Understanding Cell-Nanoparticle Interactions- Making Nanoparticles more Biocompatible

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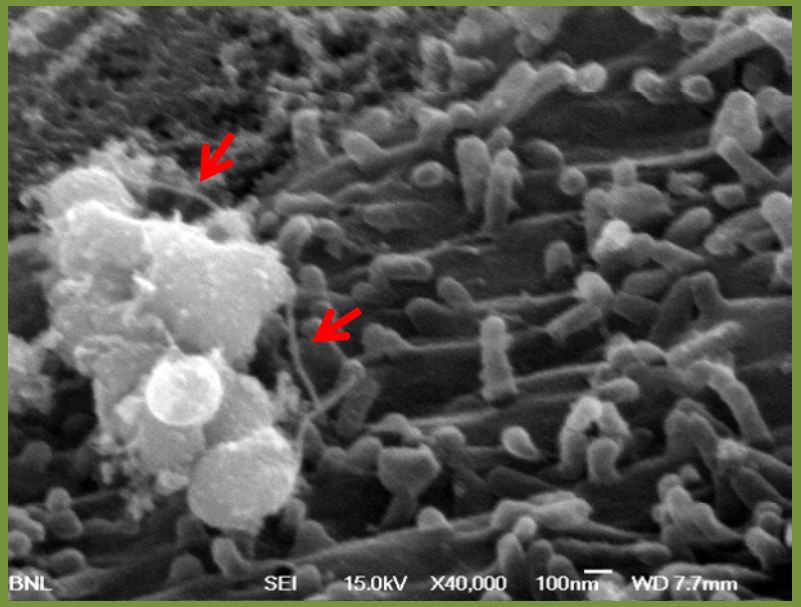

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Composition and Surface Characteristics of different Carbolex Carbon Nanotube Preparations alters Nanoparticle Reactivity and Toxicity

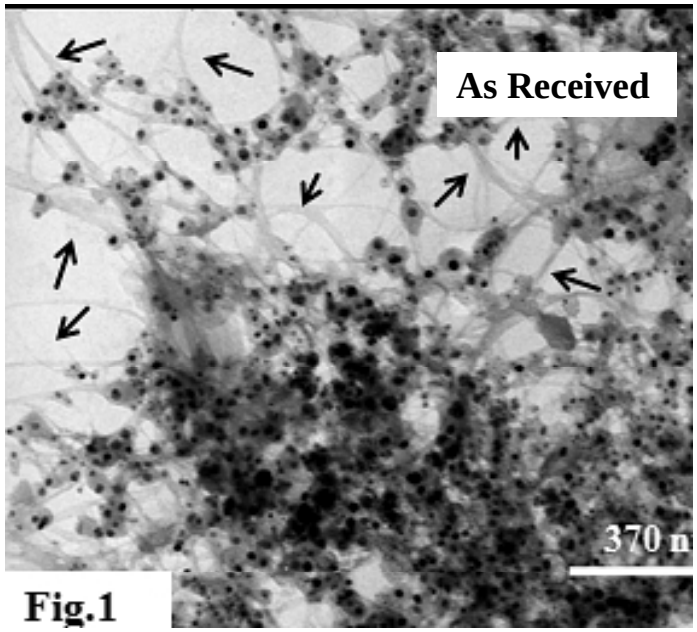


Fig.1

Carbolex material 'as received'(Fig.1) contained long SWCNTs (arrows), metal catalyst (Ni, Y), carbon black, non-tubular graphene and amorphous carbon.

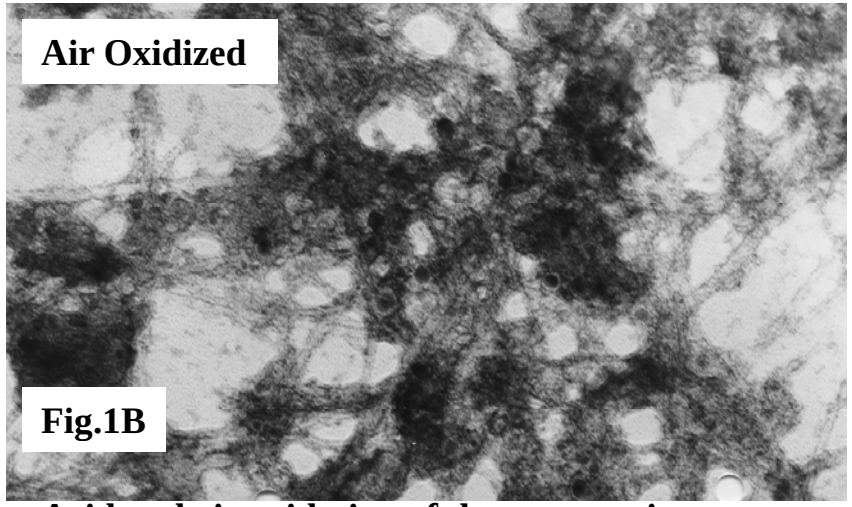


Fig.1B

Acid and air oxidation of the preparation (Fig.1B) removed all of the Y and most Ni, carbon black and nontubular graphene, leaving SWCNTs, amorphous carbon and nanotube fragments. By FTIR these SWCNTs show some OH groups (oxidation).

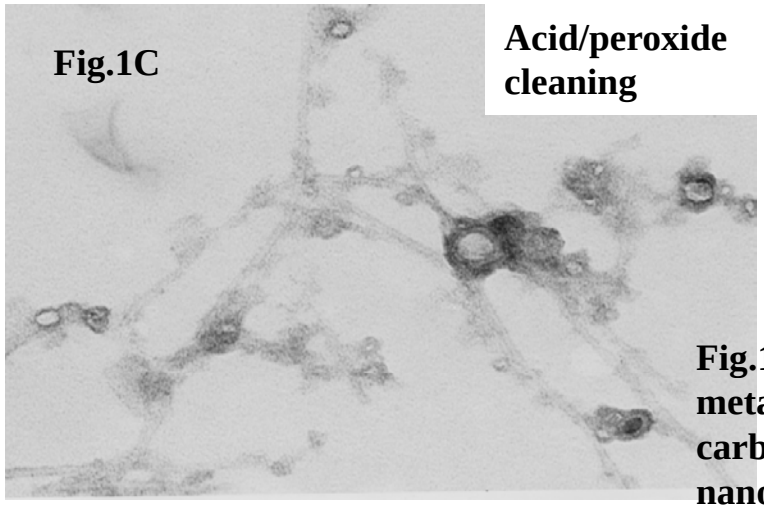
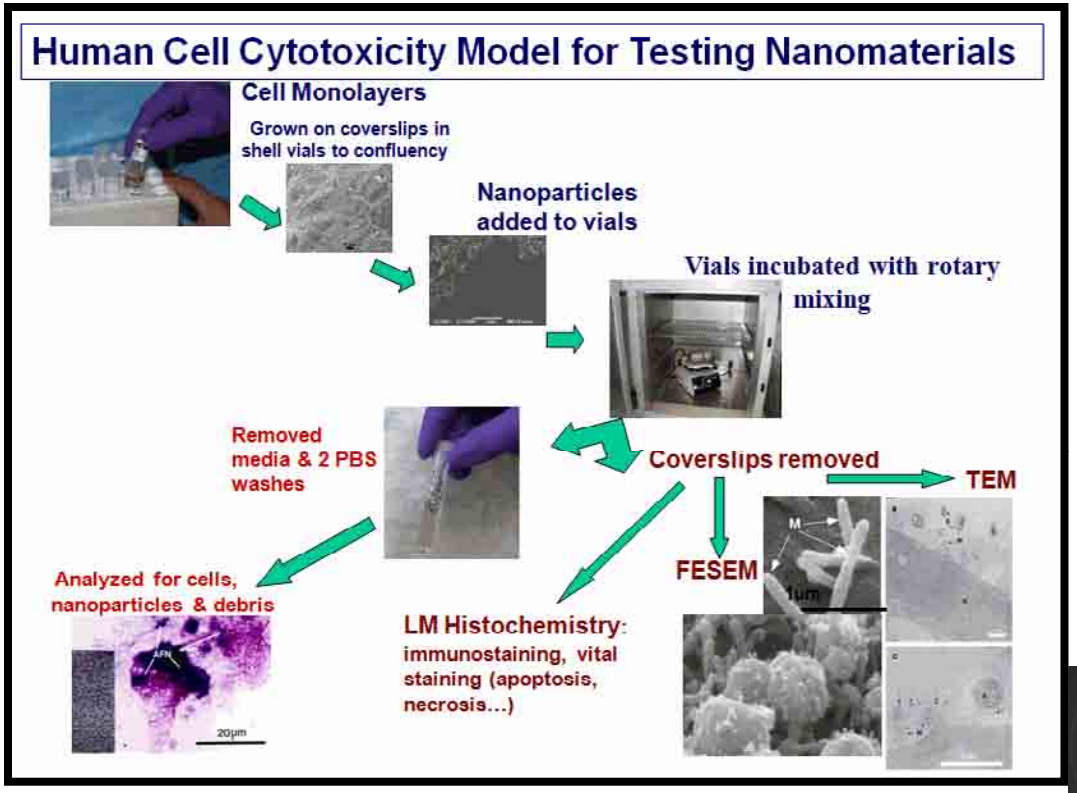


Fig.1C. Carbolex material sonicated in H_2SO_4/H_2O_2 to remove all metal catalyst, and debris leaving only SWCNTs, some amorphous carbon and graphite loops. Uranyl acetate stained. By FTIR these nanotubes show considerable OH groups.

Carbon Nanoparticle Testing



Nanoparticle Characterization:

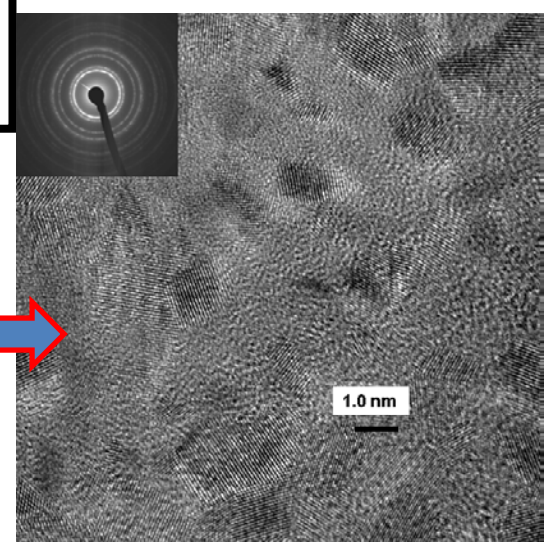
Morphology [particulates, size, shape]:
TEM, FESEM

Reactive groups & Charge:
FT-IR, Zeta analysis

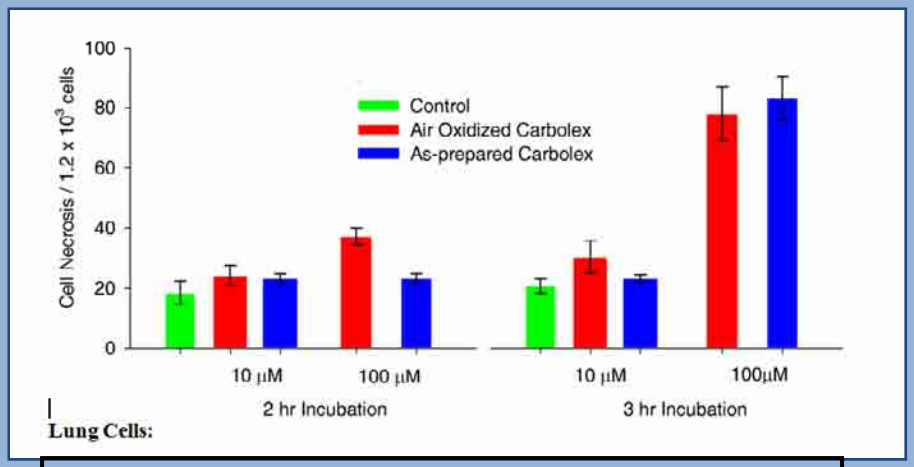
Composition & concentration:
FESEM X-ray microanalysis, UV-Vis
Raman spectroscopy

Aggregation:
TEM, Dynamic Light Scattering

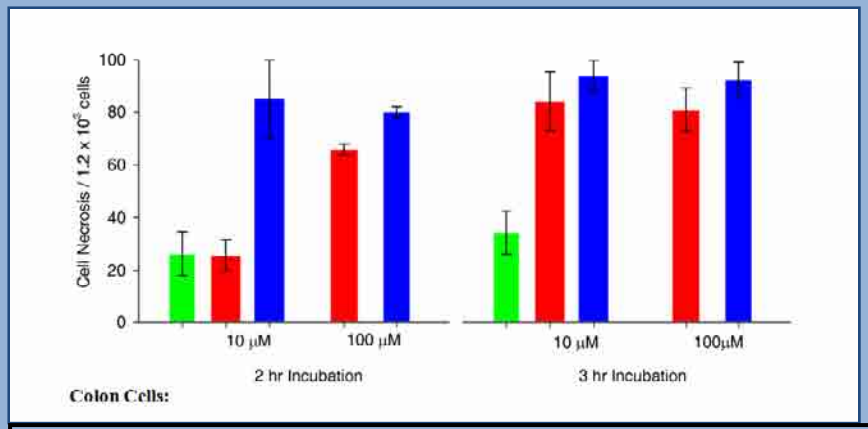
Specimens that were examined by FESEM were sputter coated with 3-4nm Pt using a turbo pumped instrument with a peltier cooled specimen stage. The metal films were found to be continuous, uniform and polycrystalline when examined by electron diffraction using a JEOL HRTEM at 300kV.



Lung & Colon Cell Viability: 2 or 3 hr exposure to Carbolex in buffered saline

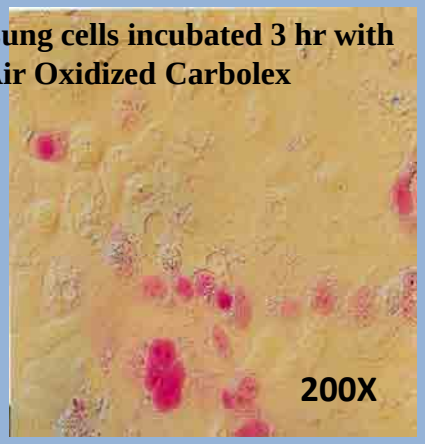


Lung cells showed most damage at higher concentrations & longest exposure times.

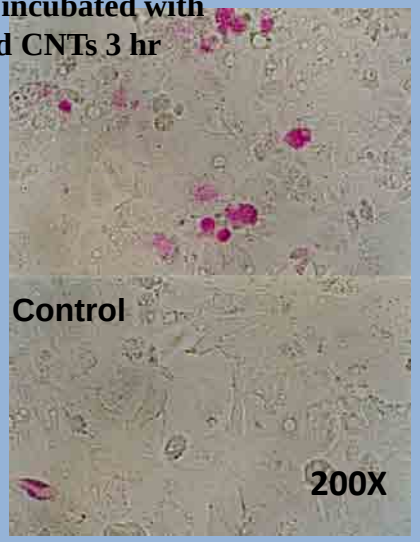


Colon cells were more sensitive to CNP exposure, showing high cell death rate at high CNP exposure time & concentration.

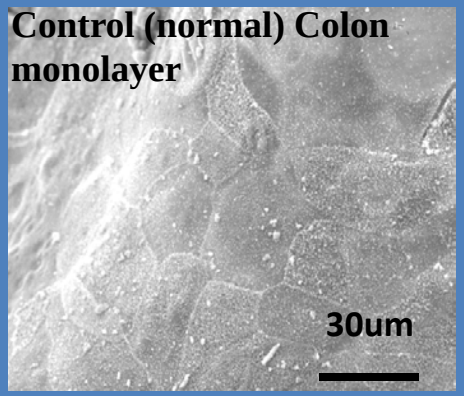
Lung cells incubated 3 hr with Air Oxidized Carbolex



Colon Cells incubated with Air Oxidized CNTs 3 hr

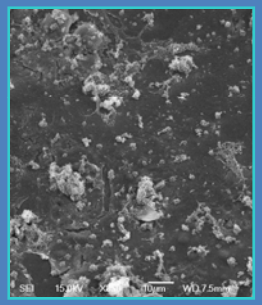


Colon Cell response to 2 hr Carbolex exposure at 10 µM and 100 µM dose.

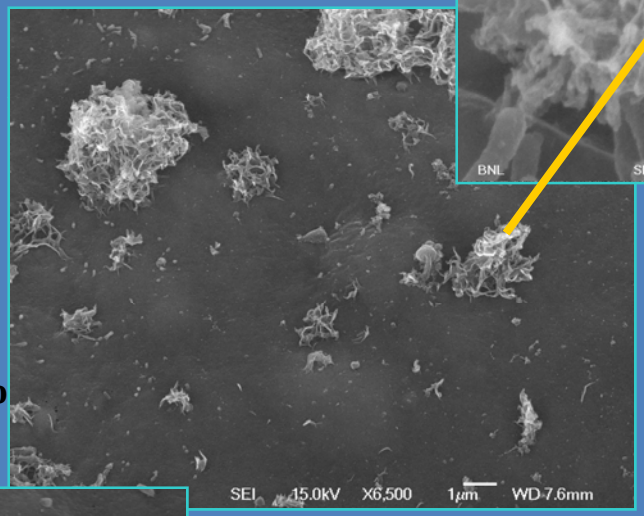


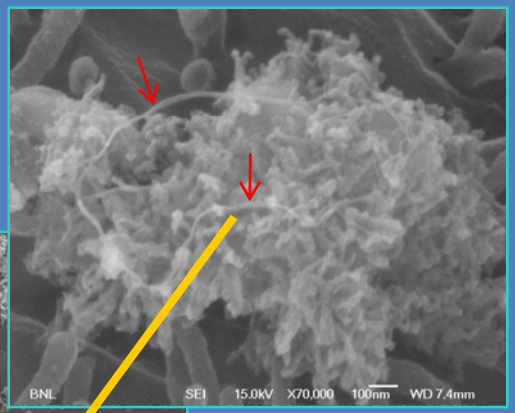
Control (normal) Colon monolayer

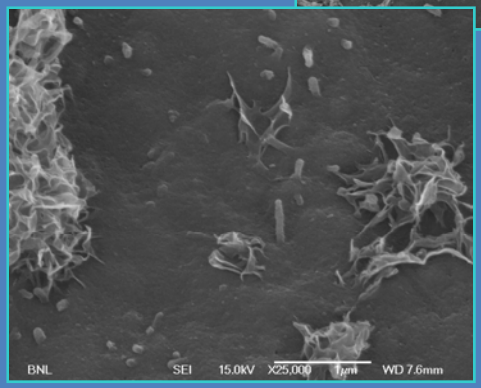
10µM 2 hr



Cells attached to one another and to substrate.

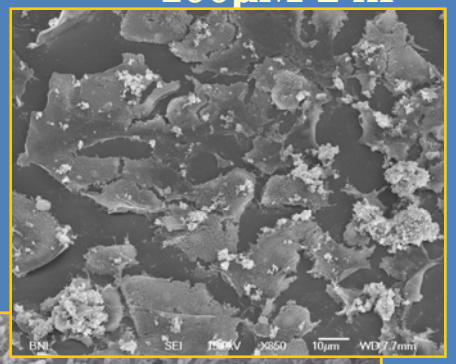


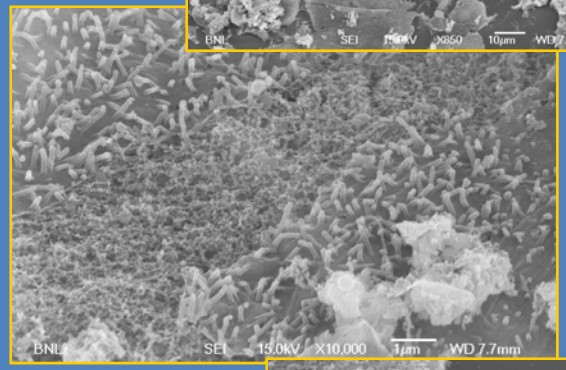




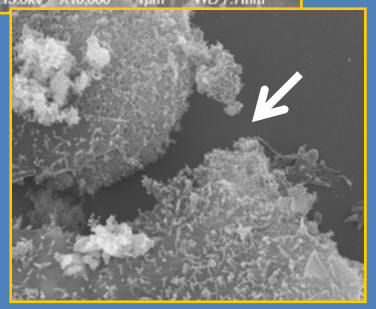
Monolayer intact. Microvilli & plasma membrane appear normal. Cells show surface mucoid material and ruffles.

100µM 2 hr



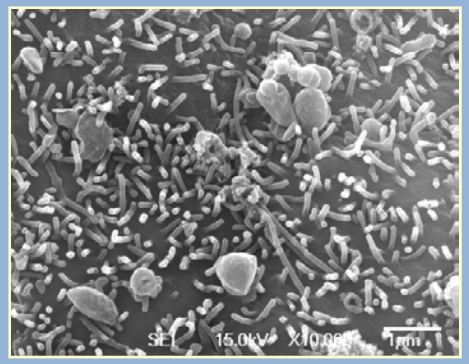


Cells separating & detaching from coverslip. Apical membrane damage

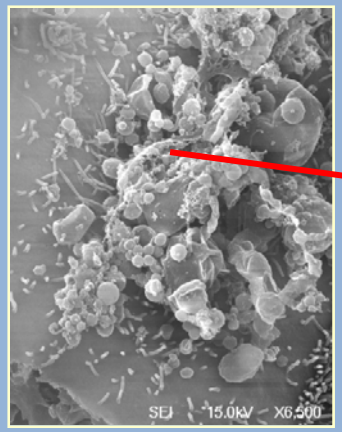


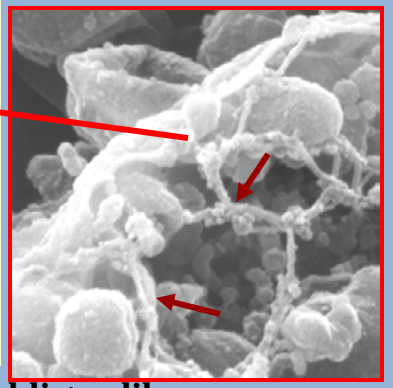
Human Colon Cell Response to 4 hr Exposure to Air Oxidized Carbon Nanotubes in buffered Saline with increasing dose.

2.2 µM



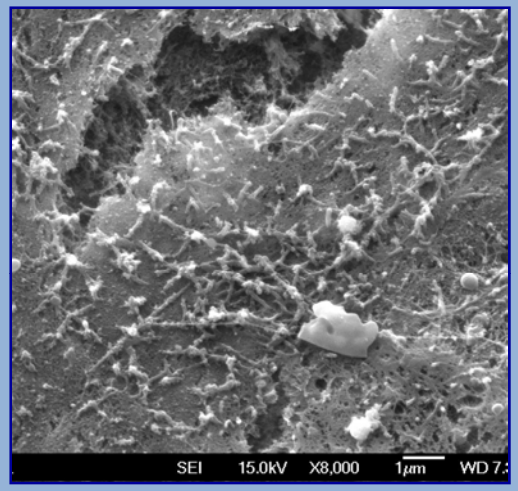
Colon cells show glisters or blebs on the membrane surface.





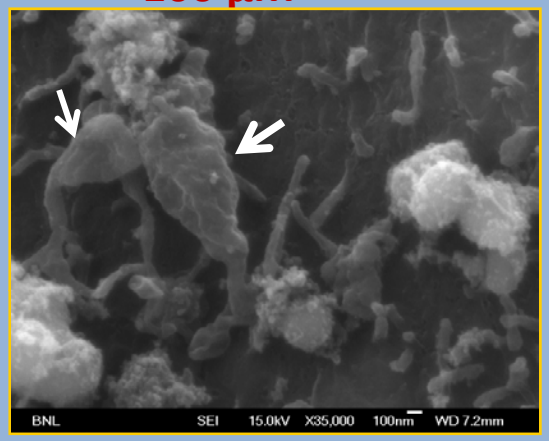
Nanotubes seen in blister-like eruptions on membrane surface.

10µM



Small and large holes in cell membranes where nanotubes and nanoropes attached.

100 µM



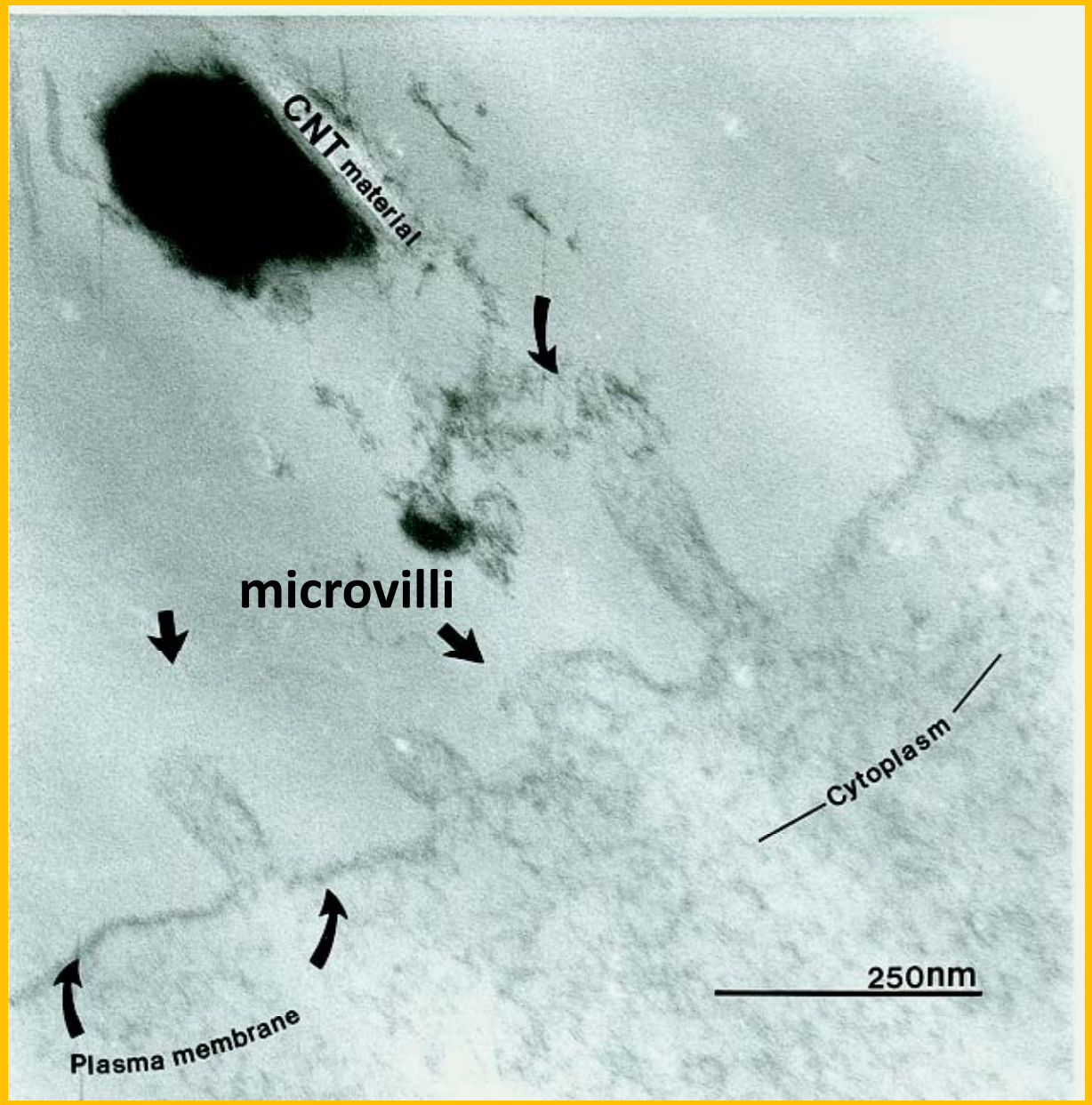
Microvilli show severe swelling where nanoparticles touch them.

Small holes in plasma membrane .

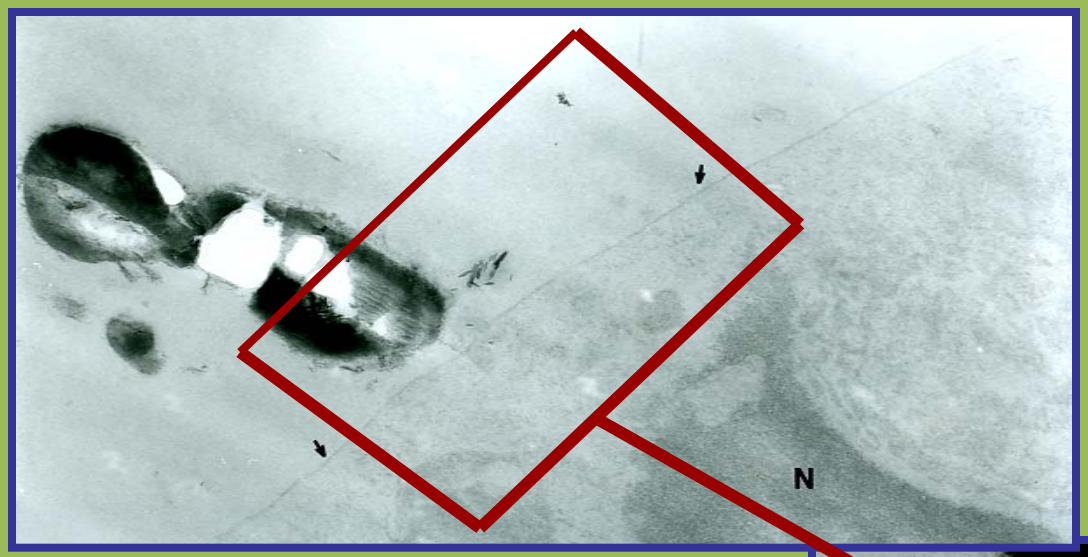
Human Colon Cells

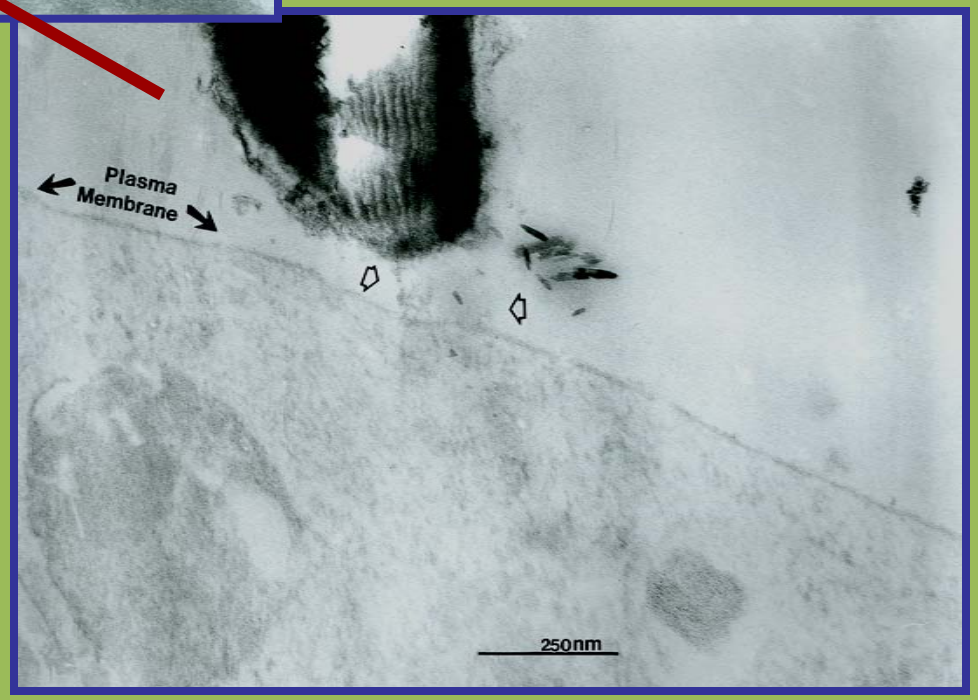
10µM Air Oxidized Carbon Nanotubes incubated for 2.5 hrs.

Nanoparticle contact with the surface membrane of the microvilli, caused the ultimate destruction of these structures.



Human Colon Cells Incubated with Carbolex 100µM (4 hr)



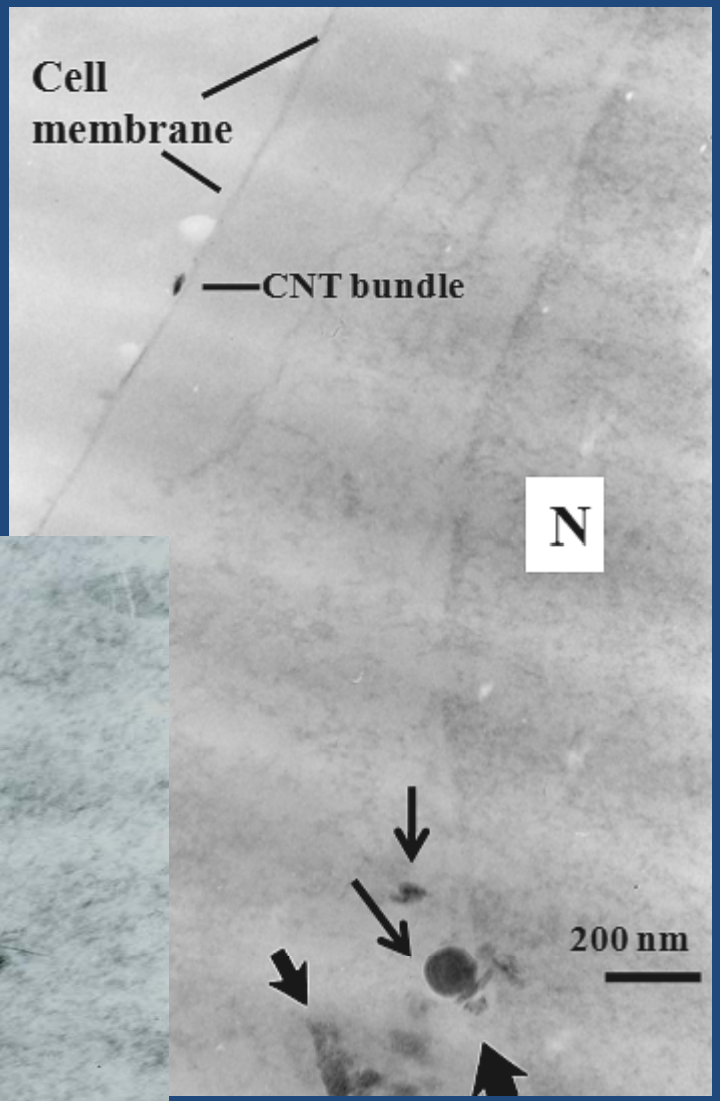


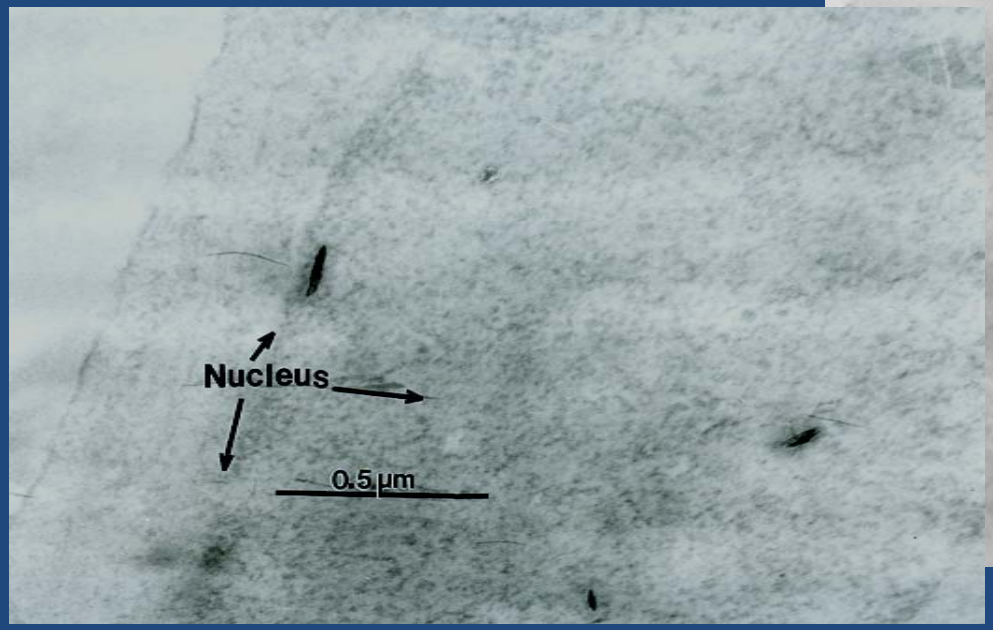
Carbolex material adjacent to colon cell surface shows electron dense carbon nanoparticles and aggregates at the cell surface. In this region there are small breaks in the plasma membrane, which may represent the type of membrane lipid peroxidation reported by Sayes et al.(2005).

Small Carbon Nanoparticles can enter Human Colon cells if the outer cell membrane is compromised

Carbon Nanoparticles enter the Colon Cells and travel freely through the cytoplasm (A) to the Nucleus (N) within 1 hr 45 min.

A.

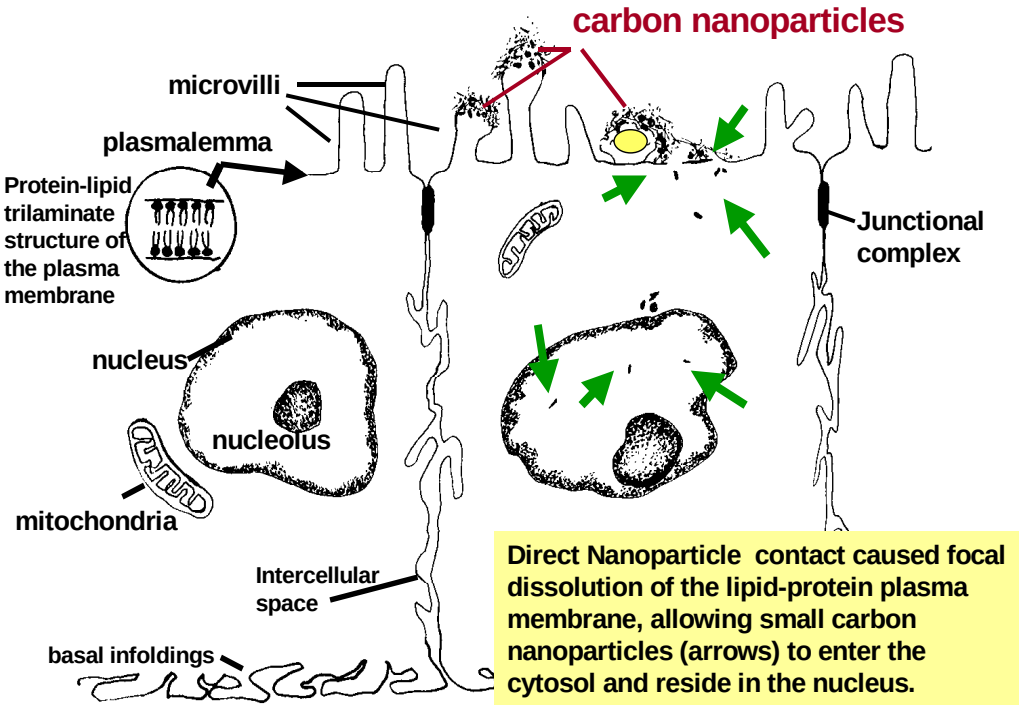




B.

B. Some particles enter the nucleus and remain within the nucleoplasm.

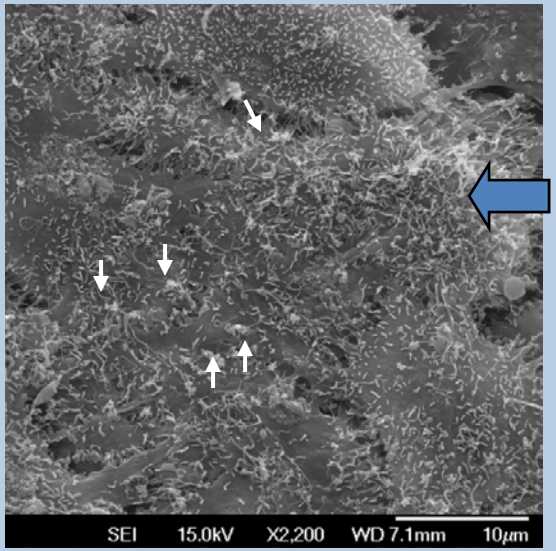
Carbon Nanoparticle Interaction with Human Caco-2 Cells



Direct Nanoparticle contact caused focal dissolution of the lipid-protein plasma membrane, allowing small carbon nanoparticles (arrows) to enter the cytosol and reside in the nucleus.

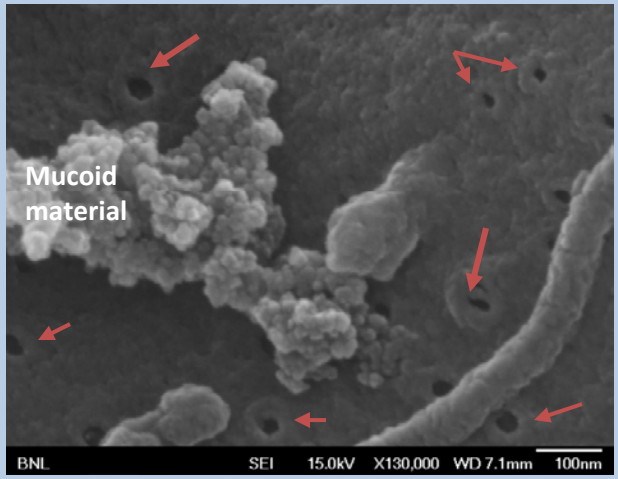
Panessa-Warren et al., 2006,J. Phys. Condens. Matter 18, S2185-S2201.

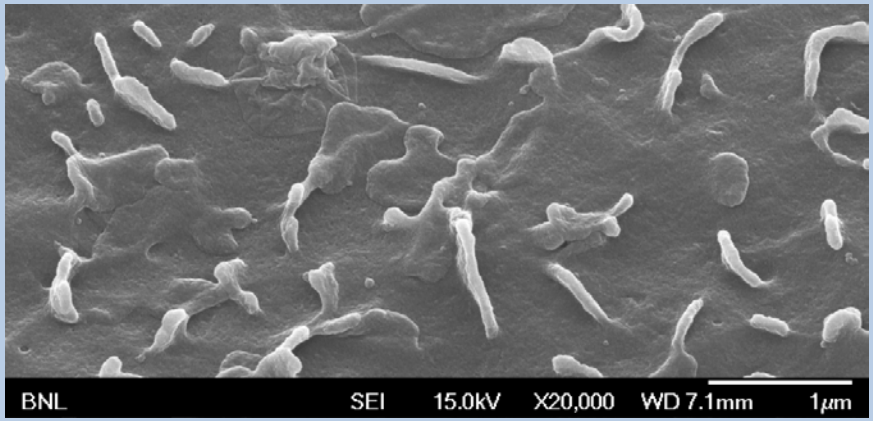
Normal Lung Cell Monolayers 2 hr and 3 hr incubation



Control cell monolayers showed continual growth, with normal cell-cell adhesion and cell substrate adhesion after 2 and 3 hr incubation.

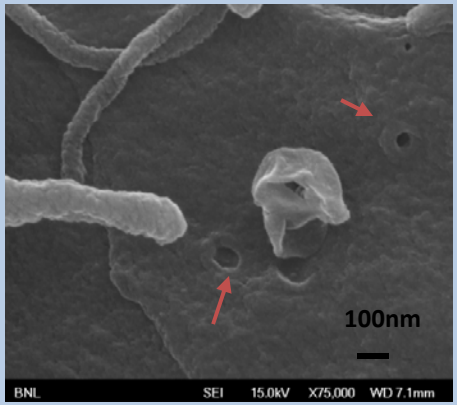
Small amounts of mucoid material (arrows) were seen on the surface membranes.



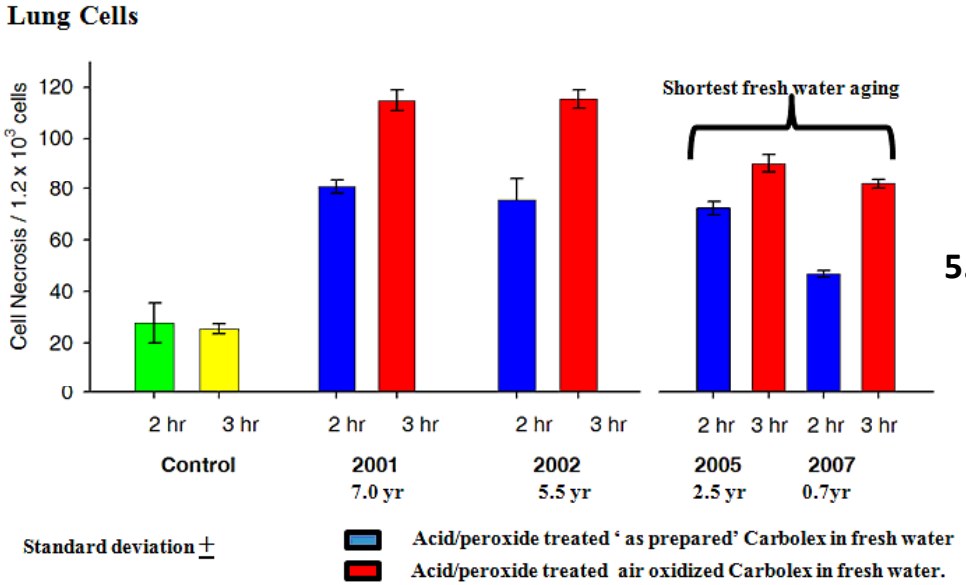


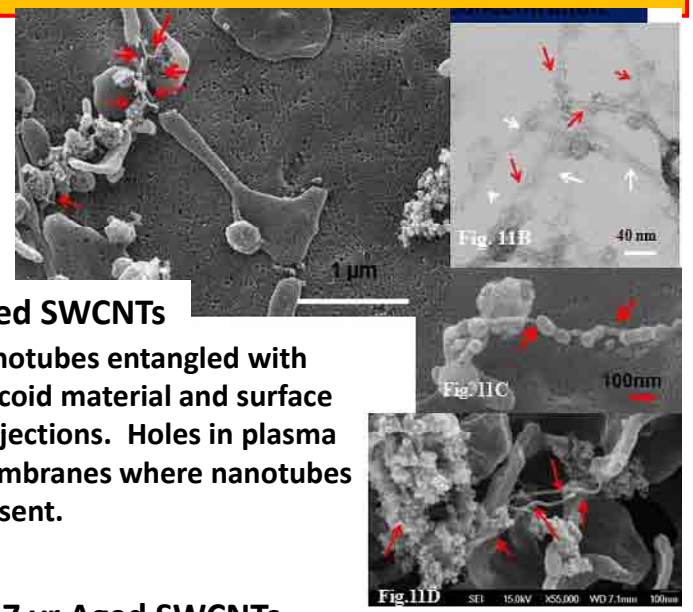
Active cells showed smooth , continuous surface membranes with cytoskeletal extensions and filliform structures with.

The apical plasma membrane was uniformly smooth with lipped openings (arrows) in the apical surface which may function in normal pinocytosis or release of the mucoid material produced by these lung cells.



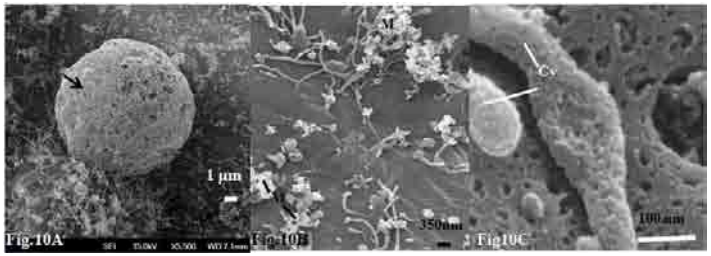
Human Lung Cell Response following 3 hr Exposure to Acid/Peroxide Cleaned Carbolex SWCNTs Aged in Fresh Water Aged 2.5, 5.5 and 7 yrs





5.5 yr Aged SWCNTs

Nanotubes entangled with mucoid material and surface projections. Holes in plasma membranes where nanotubes present.

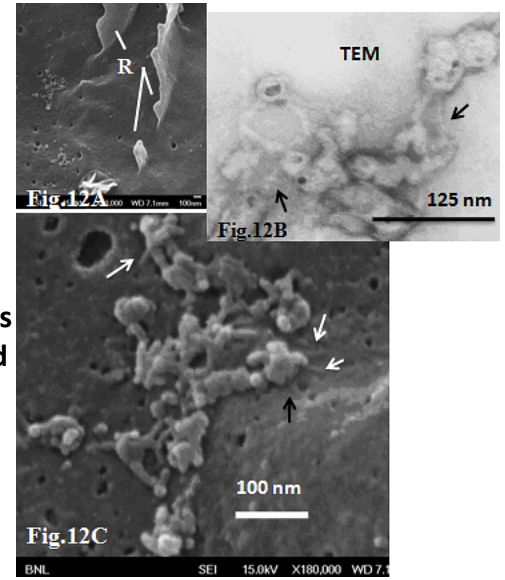


2.5 yr Aged SWCNTs

Lung cells appeared intact but showed severe membrane damage (10A, rounded cell with no plasma membrane). Some cytoskeletal appendages (Cy) showed as severe membrane damage as the plasma membrane.

7 yr Aged SWCNTs

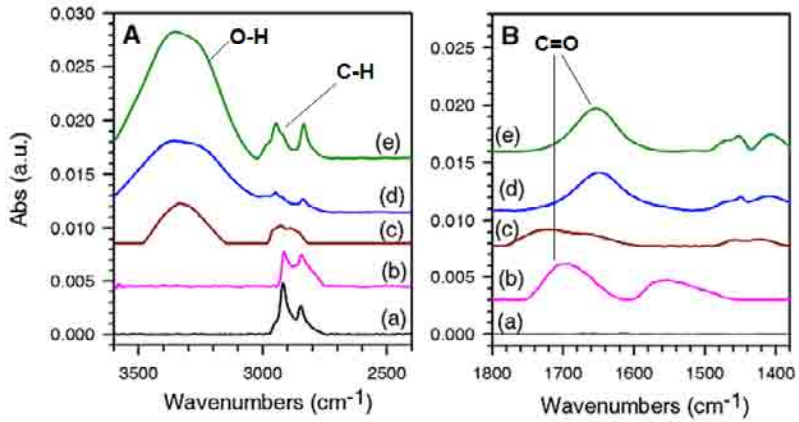
SWCNT aggregates (30-500 nm) of cut nanotubes with amorphous carbon seen by TEM were found on the apical membrane surfaces. Lung cell membranes produced ruffles and surface holes where aggregates were attached .



Panessa-Warren et al. 2008, Environ. Pollution, special issue nanoECO.

FTIR Spectra of Acid /Peroxide SWCNTs Stored in Fresh Water

Each trace represents the water, Carbon monoxide and background subtracted FTIR spectrum of the Acid/Peroxide cleaned SWCNTs aged in 18MΩ fresh water for 0.7, 2.5, 5.5 and 7.0 yrs, compared to the FTIR of the parent 'as received' material.

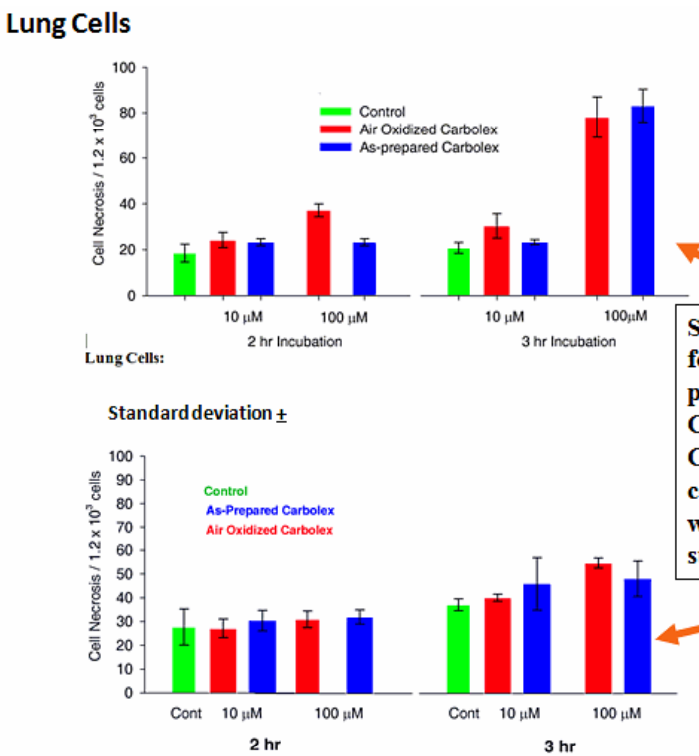


a. (black) Original 'as received' Carbolex - non-oxidized SWCNTs
b. (pink) Acid/peroxide cleaned SWCNTs in fresh water containing Fe
c. (brown) Acid/peroxide cleaned SWCNTs aged 2.5 yr in fresh water
d. (blue) Acid/peroxide cleaned SWCNTs aged0 .7 yrs in fresh water
e. (green) Acid/peroxide cleaned SWCNTs aged 7.0 yrs in fresh water

Findings:

- **The modified piranha protocol used to clean the Carbolex nanoparticles, produced SWCNTS with considerable oxidation of the cut nanotube surfaces and probably the entire lattice surface (Ziegler et al., 2005).**
- **Storing acid/peroxide treated SWCNTs in fresh water for up to 7 yrs did not decrease their surface oxidation.**
- **When SWCNTs with hydroxyl surface groups (FTIR) were incubated with lung cells, greater damage (microscopy) and cell death (viability studies) occurred than with control, non-oxidized SWCNTs.**

Lung Cells exposed to freshly prepared Carbolex Nanoparticles, and 2.5 yr Carbolex aged in buffered Saline



A. Freshly made PBS Carbolex solutions

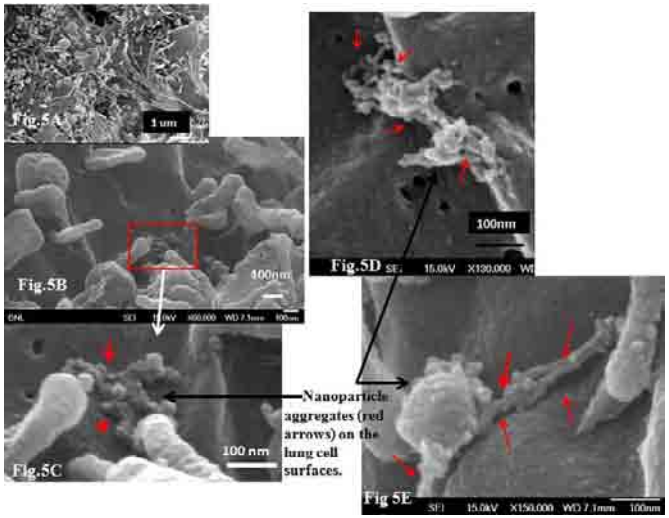
N=16

Significantly more lung cells died following incubation with freshly prepared 100 µM 'as prepared' raw Carbolex in PBS and air oxidized Carbolex in PBS (3 hr)[A], when compared to lung cells incubated with the same Carbolex solutions stored in PBS for 2.5 yrs [B].

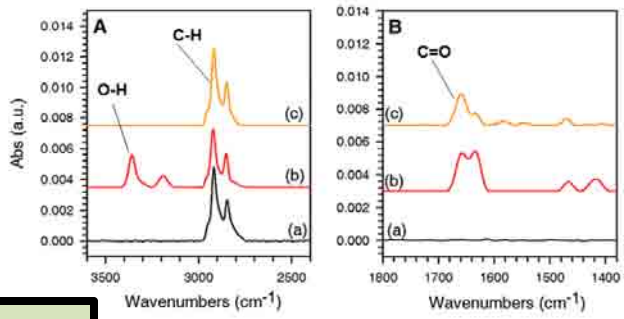
B. 2.5 yr PBS Aged

N=16

Lung cells incubated with 2.5yr Saline aged Carbolex



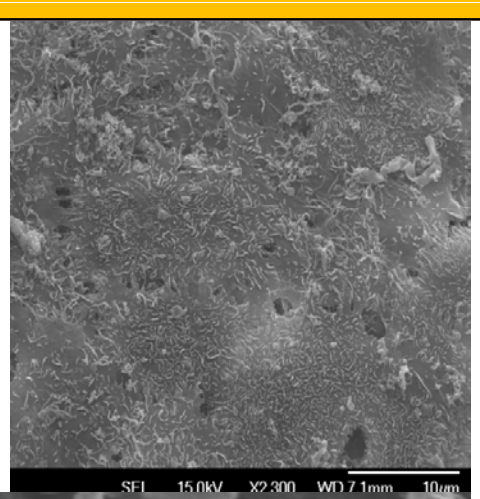
FTIR of 2.5 yr Saline aged Carbolex showed no oxidation, and only minimal oxidation was seen for the2.5 yr saline aged Air Oxidized Carbolex.

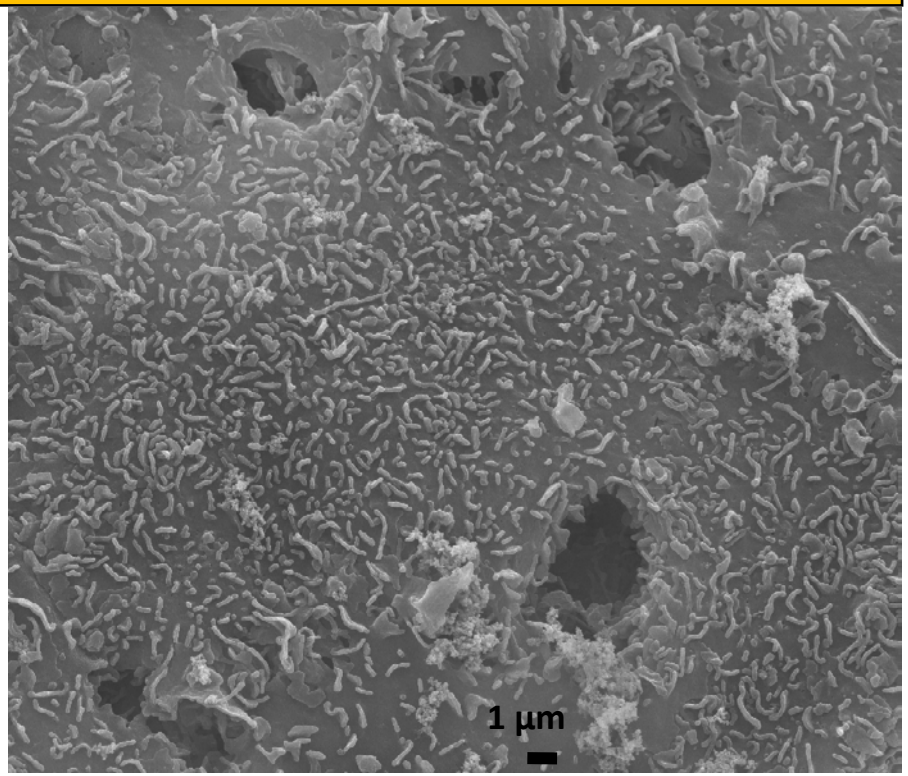


This suggests that (1)oxidized nanotubes are more toxic than non-oxidized nanotubes; and (2) toxicity can be reduced by reacting oxidized carbon nanotubes with neutral pH aqueous saline.

Human Lung Cells exposed to 100µM Air Oxidized Carbolex Aged 2.5 yrs in buffered Saline

Cells looked plump and active with some evidence of membrane activity, but good confluency.





The membrane surface at higher magnification was intact with little or any membrane damage. Nanoropes and nanoparticles did not seem to attach to the membrane surface very often, and there was reduced mucoid material produced on the cells apical surfaces.

Causes of Carbon Nanoparticle Cytotoxicity

1. Metal Catalyst particles?

- **Shevdova et al. 2003: Fe enriched SWCNTs were more cytotoxic to keratinocytes in vitro, than to Fe-chelated SWCNTs.**
- **Kagan et al. 2006: Using the same nanotubes (with and without Fe), found that RAW 264.7 lung macrophages did not produce signs of cytotoxicity when Fe was present.**
- **Panessa-Warren et al. 2008: Comparing human lung epithelial cell responses following exposure to 'as received' Carbolex and Air Oxidized Carbolex (to remove most of the metal), the lung cells showed less cytotoxicity to the metal containing material. Studies with acid/peroxide cleaned Carbolex samples (that had no metal) showed that significant oxidation of the carbon lattice produced the most lung cell damage and necrosis.**
- **All cell types are different, and may respond differently to specific metals…**

2. Exposure Time and Dose:

- **Increasing exposure time to carbon nanoparticles usually increases in cell/tissue cytotoxic responses.**
- **Increasing the dose can significantly increase toxic response (due to greater nanoparticle contact with membrane surfaces, overwhelming enzyme and transport systems, bioaccumulation etc..)**

3. Contaminants in preparations, and reactive groups on nanoparticles that were thought to be clean, can interact with cells producing damage.

Metal Core Nanoparticles: Quantum Dots

The central metal core of many nanoparticles present unique problems in biocompatibility. CdSe QDs present some specific problems:

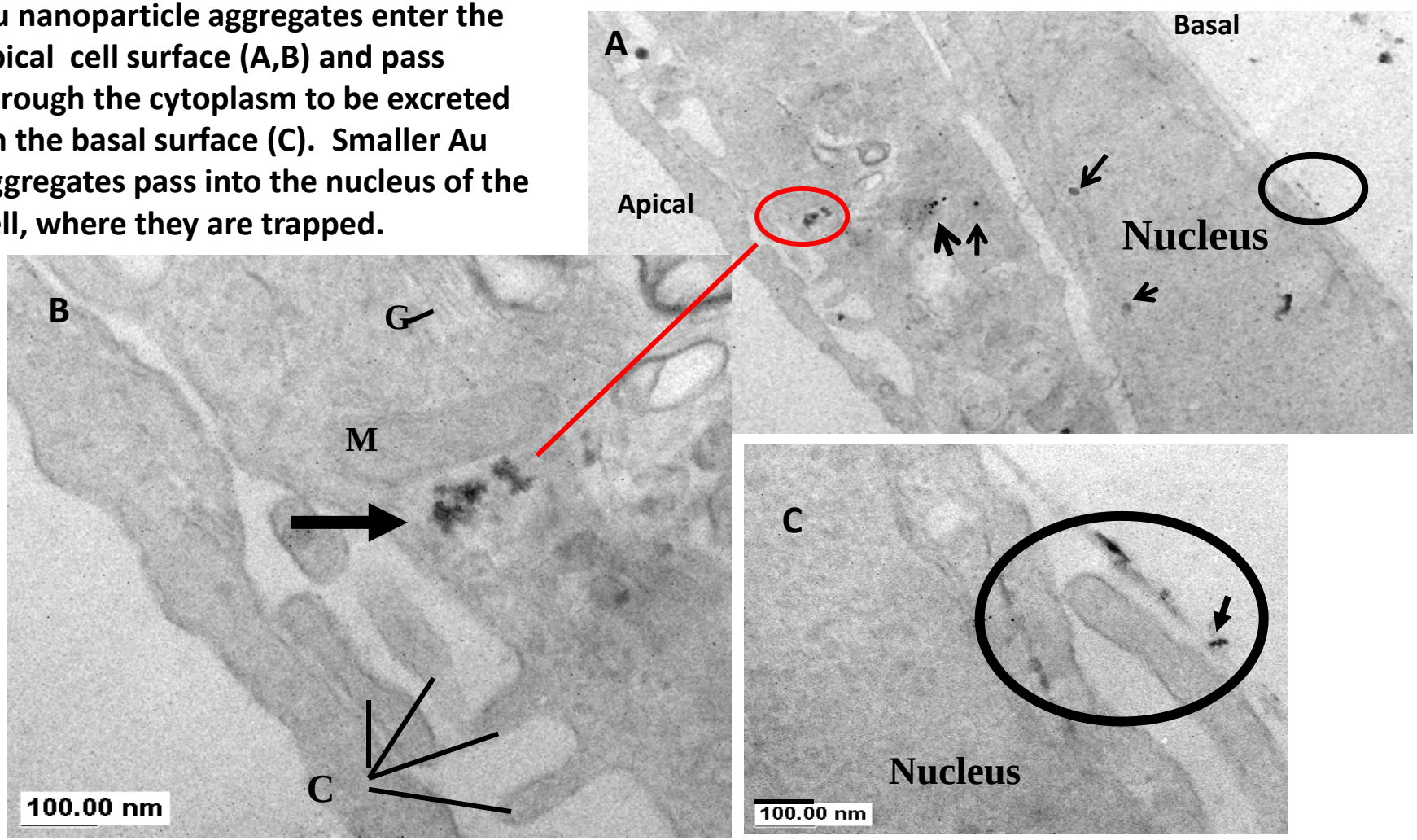
- **Derfus et al. 2004, (Nano Letters) demonstrated that CdSe core QDs were cytotoxic to hepatic primary cells *in vitro* under certain conditions (surface oxidation led to formation of reduced Cd on the QD surface and release of free Cd ions causing cell death).**
- **<u>Surface coatings (BSA, ZnS and PEG) significantly reduce, but do not eliminate cytotoxicity.</u>**
- **Ackerman et al. 2002 (PNAS) and Larson et al., 2003(Sci.) described 3 fates of CdSe/ZnS QDs in mice:**
  1. **Clearance of particles via the kidney**
  2. **breakdown into smaller particles**
  3. **QDs sequestered within vacuoles or endosomes**
- **Cracks in capping material or outer shell due to biological activity (enzymes, pH)**
- **Cd debris from partially cleaned preparations can poison cells**
- **Specific sized QDs can get lodged in tissues due to their size (2 nm-10-15 nm cores), and they may not readily leave the tissue- presenting a problem when in future the sequestered nanoparticles may biodegrade into toxic components.**

Strategies that can Mitigate Nanoparticle Toxicity in Biological Systems

1. Highly Protective surface coatings(i.e. polymer coatings, lipid micelles with protein cross linking etc…) improve QD safety.
2. Derfus et al. (2004) found that even with multiple inorganic/organic surface coatings, oxidation of the particles could occur releasing Cd.
3. Using a low dose of QDs having a nanoparticle diameter that will not get stuck within filtering pores , podocytes or slits in biological membranes or barrier systems-
   - Using too high a dose of metal core nanoparticles can overwhelm transport systems, resulting in bioaccumulation of nanoparticles within cells or tissues.
   - Sun et al., 2005 (Amer. Chem. Soc) reported that flexible core nanoparticles that permit shape adaptability can successfully traverse sinusoidal capillaries. They also found that the hydrodynamic diameter of the nanoparticle played an important role in biodistribution and needs to be considered during the synthesis-engineering phase of QD design to reduce toxic bioaccumulation.

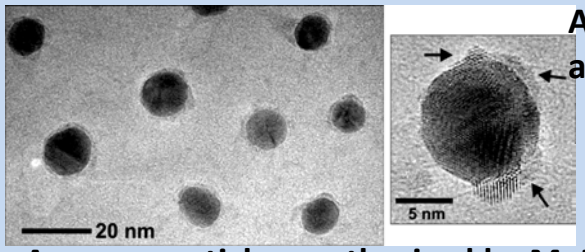
Human Lung Cells exposed to 2nm Au-citrate capped nanoparticles.

Au nanoparticle aggregates enter the apical cell surface (A,B) and pass through the cytoplasm to be excreted on the basal surface (C). Smaller Au aggregates pass into the nucleus of the cell, where they are trapped.



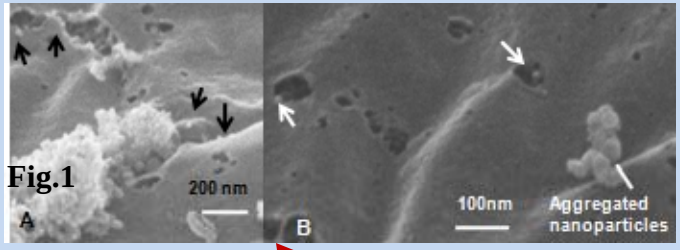
Panessa-Warren et al., 2008, Int .J. Nanotechnol. 5 (1):55-92

Au Nanoparticles: Reduced Colon Cell Cytotoxicity with DNA functionalized (100% covered) gold nanoparticles.



Arrows point to DNA around outside of gold core

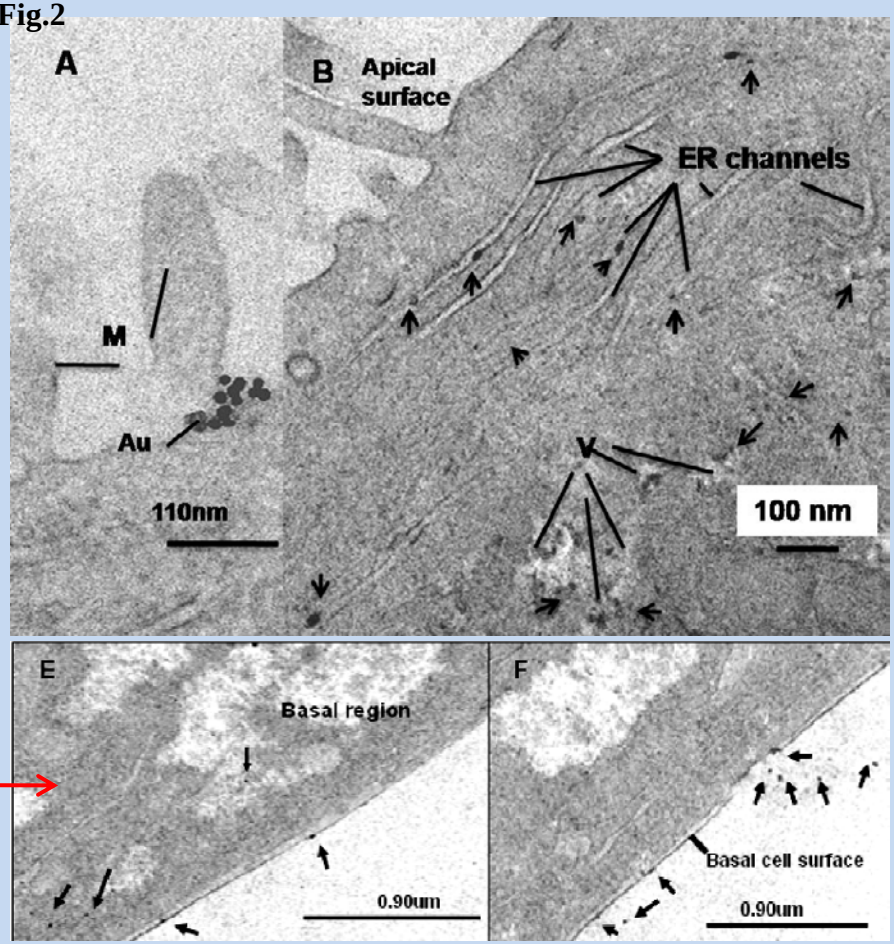
Au nanoparticles synthesized by M. Maye Brookhaven National Laboratory. Maye et al.2006, J. Am. Chem. Soc. 128: 14020.



When human colon cells were exposed to these Au nanoparticles they readily incorporated them, transporting them through their plasma membrane(1A,B), and (2A,B)cytoplasm- excreting them at the basal cell surface(2E,F).

Panessa-Warren et al., 2008, Int. J. Nanotechnol., 5 (1):55-92; and Panessa-Warren , 2008, Lecture Notes in Physics, Springer Verlag, Heidelberg, Germany.

Fig.2



Engineering Safer and more Biocompatible Nanoparticles

This review touched on many of the characteristics of engineered nanoparticles that cause toxicity to cells, tissues and animals. To have safer nanoparticles and reduce toxic affects:

1. Reduce the nanoparticle concentration (dose) and duration of exposure.

2. Avoid techniques that increase surface Oxidation of both metal core nanoparticles and carbon nanotubes (sonication causes oxidation just as readily as acid-piranha cleaning protocols). Also many nanoparticles are affected by light and change reactive characteristics during light exsposure.

3. Make sure capping, and functionalization layers cover the entire carbon nanotube or metal core, and that this coating will survive the treatment protocol or biological procedure for which it is intended.

4. Do not use old nanoparticles that may have damaged outer protective coatings, or functionalizations which have interruptions in the protective coating. Capping material & Functionalized groups change with time and storage- use freshly prepared nanoparticles when possible.

5. Design nanoparticles with a size that does not put the biological test system, or the designer of the nanoparticle, at risk (very small nanoparticles can get into organellar compartments and pass into cells readily and sometimes irreversibly).

6. Storing nanoparticles in fresh water may increase oxidation and nanoparticle degradation into reactive, toxic components. Storing some nanoparticles in salt solutions binds oxidized sites reducing reactivity and making a more biocompatible, less toxic nanomaterial.