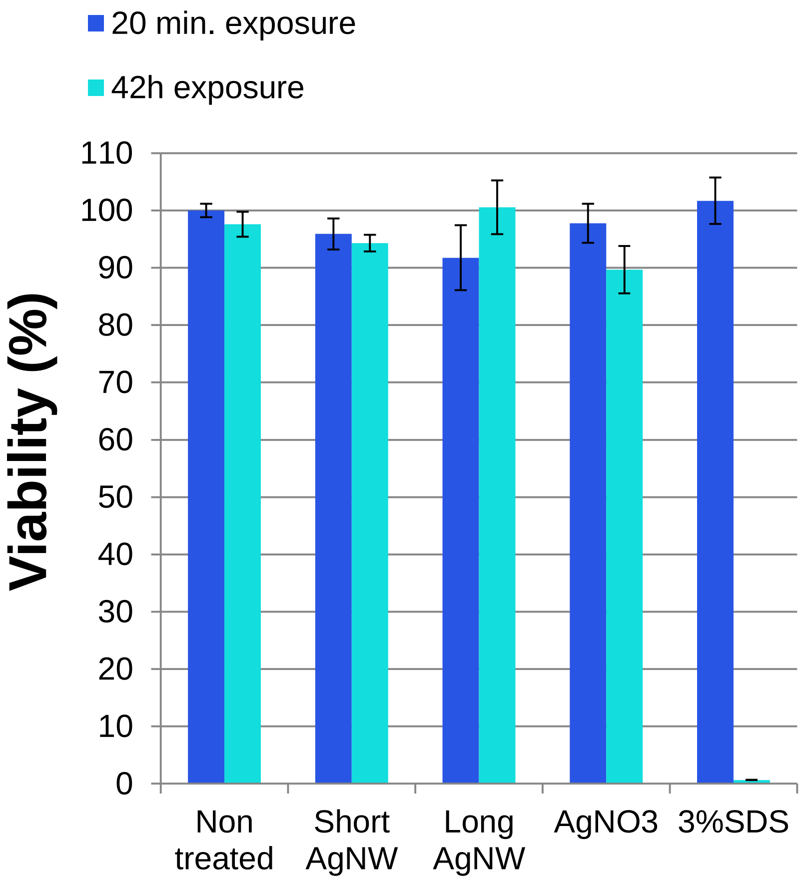
Normal human primary keratinocytes
20–µm Ag NW



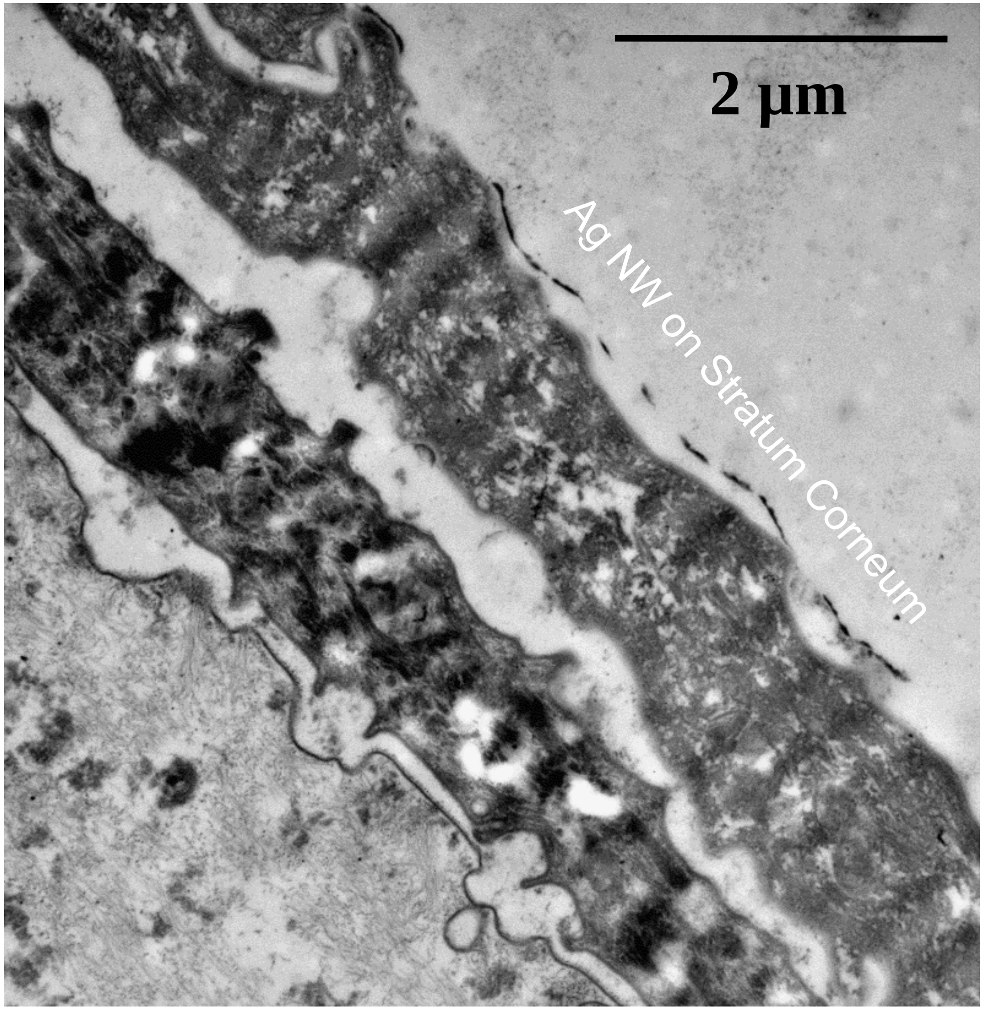
3D Model for Skin Exposure

3D human reconstructed epidermis (epiCS)

No toxicity in epidermal layer



Transmission electron microscopy



Conclusions

Intrinisic toxicity of Ag NW

High toxicity to macrophages and fibroblasts

Less toxicity to fibroblasts and no penetration through 3D skin model

Do static models correctly reproduce likely exposure?

Safer by Design approach considering NW dimensions

Length and diameter are both important Materials Parameters

Significant reduction in intrinsic harm achievable without reducing performance

We still lack a full model of intracellular interactions leading to toxicity

Safer by Design approach considering surface coatings

2017 goal will be to alter Ag NW coating to minimize Ag^+ release

Thanks

Group publications on silver nanowires

Scanlan *et al*. ACS Nano 12, 10681 (2012) *Daphnia magna*

Silva *et al*. Particle Fiber Toxicology 11 (2014) Rat model

Tagmount *et al*. in preparation Cytotoxicity in rat and other cell cultures

Lehmann *et al.* in preparation Primary human keratinocytes & skin model

Viau *et al*. in preparation Primary human fibroblasts *vs* Ag NW diameter