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Use of 3D Tissue Models EpiDerm & EpiAirway for Nanotoxicology Applications

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OUTLINE

- Background:
  - Need for in vitro nanoparticle toxicology models
  - Organotypic models
  - Special considerations for working with nanoparticles
- Results obtained in penetration and genotoxicity studies with EpiDerm and EpiAirway
- Examples of other published nanotoxicology applications using EpiDerm, EpiDerm-FT and EpiAirway tissues
- Conclusion

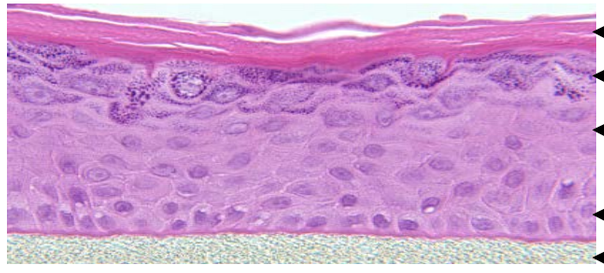
Need for in vitro nanoparticle toxicology models

- nanotechnology - a revolutionary impact on biology and medicine
- very little information regarding the toxicity of nanomaterials
- nanomaterials are utilized in numerous commercial applications where dermal contact, inhalation, or oral ingestion is likely
- potential adverse effects of nanoparticle exposure include allergenicity, cytotoxicity, and genotoxicity
- there are no accurate in vitro tools available to predict or assess the risks of nanomaterials. Existing in vitro monolayer cell culture models provide little correlation with in vivo situation.
- urgent need for in vitro tissue models that can be utilized for toxicological evaluation of nanoparticle materials.

Reconstructed Human Tissue Models

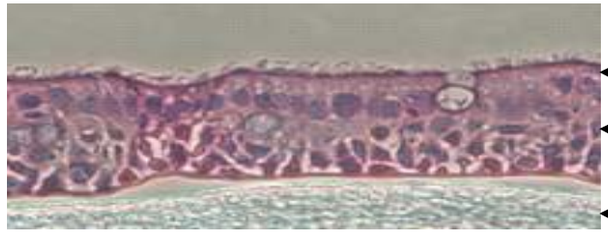
- Normal (non-immortal) human cells
- Organotypic structure
- Barrier function
- Real-life exposure conditions
- Xenobiotic metabolizing capabilities
- Co-culture models for epithelial-stromal interactions

1993 EpiDerm

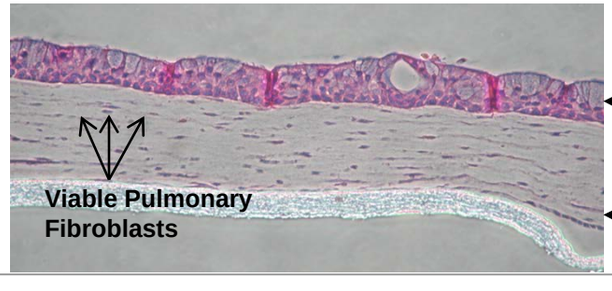


2002 EpiDerm FT

2000 EpiAirway

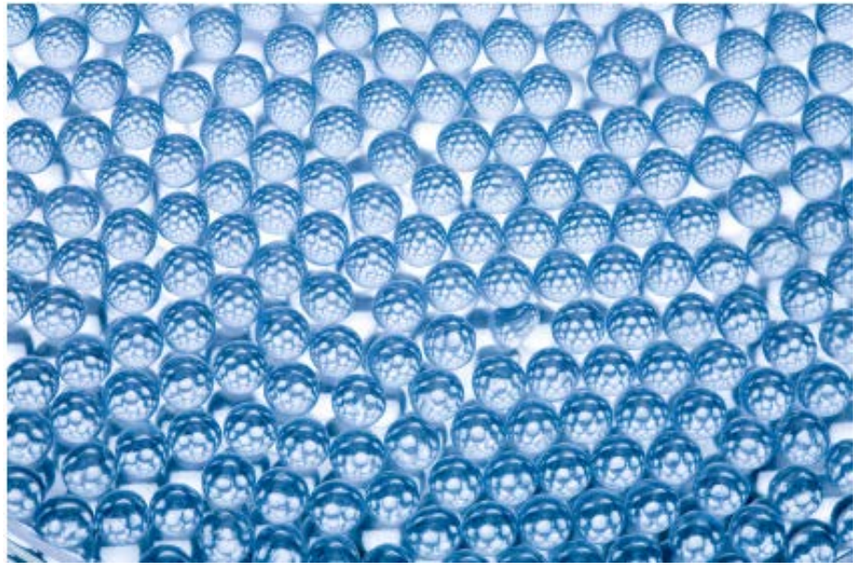


2003 EpiAirway - FT

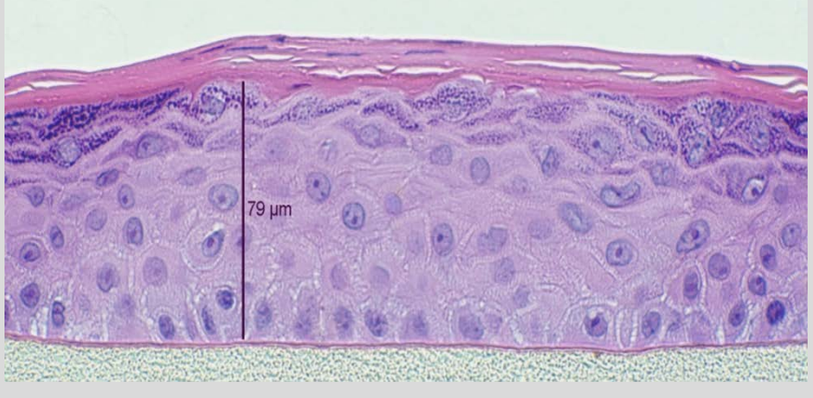


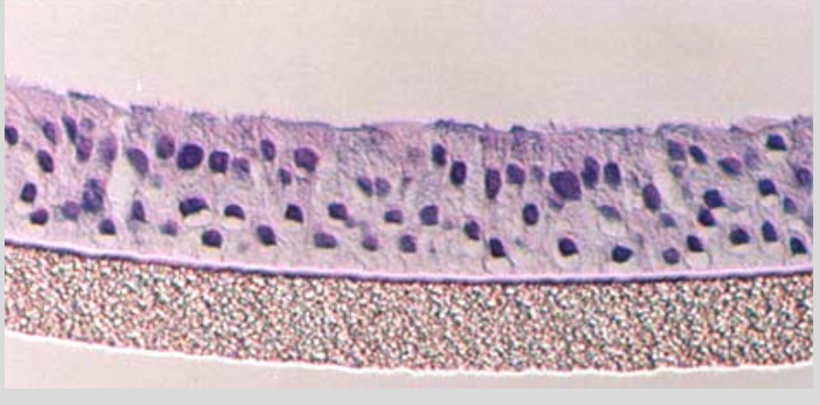
Special considerations for working with nanoparticles

- Nanoparticle properties and their toxic effect will be highly formulation dependent.
- In vitro exposure conditions should match in vivo exposure conditions as closely as possible in order to draw correct conclusions.
- Don’t want to underpredict or overpredict toxicity.

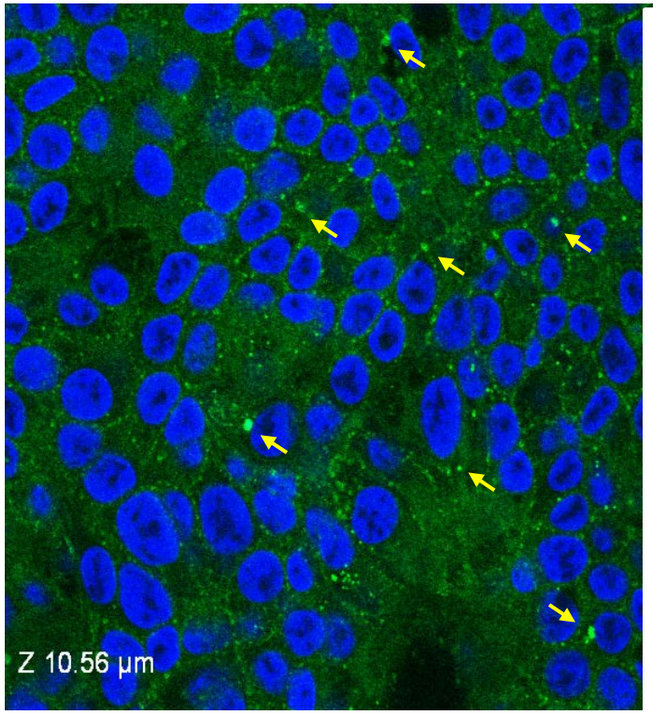


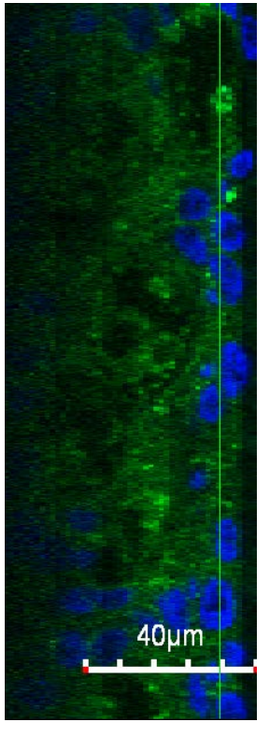
Results obtained in penetration and genotoxicity studies with EpiDerm and EpiAirway

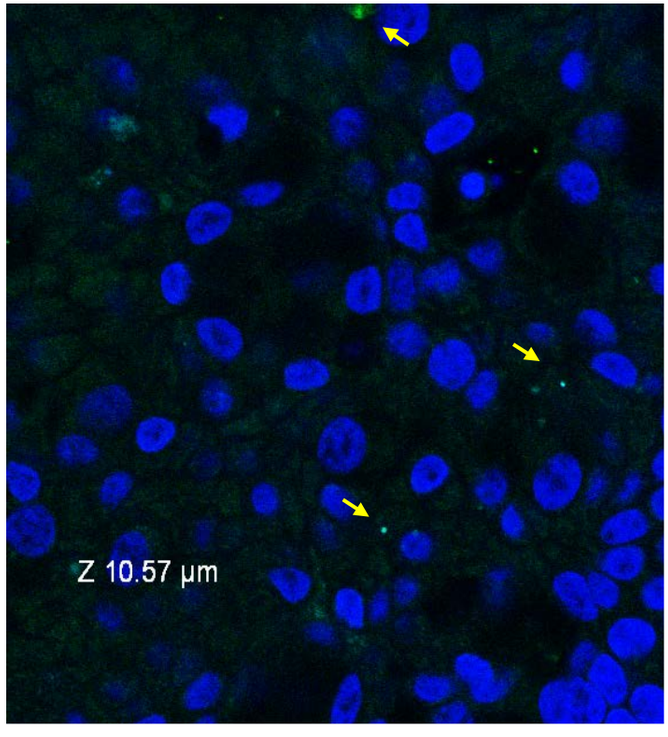


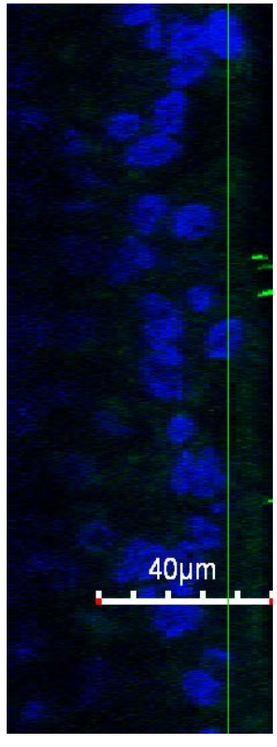


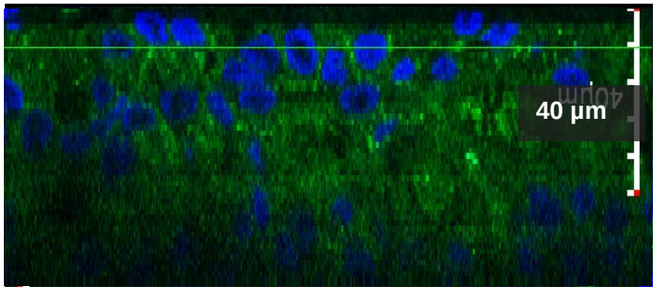
EpiAirway – Lung penetration of nanoparticles





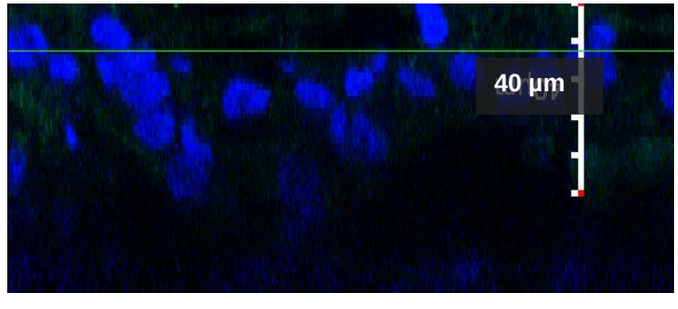






Apical surface

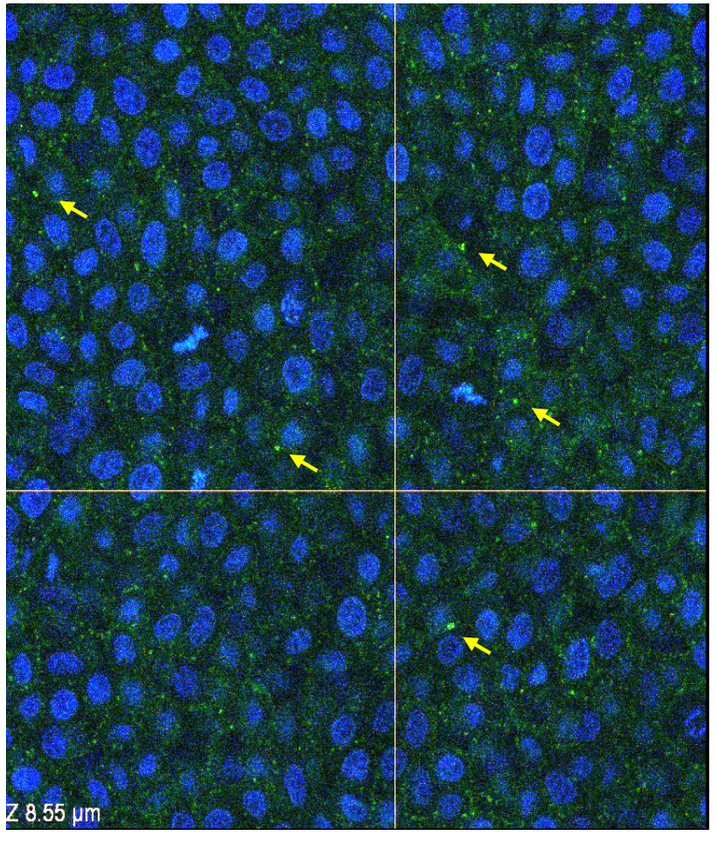
49 nm Nanoparticles (24 hr exposure)
10.5 µm below apical surface



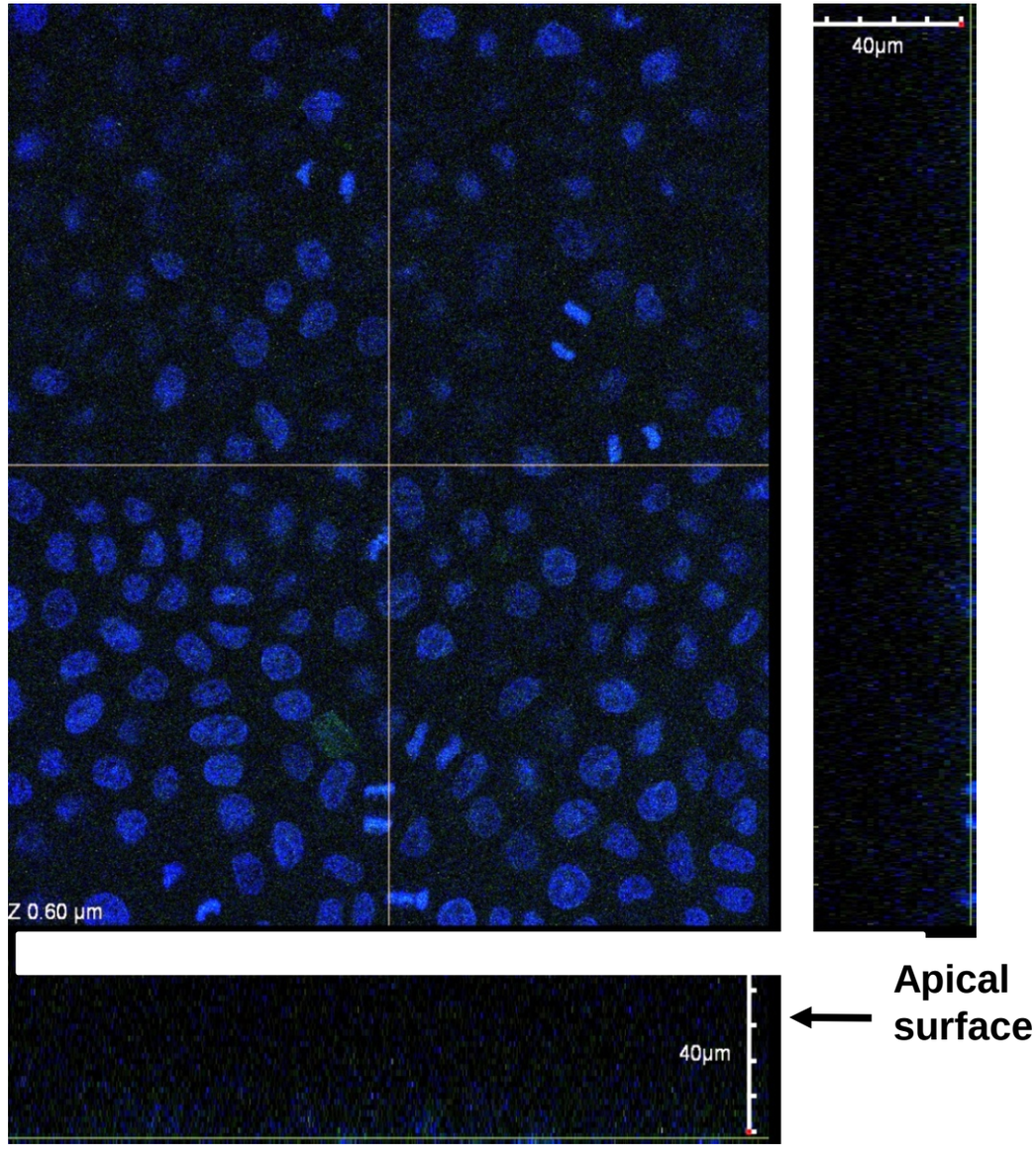
Apical surface

100 nm Nanoparticles (24 hr exposure)
10.5 µm below apical surface

EpiDerm – Skin penetration of nanoparticles

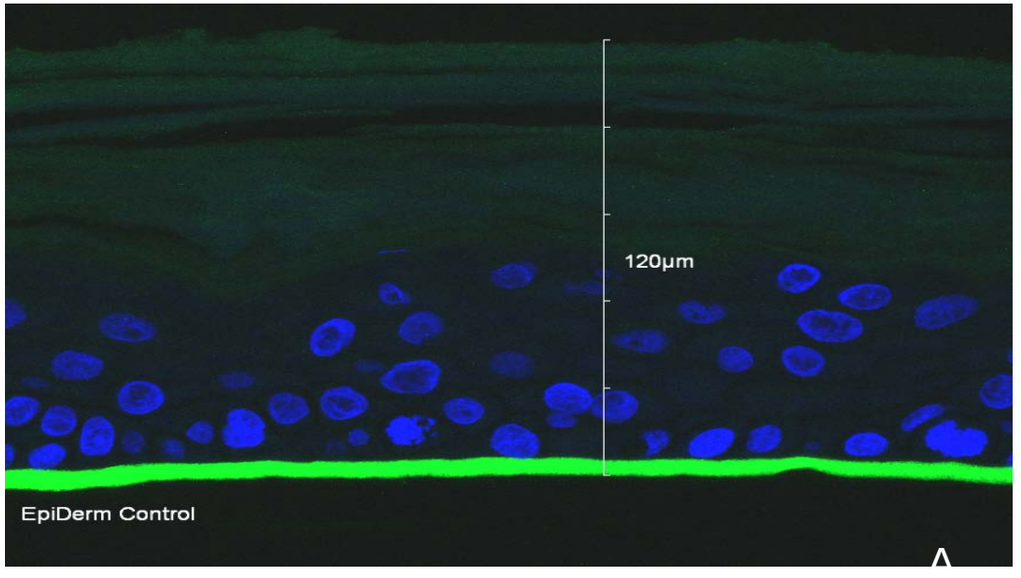


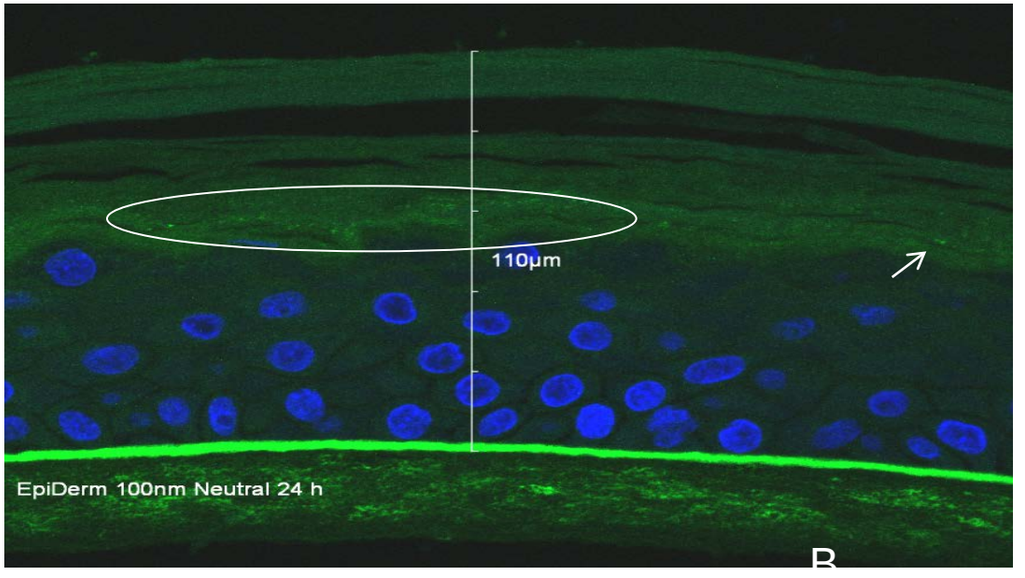
49 nm Nanoparticles (48 hr exposure)
8.55 µm above basal layer

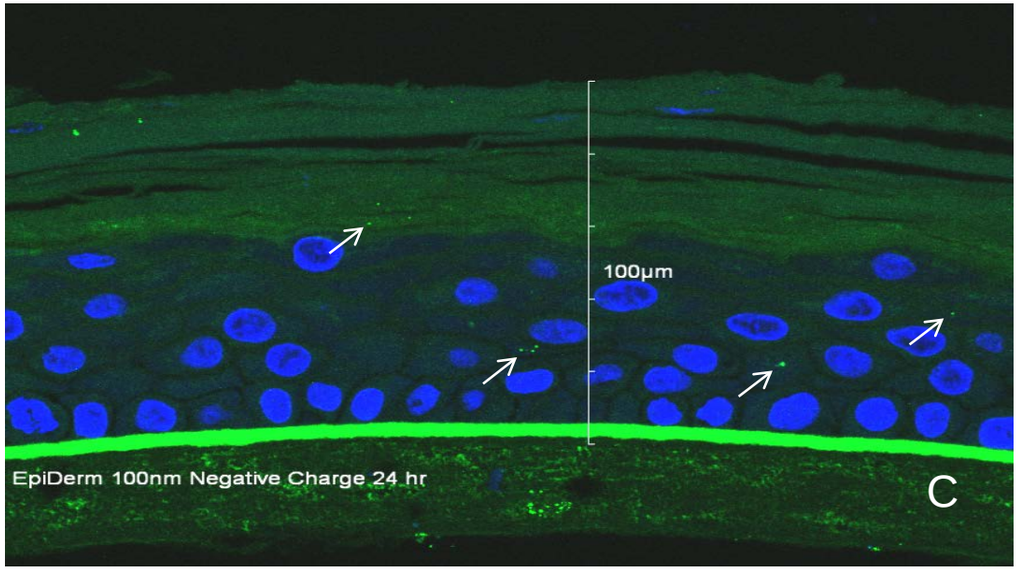


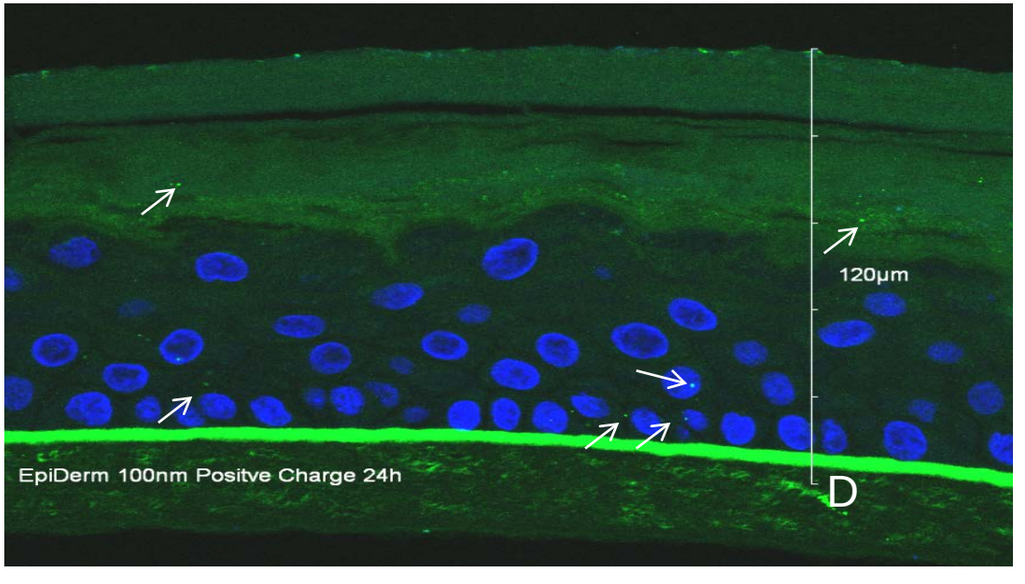
100 nm Nanoparticles (48 hr exposure)
0.60 µm above basal layer

EpiDerm – Skin penetration of nanoparticles



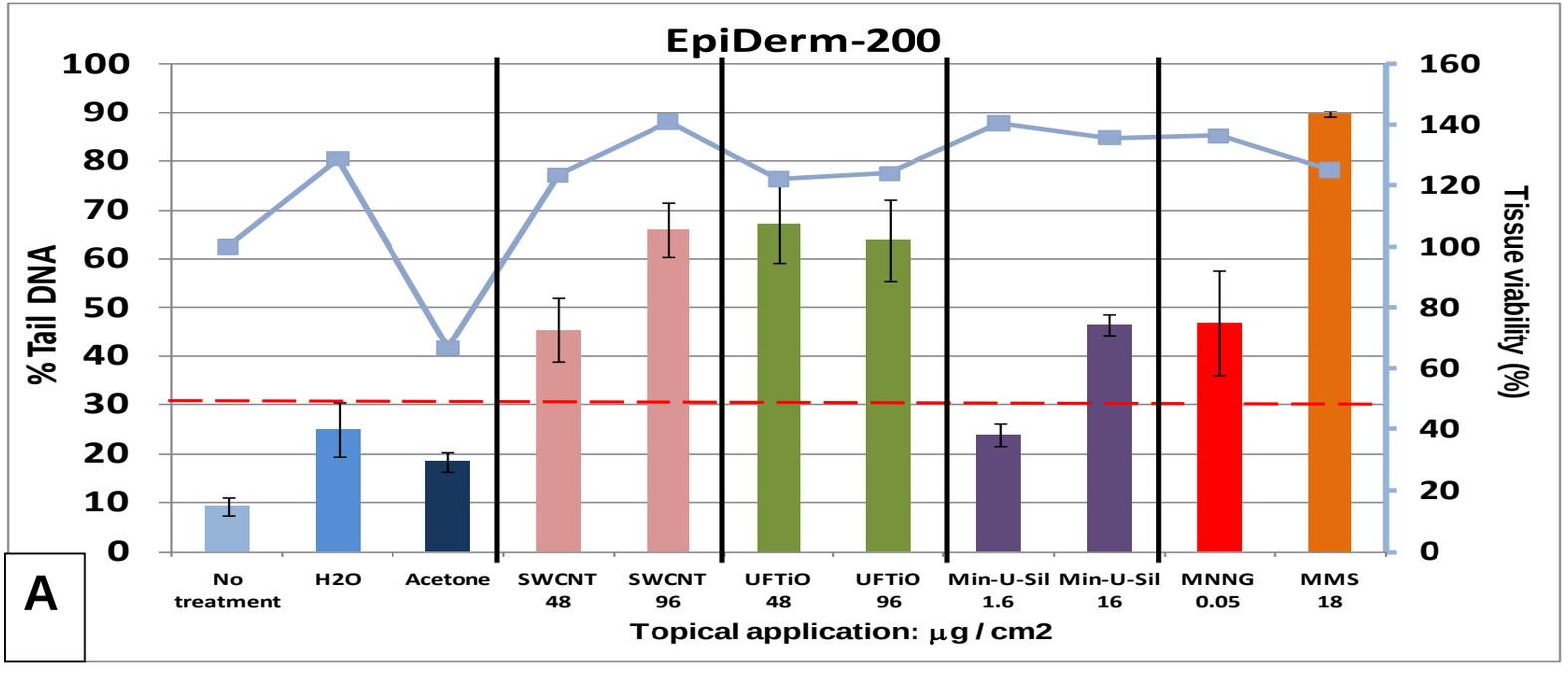






A. Control EpiDerm tissues,
B. Neutral particles,
C. Negatively charged particles,
D. Positively charged particles.

EpiDerm – Genotoxicity of nanoparticles



SWCNT = single wall carbon nanotubes

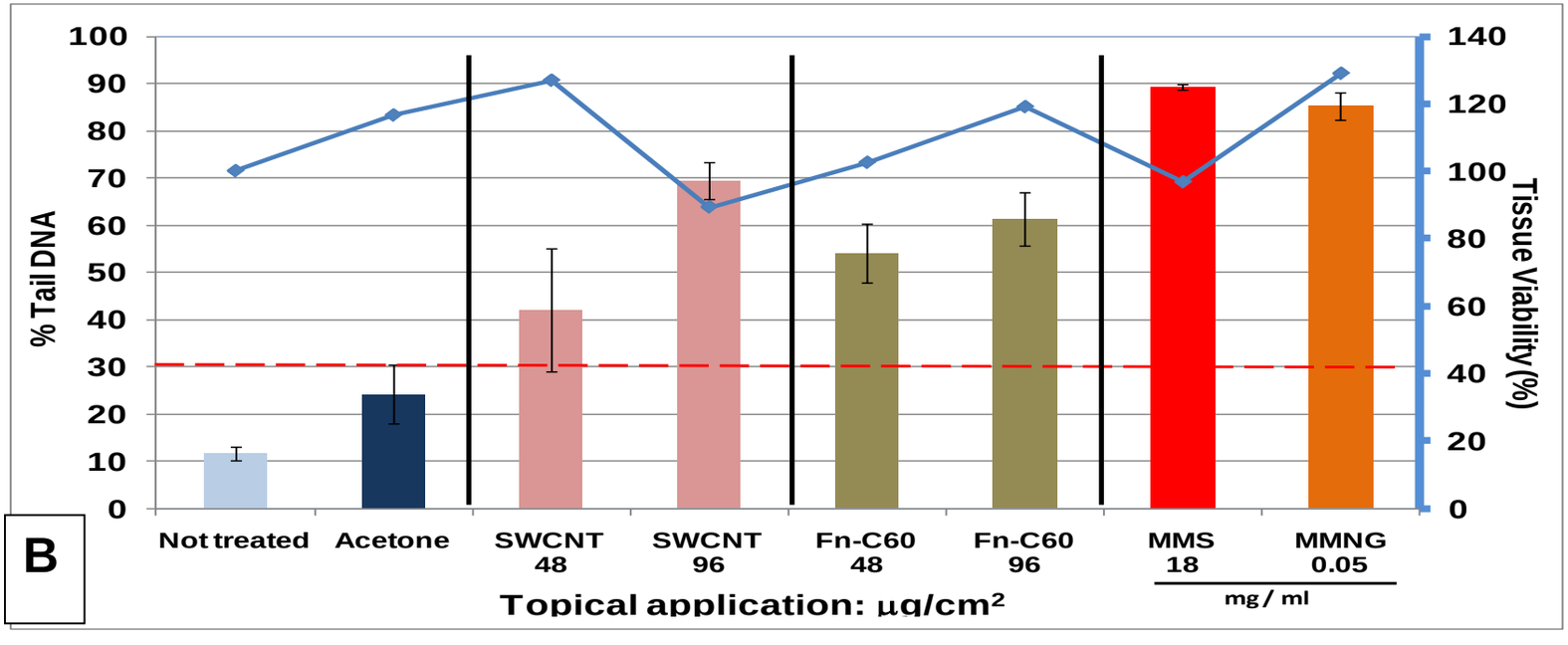
UFTiO = ultra-fine Titanium Oxide

Min-U-Sil = crystalline silica

Fn-C60 = fullerenes C60

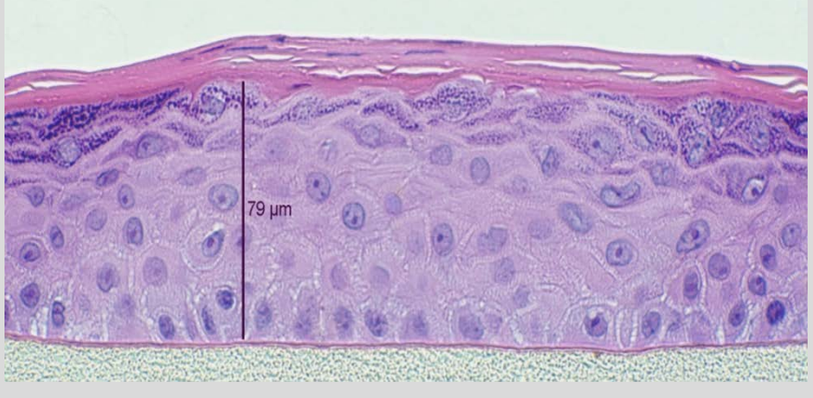
MNNG = methyl nitro-nitrosoguanidine

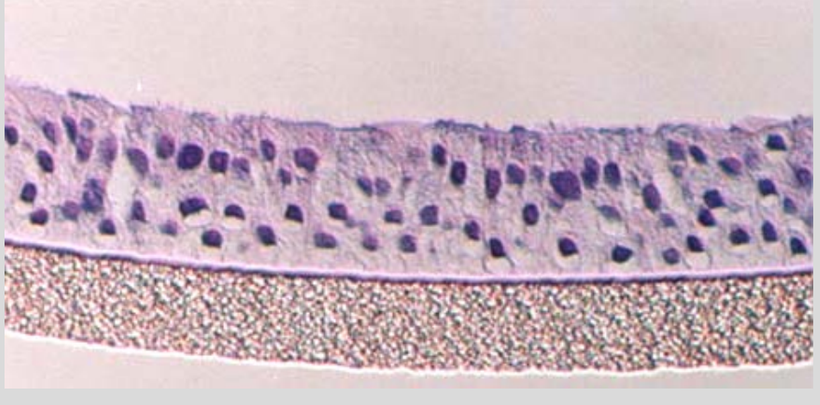
MMS = methyl methane sulphonate



Average particle size:
1 – 4 nm for SWCNT,
120 nm for Fn-C60,
3-10 nm for UFTiO ,
450 nm for Min-U-Sil

Examples of other published nanotoxicology applications utilizing EpiDerm, EpiDerm-FT and EpiAirway tissues





EpiDerm – Skin Irritation Screening of Nanoparticulate Cerium Oxide

SafePharm Laboratories, UK

Particle and Fibre Toxicology

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Research

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Initial in vitro screening approach to investigate the potential health and environmental hazards of Envirox™ – a nanoparticulate cerium oxide diesel fuel additive

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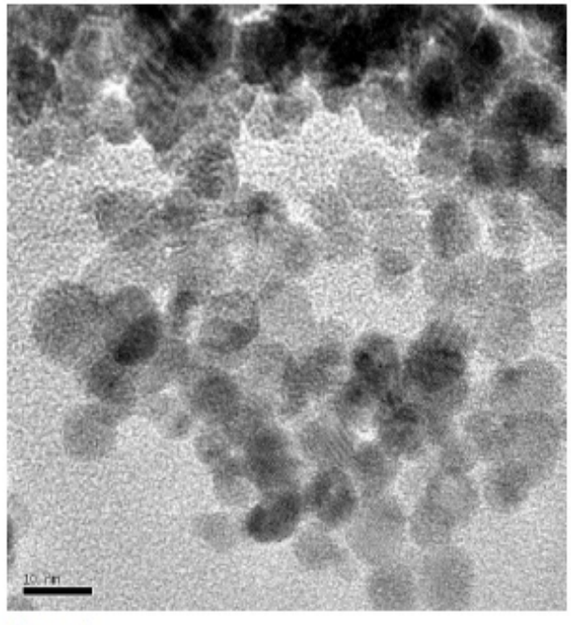

Figure 1
Nanoparticulate cerium oxide.

- Results of the in vitro screening approach reported here with a limited number of assays are clearly negative.
- Based on the available hazard data, these studies do not raise any significant concerns for potential adverse human health or environmental effects as a result of limited localized exposure of cells or aquatic organisms to nano cerion oxide.

Table 2: Results of the Epiderm™ skin model study

Test Materials	Exposure time (mins.)	Mean OD_{540}	% Viability	ET_{50} mins.	MIP
NEGATIVE CONTROL	960	1.685	100		
	1440				
NANO-CeO_2	960	2.010	119.29	1517.18	<0.01
	1200	1.138	67.54		
	1440	1.089	64.63		
NON-NANO CeO_2	960	1.994	92.87	> 1440	0.03
	1200	1.975	97.36		
	1440	1.729	85.23		
POSITIVE CONTROL 1% TX_{100}	240	1.035	61.42	260.10	<0.18
	360	0.212	12.58		
	480	0.095	5.64		
REFERENCE MATERIAL 20% SLS	15	0.983	58.34	20.68	1.00
	30	0.710	42.14		
	60	0.273	16.20		
	120	0.100	5.93		

EpiDerm – Skin Irritation Screening of Nanosilica

Korea University, College of Medicine

Toxicology 267 (2010) 178–181

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Short communication

Assessment of dermal toxicity of nanosilica using cultured keratinocytes, a human skin equivalent model and an *in vivo* model

Yoon-Hee Park[a,1], Ji Na Kim[a,1], Sang Hoon Jeong[a], Jae Eun Choi[a], Seung-Ho Lee[b], Byeong Hyeok Choi[a], Jung Pyo Lee[c], Kyung Hee Sohn[c], Kui Lea Park[c], Meyoung-Kon Kim[b], Sang Wook Son[a,*]

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[c] National Institute of Toxicological Research, Seoul, South Korea

- This study was designed to determine whether nanosilica has the potential to cause acute cutaneous toxicity, using cultured HaCaT keratinocytes (CHK), a human skin equivalent model (HSEM) (EpiDerm), and in vivo model.
- Nanosilicas reduced the cell viabilities of CHKs in a dose-dependent manner. However, the HSEM revealed no irritation at 500μg/ml of nanosilica. Furthermore, this result concurred with Draize skin irritation test findings.
- The present study data indicate that nanosilica does not cause acute cutaneous irritation.
- Furthermore, this study shows that the HSEM used provides more useful screening data than the conventional cell culture model on the relative toxicities of NPs.

EpiDerm -FT– Skin Irritation Screening of Single Walled Carbon Nanotubes (SWCNTs)

NIOSH, USA

Toxicology 257 (2009) 161–171

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes

A.R. Murray[a,b,*], E. Kisin[a], S.S. Leonard[a], S.H. Young[a], C. Kommineni[a], V.E. Kagan[c], V. Castranova[a,b], A.A. Shvedova[a,b,*]

- The authors hypothesize that SWCNT may be toxic to the skin (due to iron impurities).
- To test this hypothesis, the effects of SWCNT were assessed both in vitro and in vivo using EpiDerm FT engineered skin, murine epidermal cells (JB6 P+), and immune-competent hairless SKH-1 mice.
- Engineered skin exposed to SWCNT showed increased epidermal thickness and accumulation and activation of dermal fibroblasts which resulted in increased collagen as well as release of pro-inflammatory cytokines.
- These data indicated that topical exposure to unpurified SWCNT, induced free radical generation, oxidative stress, and inflammation, thus causing dermal toxicity.

Other Literature related to NP research & reconstructed tissue models

- Jeong, S.H., Kim ,J.H., Lee, S., Lee, J.P., Kim, J.H., Sohn, K.H., Park, K. L., Kim, M-K., Son, S.W. 2010. **Assessment of penetration of quantum dots through in vitro and in vivo human skin using the human skin equivalent model and the tape stripping method.** Biochemical and Biophysical Research Communications. 394 (3), 612-5.

- Carter, J.M., Kennedy, J.M., Oberdorster, G., and Clark, E.D. **The Use of Fluorescently Labeled Nanoparticles to Determine the Effect of Particle Size on Translocation From the Lung.** In: The 43rd Annual Meeting of the Society of Toxicology; 2004 Mar 21-25. The Toxicologist, 78 (S1), 440. (MatTek TR # 347 – Available per rerequest).

- BeruBe, K., Balharry, D., Jones, T., Moreno, T., Hayden, P., Sexton, K., Hicks, M., Merolla, L., Timblin, C., Shukla, A., Mossman, B. 2009. **Characterization of Airborne Particulate Matter and Related Mechanisms of Toxicity**: An Experimental Approach. In: Maynard, R.L. editor. Air Pollution and Health. London, UK: Imperial College Press. p. 69-110.

- Chalupowicz, D.G., Frankowski, R., Sanchez, Y., Kou, J., Barnette, M., and Walsh, P.T. **Cellular Responses of Primary Human Bronchial Epithelial Cells to Whole Cigarette Smoke Exposure**. American Journal of Respiratory and Critical Care Medicine, Abstracts Issue 177, Abstract nr PA199. (MatTek TR # 482 – Available per rerequest).

- Walsh, P.T., Chalupowicz, D.G., Frankowski, R., Barnette, M. **Exposure of a Multi-Cellular In Vitro Model of Human Airway Tissue to Whole Cigarette Smoke.** American Journal of Respiratory and Critical Care Medicine, Abstracts Issue 177, Abstract nr PA728. (MatTek TR # 481 – Available per rerequest).

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CONCLUSIONS

1. Nanomaterials are able to penetrate into NHu-3D tissue models EpiAirway and EpiDerm.
2. Nanoparticle penetration into EpiDerm tissues is charge-dependent.
3. We observed dose-dependent increase in %Tail DNA after treatment EpiDerm tissues with: single wall carbon nanotubes (SWCNT), Fullerenes C60 (Fn-C60), ultra fine titanium oxide (UF-TiO_2) and Min-U-SIL-5.
4. Organotypic in vitro models of human skin (EpiDerm, EpiDerm-FT) and airway (EpiAirway, EpiAirway-FT) have been successfully utilized for a variety of nanoparticle research applications.
5. Results suggest good correlation with in vivo data.
6. In vitro organotypic models appear promising for in vitro nanotoxicology applications.

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Thank you for your attention!

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