Nanotechnology Challenges and Fears

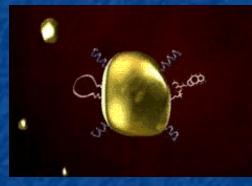
Today we'll focus on health & environmental challenges posed by nanotechnology This will be based on "case studies" drawn from research and activist literature For which general tone will be cautionary, or even downright negative So before I begin, I want to offer three caveats / qualifications: Caveat 1) All of what follows should be taken with reservations In the spirit of sounding warnings, I have **sought out** alarming data But these data are drawn largely from medical studies: No other field takes longer, or has to work harder, to reach understanding & consensus Because in medicine it's virtually impossible to set up the sort of single variable well controlled experiment we physical scientists have the luxury of using

Caveat #2) I am not a medical or even a biological researcher Reinforcing caveat #1's suggestion that you reserve final judgment And emphasizing YOUR need to study further Which I've tried to facilitate by collecting dozens of MY source papers on the accompanying webpage: Nano Challenge and Fears - Supporting Materials Caveat #3) Bear in mind that nano's most POSITIVE impact may yet be in medicine! Certain beneficial medical applications are already easy to anticipate: Such as possible use of Buckyballs to safely contain metal MRI contrast agents But others follow from nano's unexpected interactions with our body and cells: For instance, evidence Buckyballs may also stimulate creation of new hair follicles And the most dramatic may ultimately be found in nano's use for cancer treatment:

Case Study #1) Use of nano gold in chemotherapy: From the spring 2008 business section of the Washington Post: CytImmune Inc. One of a number of companies looking to nano for chemotherapy: Goal: Deliver chemotherapy toxins directly (and specifically) to human tumors!

Approach?

1) Make gold nanoparticles and attach chemotoxins . . .



2) Inject into bloodstream

3) Flawed tumor blood vessels



Supporting webpage with embedded animations: Supporting Materials - CytImmune

Of course, it's never quite that simple:

First, the body has mechanisms for encapsulating and eliminating foreign bodies

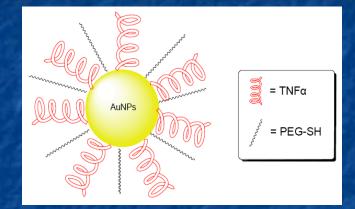
But many of these kick in only if object is $> \sim 1/3$ um

Likely targeting bacterial invaders

NANO particles (NPs) ARE much smaller

UNLESS they clump together!

To prevent clumping, NPs must be hydrophillic



See: Chen et al. "Gold Nanoparticles from Nanomedicine to Nanosensing (link)

Common trick is to add polarized surface layers or molecules

Such as poly-ethylene glycol ("PEG")

Which, for non-reactive gold, is attached via S-H ("thiol") groups

Then similarly attach the chemotherapy toxin: Tumor necrosis factor (TNF)

But there is a (serious!) complication:

This toxin (and most chemotherapy drugs) is not just toxic to cancer cells! That's why they are trying to "target" delivery Here, by sizing gold NPs to penetrate sloppy blood vessel walls of tumors! More generally, they're trying to target any cell exhibiting unusually rapid growth It's why chemotherapy affects hair, lining of digestive tract and testicles Because they contain body's most rapidly growing **normal** cells! But to use really **strong** drugs, targeting must be almost 100% effective: Not just to minimize "peripheral damage" but because trace concentrations of the same toxin can **INDUCE** cancer elsewhere! So, here, we cannot be satisfied with **most** of toxin-laden gold going to the tumor Must ensure that virtually ALL chemotoxin goes to the tumor **OR** is rapidly excreted **OR** is rapidly degraded/metabolized THIS is problem holding up deployment of such therapies! But it also suggests huge, perhaps surprising, potential financial benefit: Drug companies spend BILLIONS on ultimately "ineffective" cancer drugs But many of these "ineffective" drugs do a **great** job of killing cancer cells! Problem was that they ASLO killed healthy cells they encountered Truly "targeted" delivery would allow companies to resurrect abandoned drugs! Recouping their investments and (we'd hope) also saving us money

But the use of gold is straightforward, right?

After all:

Gold IS "golden" because of its almost complete lack of chemical reactivity This is why, unlike silver (or other metals), gold does not tarnish or oxidize In fact, chemists have to take very special measures to bind anything to gold Their stock trick (used above) is to add a S-H (thiol) chemical attachment group Lack of reactivity makes gold the **first choice** when need to put metal into body: Preferred for everything from pierced earring posts to pacemaker wiring However, in the body, it's not just about chemical reactivity It's also about shape, and the often strange interaction of shape and chemistry

How **might** nanoparticles acquire unexpected toxicity? 1) SURFACE TO VOLUME ratios are vastly higher for nanoparticles: - Much larger fraction of particle's atoms/molecules exposed to environment - Then, if constituents are soluble in environment, will also dissolve much more quickly What IS total surface area of 1 microgram of material with density of 3 gm /cc? Particle Mass # of Particles **Particle Diameters Total Surface Area** 121 microns .0002 cm² 1 microgram 1 103 12.1 microns 1 nanogram .002 cm² 10⁻¹² grams 106 1.21 microns .023 cm² 10⁻¹⁵ grams 109 121 nanometers .235 cm²

With 12 nm particle diameters, 1 microgram of material => 2.3 cm² surface area!

12 nanometers

2.350 cm²

1012

10⁻¹⁸ grams

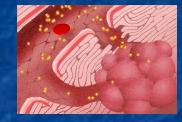
2) QUANTUM SIZE EFFECT can alter fundamental properties

Electron standing waves are induced by confinement in particle (lecture 3) They have altered size-dependent energies

Those altered energies dictate nanoparticle's optical and chemical properties

3) SIZE AND SHAPE may yield new biological interactions

For instance, the above ability of properly sized gold nanoparticles to penetrate blood tissue barrier:



But in the case of nano gold, is there ANY evidence of altered properties? YES: Discovered in 1930's that injected colloidal gold relieved rheumatoid arthritis

Via a mechanism STILL not fully understood!

Along with more recent studies on nano gold:

"Effect of gold nanoparticles on spermatozoa" (link)

Gold nanoparticles observed to penetrate into heads and tails of spermatozoa Result: Motilty fell from 95% to 75%

Mechanism? Article didn't provide, but did note nanogold can be more reactive Manifestation of "Quantum Size Effect" (standing waves) of lecture 3?

Also cited research on gold-induced male sterility in manufacturing environments

May seem like rather limited/specific problem

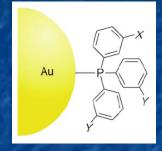
But reproductive cells can be particularly sensitive indicators: "The Canary in the coal mine"

And to the above report, add:

"Size-dependent cytotoxicity of gold nanoparticles" (link)

Used gold nanoparticles of varied sizes, stabilized by triphenyl phosphine ligands

"Stabilization" = enhancing solubility, suppressing aggregation:

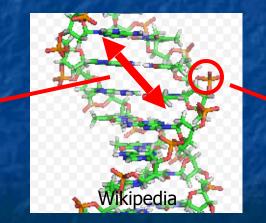


Exposed connective, epithelial, microphage and melanoma cells to these particles

Observed that for specific gold nanoparticles sizes:

Gold nanoparticles attached to DNA in its "major groove"

Physically obstructing DNA expression?



Gold's triphenyl phosphine groups partially replaced DNA backbone phosphate groups

Rebuilding (a.k.a. mutating) DNA!

Likely to interfere with DNA coding and/or expression!

Reminding us of course's title: "We're not in Kansas Anymore!" Things (here normally innocuous gold) act VERY DIFFERENTLY at the nanoscale!! But going back to this lecture's title: While CHALLENGES of gold nanoparticle-based medicine are abundantly clear In my opinion FEAR of the above emerging nanotechnology is **not** called for Why? Goes back to the FIRST principle of medicine: "Do No Harm!" That is: Risk assessment is an integral part of **any** investigation of medical benefit Indeed, I easily found a rich literature on gold nanoparticle risk vs. benefit So I'd instead reserve my personal "FEAR" category for situations Where nanotechnology is being deployed with little or no assessment of risk!

Case Study #2) Nano metal oxides in sunblock Sunblocks traditionally consist of metal oxide powders suspended in an oil The metal powders are chosen for ability to absorb damaging UV ranges of sunlight Classic is zinc oxide, but many other metal oxides are also used Such sunblocks have long been used without problems, thus judged to be safe: Because their metals ARE already fully oxidized, they **tend** to be chemically inactive And, due to the strength of oxide bonds, metal oxides do not readily dissolve **HOWEVER:** When they absorb UV they can disassociate/ionize to become ROS's (ROS's = Reactive Oxidizing Species) Nevertheless thought "safe" because they are only put on skin surface Skin = Body's Spacesuit: Its job is to largely isolate us from our environment Skin surface is dead cells (therefore hard to damage) Which are continuously shed (renewing barrier + releasing embedded chemicals)

But sunblocks can have a **cosmetic** problem:

In addition to UV light absorption . . .

Particles also scatter light of any color

Result: Powders tend to appear white (= all colors scattered) Fine for flour, talc . . . But can look kinda weird on your face:

Solution comes right out of this class's water ripple tank labs: When light wavelength > particle size, get far less light scattering

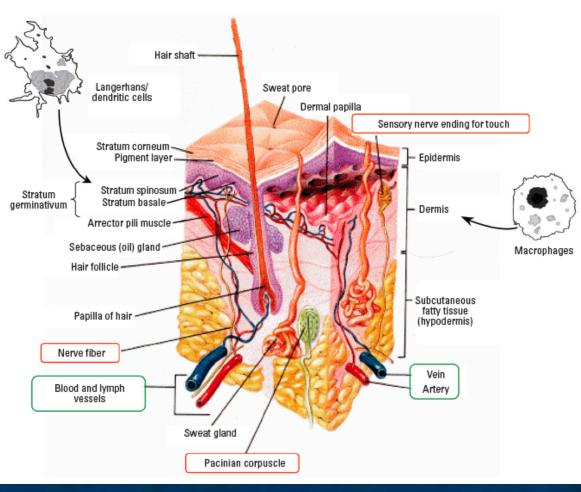
So reduce sun block metal oxide particles to < λ visible light ~ 300 nm

Versus:



However, skin is hardly monolithic armor:

It is instead penetrated by a rich assortment of pores, glands, hairs, nerves, blood vessels . . .



From Oberdorster et al. (link)

So can we assume that skin blocks **nano** metal particles? Classic assessment: Scotch Tape removal of deeper and deeper skin cell layers Then analyze successive tape pieces for presence of metal in question For nanometal sunblocks, detectable metal signals guickly diminish to zero But the key (often ignored) word is "detectable" However, for instruments using electrons and photons to analyze intact samples: I know, from personal experience, that sensitivity is likely no better than 0.1 to 1%. For better sensitivity must have significant 3D volume of sample, and take it apart: As done in mass spectroscopies or chromatographies that actually count atoms/molecules Likely that Scotch Tape analysis is limited to 0.1 - 1% surface analysis techniques So "undetectable metal oxide skin penetration" may ONLY mean < 1 part per 1000

But "less than one part per thousand" **sounds** pretty low Concentrations may not **stay** low if metal oxides make it into the blood stream At that point metal oxides could end up anywhere in body And, most importantly, size, shape or chemistry, could drive local accumulations In which case there are, again, reports of possible cell damage:

- Studies by EPA showing that nano TiO₂ affected mice immune cells (link)
- Other reports of metal oxide cell mitochondrial damage or even DNA mutation (link)

But don't I need to balance these **possible** risks against the near **certainty** of developing possibly fatal melanoma if I don't use sunblocks? Absolutely!

And anyway, in this case, as a consumer don't I have a simple personal option?

To play it safe just avoid nano sunblock brands! (right?) Then along comes the December 2008 issue of Consumer Reports (link): Tested six sunblocks specifically claiming they contained no nano sunblock ingredients For which CR tests showed all but one DID contain nano zinc oxide or nano titanium oxide

Are vendors just lying to consumers?

Or is it possible **they** are ignorant about ingredients?

Such ignorance would have seemed unlikely



At least until recent rash of news stories on product adulteration, in particular: NH_2 NH_2

(Over last decade, FDA & Consumer Product Safety Commission have been essentially silent!)

So I put nano sunblock on my "Fears" list

NOT because there IS strong evidence of current hazard:

Existing evidence of skin penetration IS weak

Existing evidence of biological toxicity IS limited

But we DON'T have better data because proper testing has not been done! So my first reason for "fear" (or at least caution) is lack of information

Second reason for fear/caution is near total lack of control:

Nano metal oxides have been widely introduced into sunblocks, without labeling Even when labeled non-nano alternatives appeared, CR tests indicate false labeling

So as a consumer I am left with inadequate information AND inadequate control!!

Case Study #3) Nano silver impregnated plastics

Even the ancients knew that silver could have antibacterial & antifungal effects It's why (at least the richest of them) used it for wine and water storage

Also why they used it to treat syphilis and gonorrhea

And led them to think that a silver coin held in the mouth might hinder infection by plague



Many (too many?) explanations for antibacterial effects – A leading candidate: "Denaturing" (unfolding) of cell membrane/wall proteins as insertion of silver ions cleaves the hydrogen bonds that pulled different parts of the protein together

And if silver ions thereby manage to get inside the cells: Metal ions can bind to phosphate groups on DNA And to many other inter-cellular chemicals, blocking their function

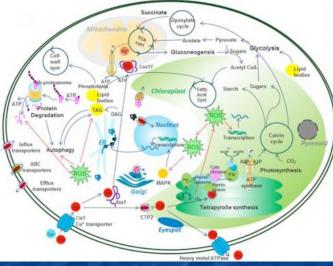
And silver has surprisingly strong/quick effect upon aquatic bacteria

= Key constituents of the aquatic / wetland ecosystems upon which we depend!

These cells naturally metabolize copper Thus readily absorb it through their cell walls Silver is so similar it can exploit same pathways Concentrating it 1000 fold inside cells! Inside cell it can **poison** cell's photosynthesis Photosynthesis rate cut in HALF after just 15 minutes exposure to DILUTE Ag!

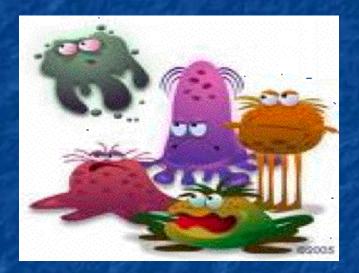
Could easily be similar effects in soil bacteria (necessary for our food growth)!

Paper: Linking toxicity and adaptive response . . . (of bacterium) exposed to silver



And now add in the modern paranoia about

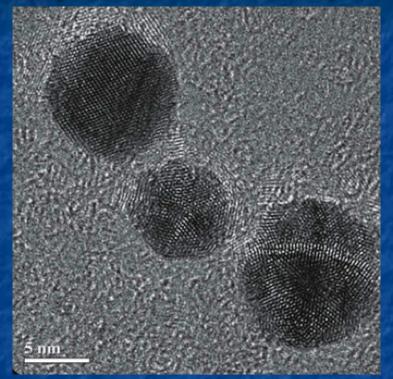
GERMS (!!!!):



www.oralabs.com/blog/hand-sanitizer-vs-soap-and-water/

The free market response?

Manufacturers have found ways to embed nano silver spheres in plastics:



Source: http://www.pfonline.com/articles/090701.html

And where have they ALREADY used such nano silver impregnated plastics?

Where you can expect to find nanosilver in YOUR life:

In things you wear

Sheets & pillows Towels Fabrics Uniforms Sports shirts Hats & gloves Underwear Condoms Hearing aids

Socks & Slippers Shoe inserts & cleaner Foot bath massager

Fabric softeners Laundry balls Laundry additives Soaps & detergents Washing machines

In things you ingest

Water purifiers Vegetable cleaner Toothpaste Toothbrush Denture cleaner "Health" supplements "Health" drinks Food storage containers Dishware Frying pans Teapots Cutlery handles Kitchen cutting boards Kitchen cleaners

Refrigerators

Air filters & humidifiers Air masks Air conditioners

In things to "look good" Hair dryers Hair brushes Make-up brushes Curling irons Shavers Razors & blades Cosmetics Shampoos & soaps Wipes Vaginal gel

In things for your baby

Nanbabie[™] clothing
Pacifiers & teething toys
Baby bottles
Bottle brush cleaner
Baby strollers
Children's plush toys
Baby antibacterial spray

In things you touch

Cell phones Laptop PCs Computer peripherals ATM buttons Elevator buttons Doorknobs & handrails Telephones Lamps Watches Vacuum cleaners Paper Paints & Inks

In things for your pet

Pet food & water bowls Pet water purifier Pet spray Pet Shampoo

Source: Appendix to the Center for Technology Assessment's petition to the EPA (link)

Why should I worry about a fungicide / bactericide?

Because for for truly selective toxicity you generally need complexity

Complexity => uniqueness (e.g. hormones that lock a specific insect in larval form)

But silver's biocidal mechanisms (slide 18) are instead likely to be very NON-specific

Besides, early GERM exposure strengthens immune system / suppresses allergies

Medical researchers call this the "Hygiene Hypothesis" – as reported in Smithsonian Magazine (2013):

The Unintended (and Deadly) Consequences of Living in the Industrialized World

"At birth, our immune cells make up an aggressive army with no sense of who its enemies are. But the more bad guys the immune system is exposed to during life's early years, the more discerning it gets. The immune system is programmed within the first two years of life. With less early infection, the immune system has too little to do, so it starts looking for other targets."

The article describes:

- Genetically equivalent populations living on both sides of the Finnish/Russian border

- Russians are poorer, receive inferior health care, and are less healthy in almost all respects

EXCEPT they have lower rates of asthma, allergies, diabetes and other autoimmune disorders

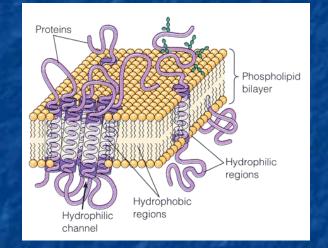
And do we even need bactericides?

Simple soaps offer a viable alternative:

Soap is NOT just a means of enhancing removal, it's also a biocide

Surfactants mix polar & non-polar => dissolution in water

INCLUDES breaking down "bilipid" cell membranes of GERMS



It's why getting soap INSIDE your protective skin layer can be such a bad idea!

We've already lost control of where nano silver ends up

It's thus VERY likely we are already ingesting nano silver (possibly in quantity)

That is why public interest groups such as the Center for Technology Assessment and the Natural Resources Defense Fund have petitioned EPA to invoke FIFRA:

FIFRA = Federal Insecticide, Fungicide or Rodenticide Act

Application of FIFRA would require testing of product for both **efficacy and safety** But at this time, EPA invokes only if vendor uses specific terms in labels or ads: Don't use words "insecticide," "fungicide" or "rodenticide" and you are in the clear!

Last time I checked, EPA had fined only ONE manufacturer for use w/o testing: Parent company of IOGEAR computer peripherals fined \$208,000 in March 2008 Despite what appears to be obvious appropriateness of applying FIFRA standard!

And so nanosilver TOO ends up on my "Fears" list

Even though, as in earlier examples, I don't claim strong proof of harm

It's instead, once again, due to:

1) A near complete lack of information, based on absence of appropriate testing

2) And lack of any personal control (widespread deployment without labeling)

But, as with earlier example, "caution" may again be a better word than "fear"

To get well into the fear category, need something more like the following:

Case study #4) Asbestos and possible nanotube parallels Asbestos is a class of mineral fibers of made from Si, Mg, Fe, Ca and Al oxides Includes a variety of names and structures, with straight or curly fibers, e.g.:

"Amosite"







"Crocodolite"

For an introduction, see the Scientific American article from which these figures are drawn (link)

The commercial attraction of asbestos was its apparent inertness:

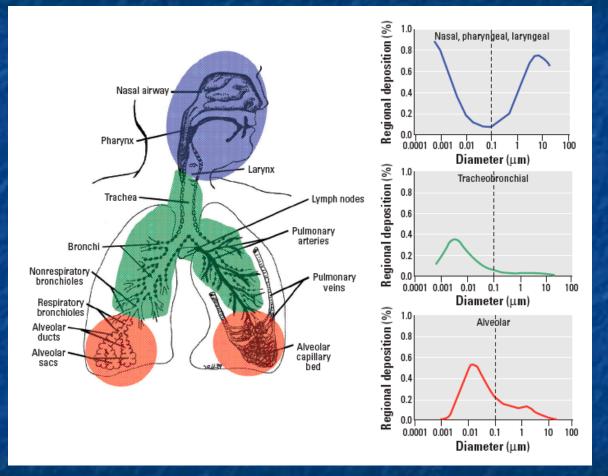
Ended up being used in consumer products including even toothpaste

But its outstanding application was as fireproof insulation

At least until workers started dying of particularly virulent cancers!

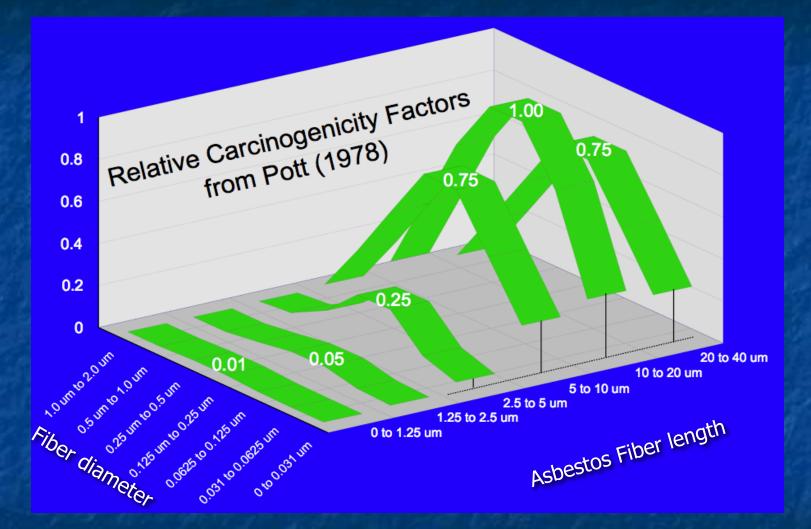
Toxicity is related to where fibers come to rest in human body:

And that resting place depends on the fiber's size and shape:



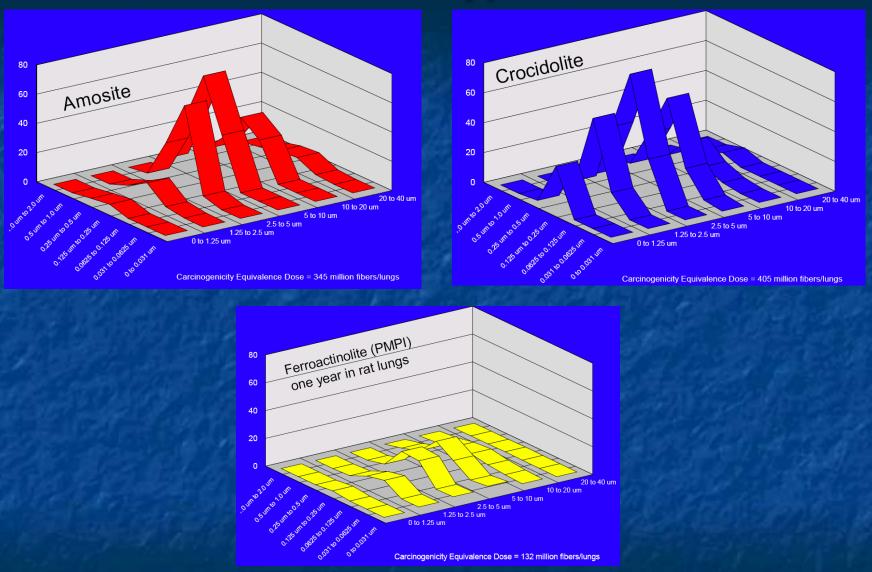
Oberdorster et al.: "Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles" Environmental Health Perspectives, vol. 113, p. 823, 2008 (<u>link</u>)

Even at particular location, toxicity is shape/size dependent:



Source: Phillip M. Cook - National Health & Environmental Effects Research Laboratory (link)

And can be differentiated by particular asbestos fiber:

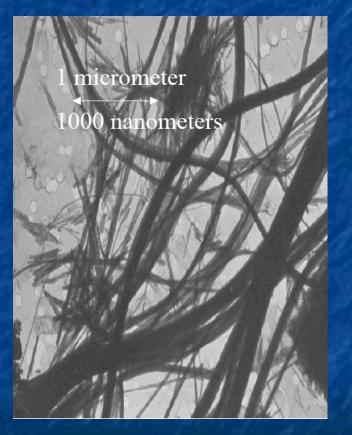


Source: Phillip M. Cook - National Health & Environmental Effects Research Laboratory (link)

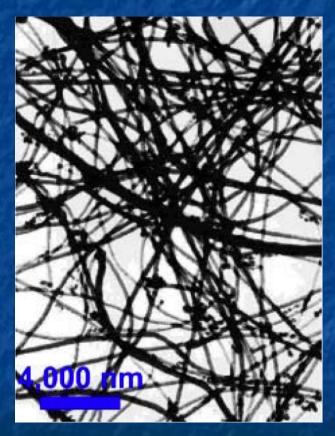
Why bring up the subject of asbestos?

Because shape similarities between asbestos & carbon nanotubes can be striking:

Chrysotile Asbestos:



Carbon nanotubes:

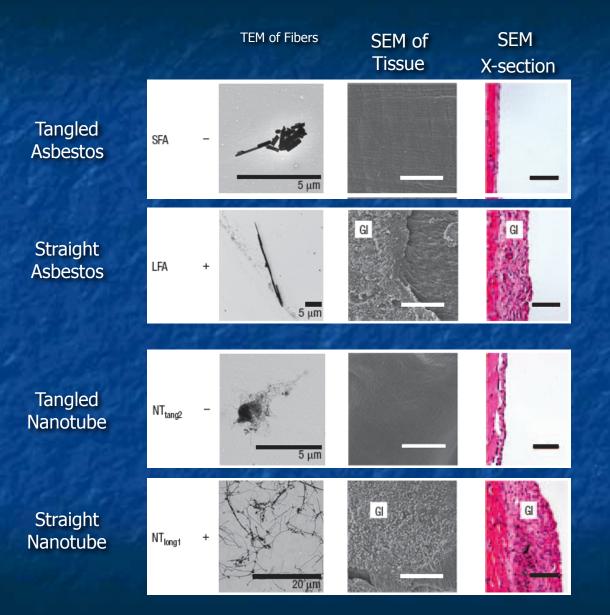


Source: Phillip M. Cook - National Health & Environmental Effects Research Laboratory (link)

But asbestos toxicity ALSO depends on its surface chemistry: Studies on animals and cell cultures indicate that embedded asbestos: Generates reactive oxygen and nitrogen species That are known to damage proteins and DNA See: "Multiple Roles of Oxidants in the Pathogenesis of Asbestos-Induced Diseases" (link) But remember possible "free-radical" like behavior of graphitic planes? (lecture 7) Electrons perpendicular to plane can pair to form resonant bonds OR as unpaired electrons could act as oxidizing "free-radical" sites Conceivably producing similar oxidative damage to proteins and DNA

Any experimental confirmation? Possibly:

"Carbon nanotubes that look like asbestos behave like asbestos"

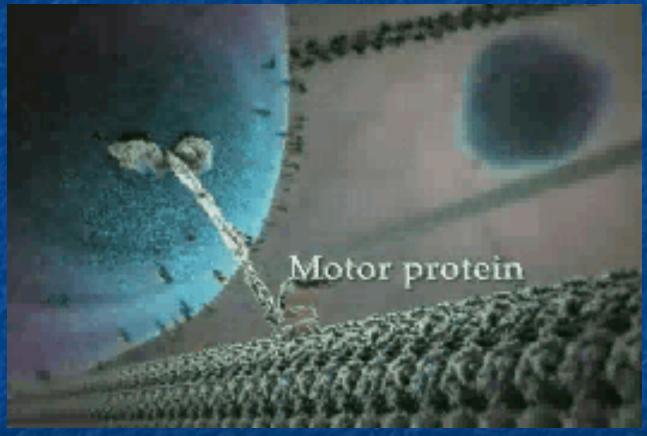


"Carbon nanotubes introduced into the abdominal cavity of mice show asbestos like pathogenicity in a pilot study"

C.A. Poland et al., Nature Nanotechnology (2008) (link)

But why are only certain cells sensitive to certain nanofibers?

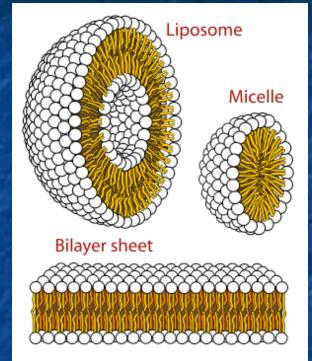
Remember those strange spheres being hauled around the interior of cells?



From Harvard University's "The Inner Life of the Cell" animation (as shown in last lecture)

They are the cell's internal cargo containers:

From Wikipedia on "Lipid Bilayers"



Liposomes:

Hydrophillic outer and inner surfaces => carry polar molecules

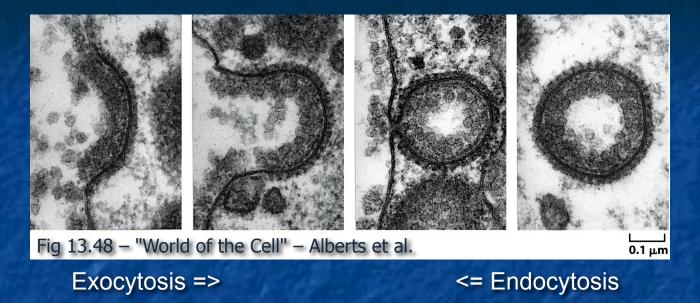
Micelles:

Hydrophillic outer / hydrophobic inner surface => carry non-polar substances

Can be formed when cell wants to haul in from outside a thing that is either too big or too polar to pass through the cell's membrane

Cell identifies these wanted "things" via chemical receptors on its exterior

Process is called "endocytosis" – looks like this:



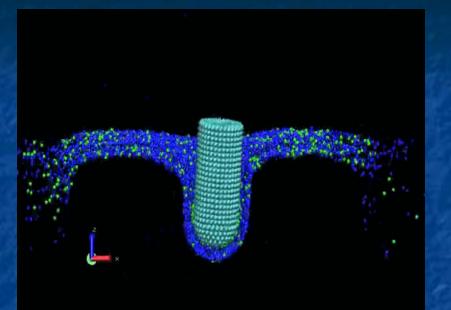
RELEVANCE?

Negative surface of ASBESTOS binds to serum protein vitronectin that, in turn, binds to receptors on surface of mesothelial and lung epithelial cells

And CARBON NANOTUBES are thought to bind directly to lectin cellular receptors

Initiating endocytosis – Despite fact that cell is too small to "swallow" a nanotube!

Computer simulation of resulting cellular indigestion:



"It's as if we would eat a lollipop that's longer than us - it would get stuck."

Huanjian Gao, Professor of Engineering, Brown University, discussing his paper "Entry of one-dimensional nanomaterials occurs by tip recognition and rotation" Nature Nanotechnology, September 2011

=> Sustained oxidative stress => Inflammation => Mutations =>Cancer

So I put this into my full "Fear" classification Especially as asbestos-induced cancers can take **20-30 years** to show up! AND there are claims that cancer could result from as little as **one** exposure Classic anecdote is of person who **once** changed asbestos-laden auto brake pads/shoes So asbestos' terrible toxicity would not have shown up in normal testing!!! Raising possibility that carbon nanotubes could pass reasonable tests But still, far down the road, turn out to be dangerous So what CAN one reasonably do? Help skin and lungs do their protective jobs! Protect them from nanoparticles with combinations of size & shape known to penetrate Or from nanoparticles with combinations of size & shape they have difficulty expelling Suggesting we may want to be really careful with certain sizes of carbon nanotube!

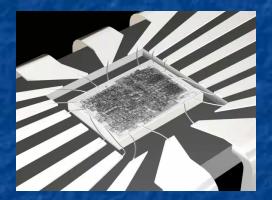
But where ELSE might nano toxic materials go?

To this point we've take a selfishly human-centric viewpoint: It's fine if it doesn't hurt me or (being cautious) penetrate me! But that is hardly the end of the story: There is that thing called **the environment**, a.k.a. all other living organisms Sizes/shapes that don't penetrate our bodies might easily penetrate other organisms Indeed many of the readings for this lecture provide evidence of this And if organism is a simple cell, we've seen it's very vulnerable to nanotoxic effects

And nano containment is a BIG potential challenge

Nanoparticles are easily carried on the winds and through the waters May even be possible that they'd percolate out of landfills through soil That was not possible with predecessor micro technology!

I've spent a career making micro/nano dimension things But when I got done, they went into something like this: A cm² sized chip, with inert SiO₂ surface



There were, of course, real issues with safety of manufacture and its wastes But when manufacture was complete: Was fairly easy to conclude product was safe And/or that the product was going nowhere other than where we last left it!

With containment being such a challenge, what are we doing?

We are putting nano metal oxides into sunblocks

That we then wash off our bodies and send into our water systems



We are embedding nano silver particles throughout our plastics

Where they can dissolve or dislodge from surfaces into our environment



Label I found on my kitchen wastebasket!

And we are gearing up to reinforce automobile tires with carbon nanotubes



This last possible application is a real gem:

Carbon nanotube reinforced tires:

- Should be stronger than "steel belted radial" tires

- And also lighter (and thus likely more fuel efficient)



But what happens when tires are used? Their tread wears away!

Top 1/4 inch or so of rubber seemingly "disappears" – But to where?

Rubber becomes fine powder distributed all around our highways and roads

Right into your own front yard!

Could "Doctor Evil" have imagined a better scheme for covering the earth with possibly carcinogenic CNT's?

Can it / should it be stopped?

I am a post WWII baby boomer who later became a physicist

So from two directions I heard the soul searching on nuclear weapons development "Someone is going to do it - so it better be us" was the favorite justification But, for better or worse, it also seemed to represent cold-hearted reality From this, and the broader history of technology, I conclude: Nano IS going to be pursued somewhere, REGARDLESS of our local beliefs, laws And from the science (e.g. nano-fluidics) I conclude: Even if "somewhere" is far away, will be impossible to contain once loose in environment So we ARE going to live with it. We should at least do so with our eyes wide open!

For nanotechnology:

Revise consumer product laws that now assume safety until proven otherwise

Move instead towards food and drug like requirement of up front safety testing

As the Europeans are now doing!

Demand labeling of nano containing products (allowing for some personal choice)

Revise health & environmental laws now naively based on only total mass exposure Instead take into account particle size and effects due to surface area enhancement 1 gm of carbon charcoal being safe, doesn't prove 1 gm of carbon nanotubes are safe ! Put some fraction of the governmental funds going into nano, into nano safety This HAS been proposed but strongly opposed (including by scientists wanting every \$) Closely monitor particularly vulnerable cells & species (recalling experience with DDT) In general: Act cautiously, particularly where we **have** viable alternative technologies

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