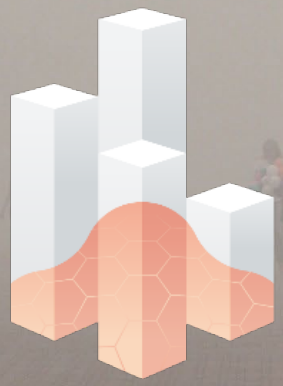
Simulating impact of diesel exhaust particles on human skin using a 3D *in vitro* epidermal model

Irini Magdelina Dijkhoff

19th of June 2019



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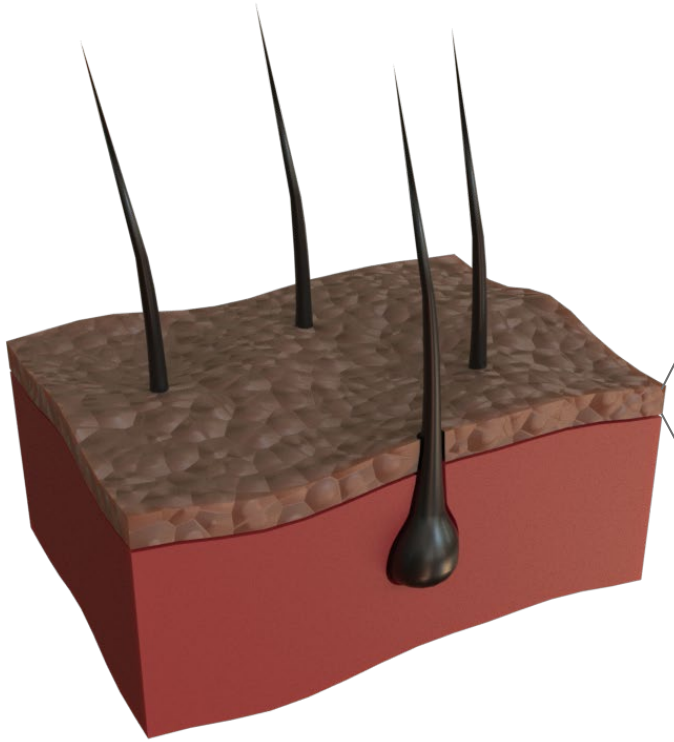
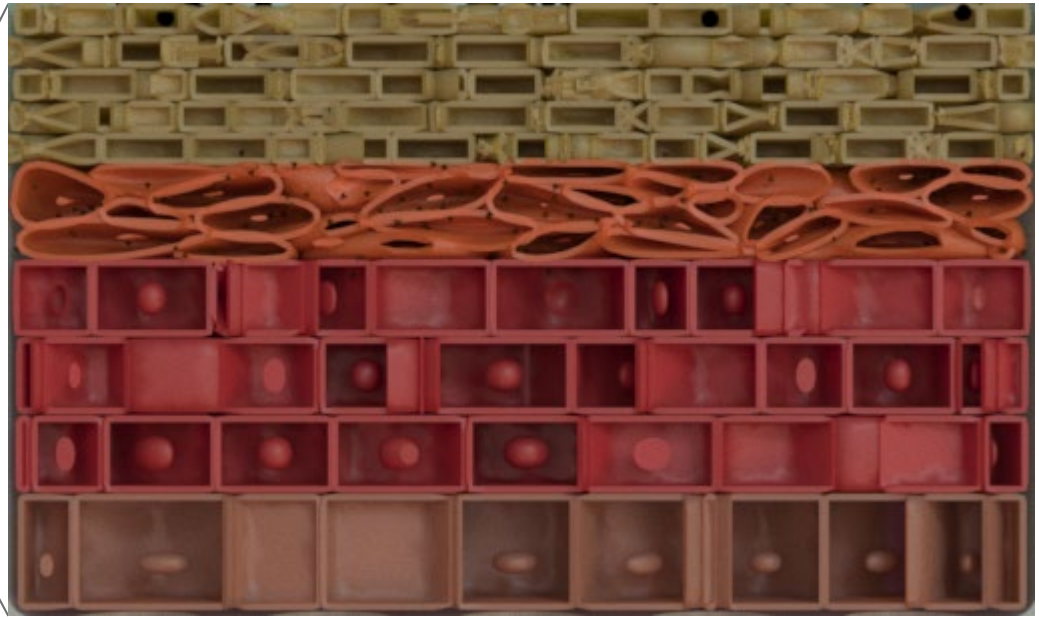
Human skin: a protective barrier



Epidermal barrier



- Differentiated keratinocytes
- Protection against outside environment

Images by Dr. M. Spuch-Calvar

Adverse effect of ambient air pollution on various skin diseases[1]

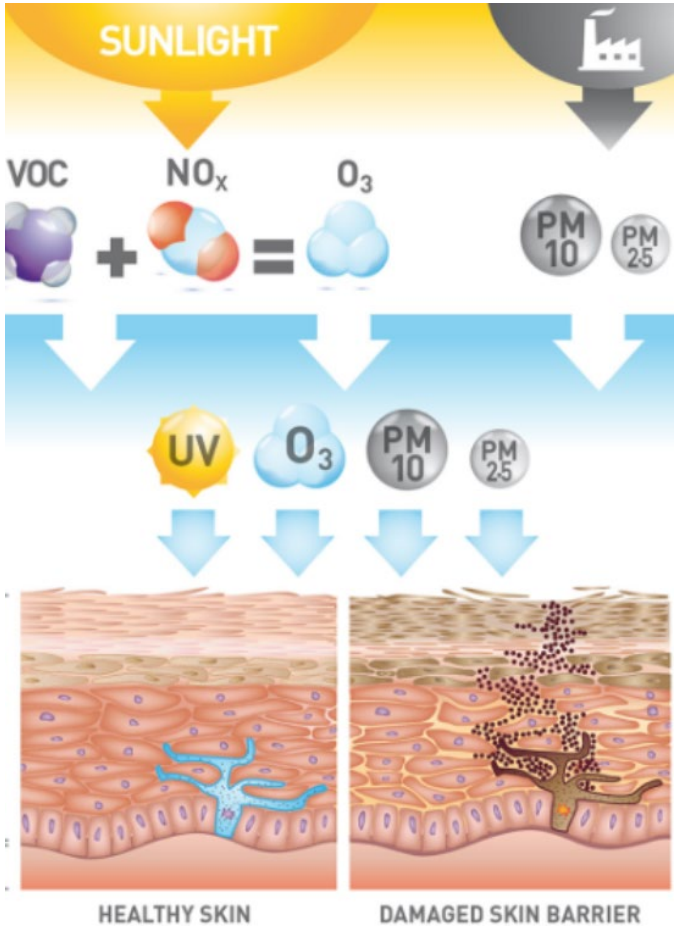
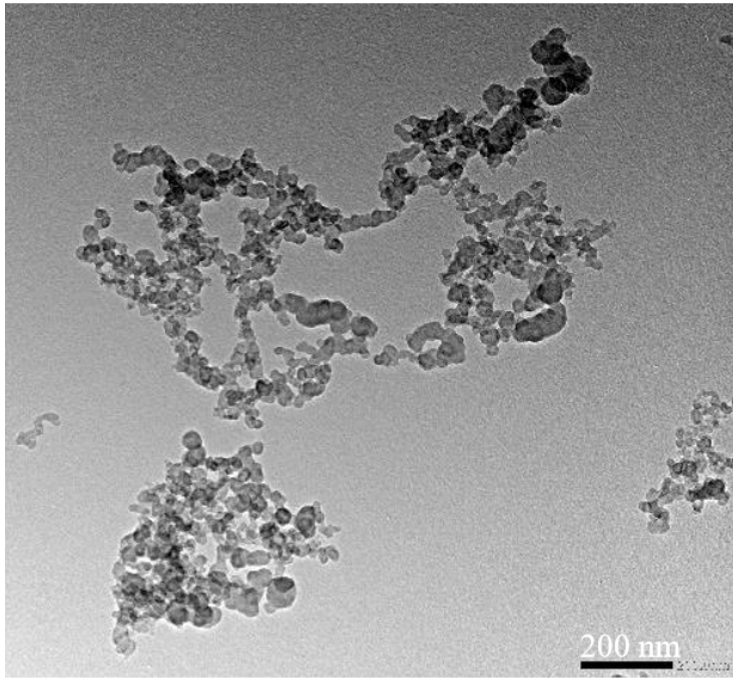


Image from Krutmann, J., et al. J Dermatol Sci. (2014)

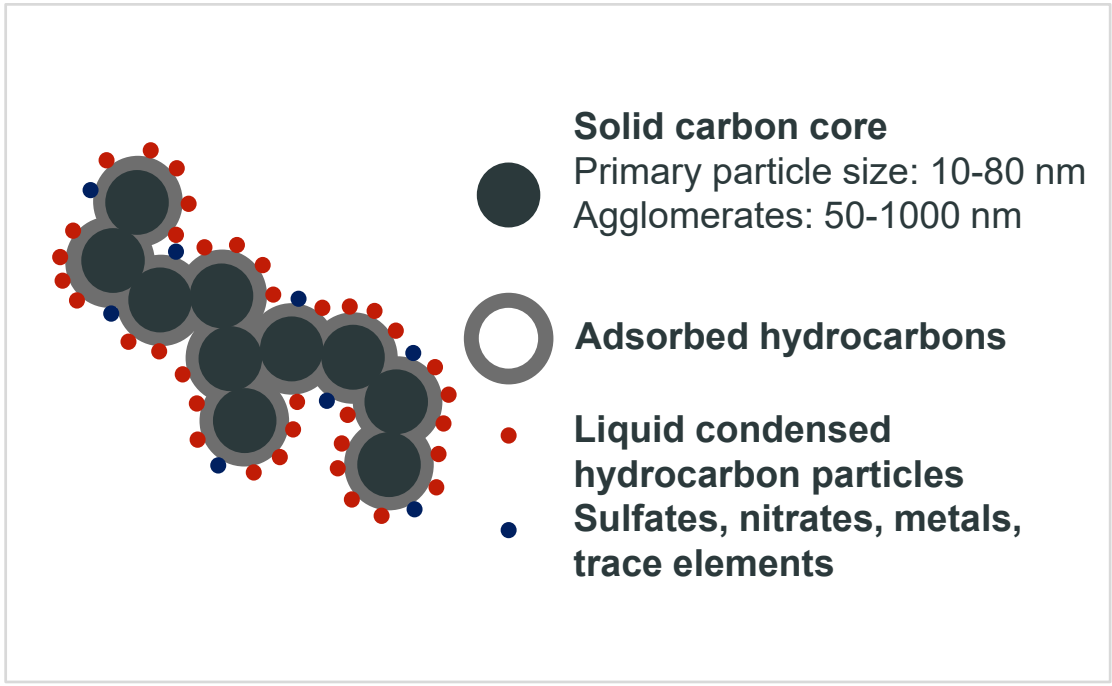
1. **Generation of oxidative stress and impairment of DNA repair**
   - → Extrinsic ageing[2, 3]
   - → Increased incidence in malignant melanoma[4]
2. **Inducing pro-inflammatory reactions and exacerbation of symptoms[5]**
   - → Atopic Eczema[6,7]
   - → Acne[8]
   - → Psoarisis

[1] Kim, KE., et al. Life Sci. (2016)
[2] Vierkötter, A., et al. J Invest Dermatol. (2010)
[3] Krutmann, J., et al. J Dermatol Sci. (2014)
[4] Puntoni, R., et al. Cancer Causes Control. (2004)
[5] Mancebo, SE., J Eur Acad Dermatol Venereol. (2015)
[6] Morgenstern, V., et al. Am J Respir Crit Care Med. (2008)
[7] Song, S., et al. Environ Res. (2011)
[8] Yang, YS., et al. Ann Dermatol. (2014)

Diesel exhaust particles (DEP)

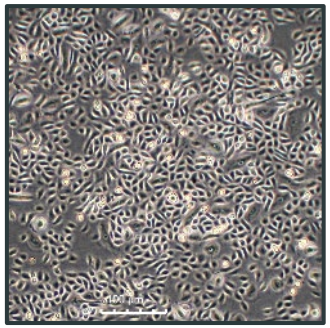
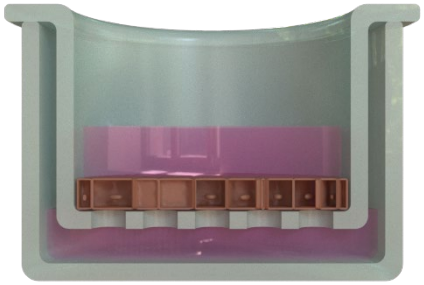
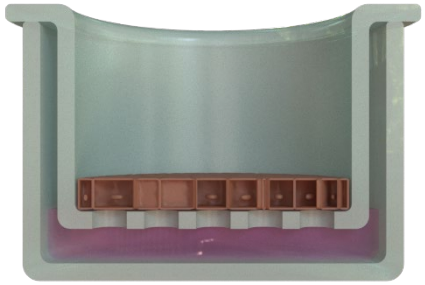
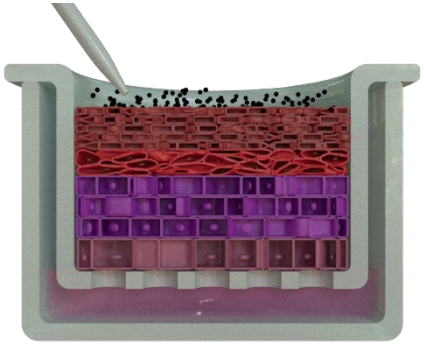


Koko, P., et al., IOP Conf. Ser.: Mater. Sci. Eng. (2019)



Adapted from Marano, F., et al. Cell Biol Toxicol. (2002)

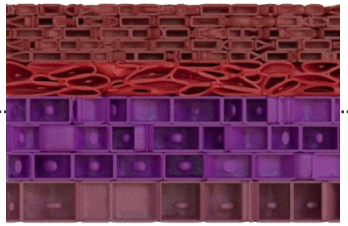
Workflow of an *in vitro* 3D epidermal model

2D culture	Submerged phase	Air-liquid interface	Treatment/ exposure
			
Day -8	-3	0	14	15/16
In low Ca^{2+} medium to stimulate proliferation	Ca^{2+} switch to stimulate differentiation	Air exposure to stimulate profilaggrin synthesis, resulting in stratification	Strong barrier is formed. Tissue ready for treatment/ exposures	Harvest of tissues

Images by Dr. M. Spuch-Calvar

End points of epidermal model after treatment

Tissue



Barrier integrity (TEER)

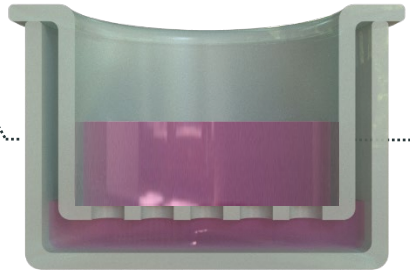
Paraffin embedding

1. Morphology (H&E staining)
2. Protein expression and localization (IF staining)
   - Barrier formation

Viability (MTT, MTS or resazurin assay)

Protein lysis

Protein expression (WB)
- Barrier formation
- Antioxidant stress response
- Xenobiotic metabolism



Supernatant

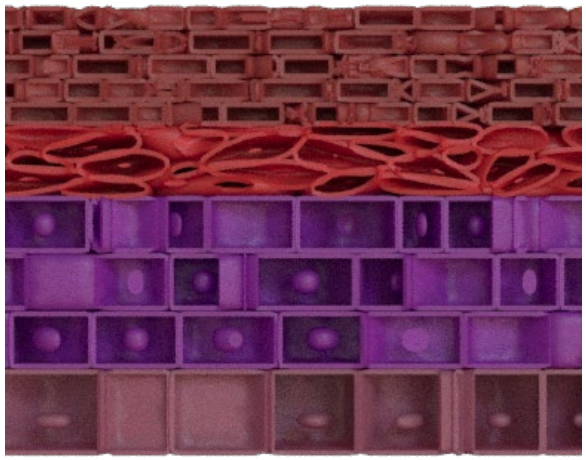
Cytotoxicity (LDH)

Pro-inflammatory response (ELISA)

Images by Dr. M. Spuch-Calvar

Morphology of an *in vitro* 3D epidermal model

Schematic



Images by Dr. M. Spuch-Calvar

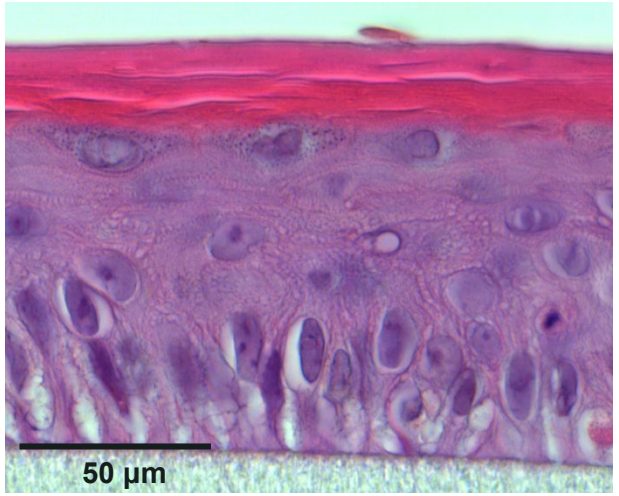
Stratum corneum

Stratum granulosum

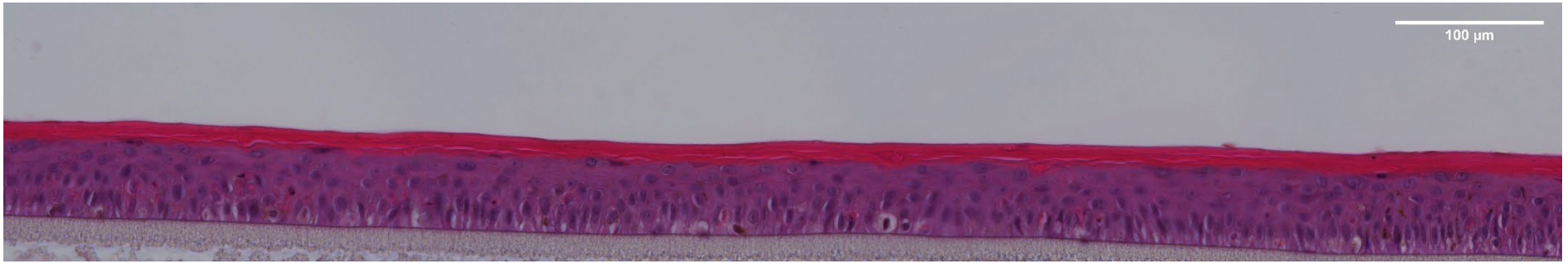
Stratum spinosum

Stratum basale

In vitro



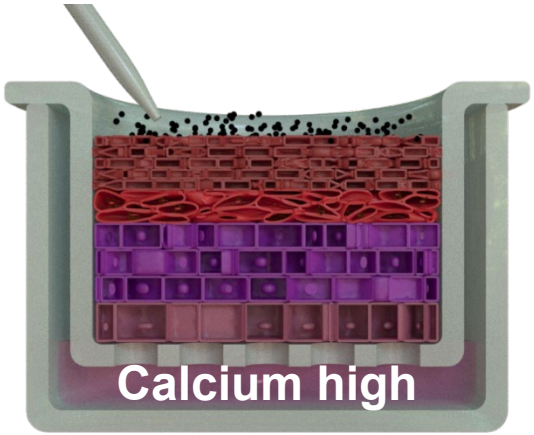
***In vitro* full tissue overview**



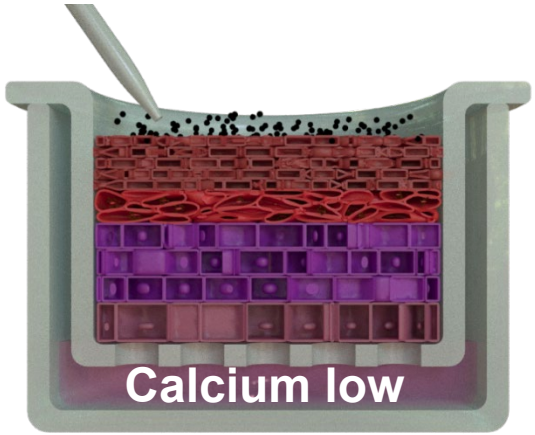
Paraffin embedded tissue sectioned at a 5 µm thickness and stained with Hematoxylin and Eosin.

Skin tissue and calcium depletion for 24 hours

Healthy skin (control)



Compromised barrier



- Barrier integrity is decreased upon calcium depletion
- Serving as a **compromised barrier model**

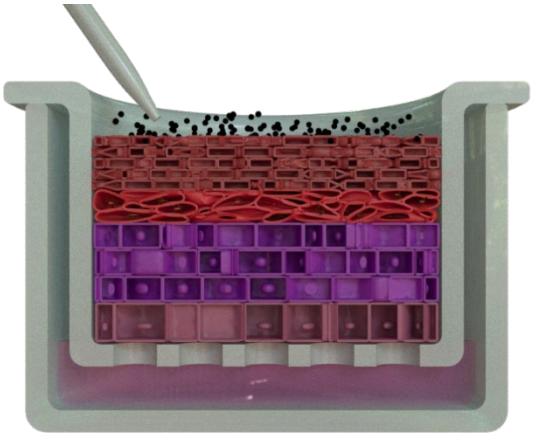
Images by Dr. M. Spuch-Calvar

Diesel exhaust particle (DEP) suspensions and application on skin

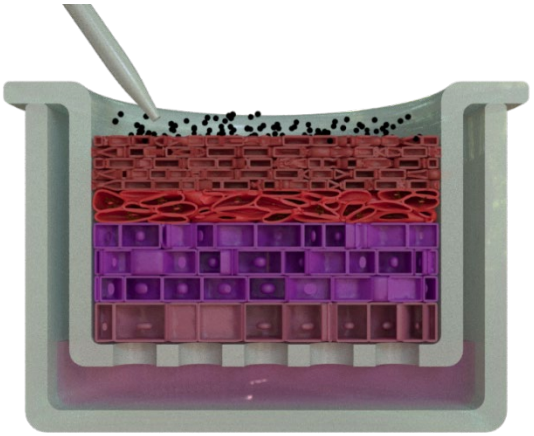
Particulate

Particles: NIST Standard Reference Material® 2975
Concentration: 0.13 mg/mL

DEP suspension



DEP suspension stabilized with 0.05% BSA



Application

Pseudo air/liquid interface: 30-80 µL/cm^2
Deposition on skin tissue: 10 µg/cm^2 DEP
Exposure time: 24 hours

Images by Dr. M. Spuch-Calvar

Response in cytotoxicity and mitochondrial acitivity

10 µg/cm² DEP

10 µg/cm² DEP + 0.05% BSA

Pro-inflammatory response

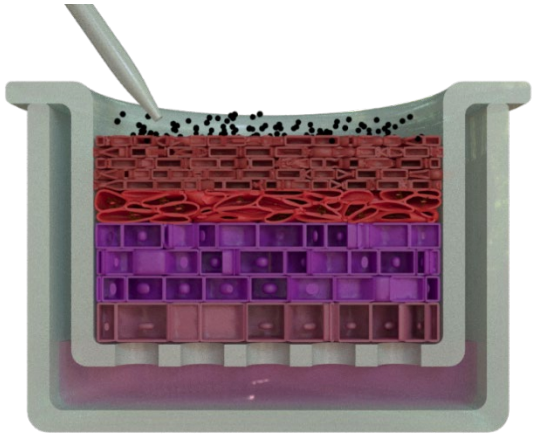
10 µg/cm² DEP

10 µg/cm² DEP + 0.05% BSA

Discussion

1. **DEP suspensions are inducing a pro-inflammatory response in skin model with compromised barrier**
   - **Slight increase in cytotoxitiy and decrease in mitochondrial activity** upon exposure to DEP suspensions
   - **Increase in pro-inflammatory response (IL-8)** upon exposure to DEP suspensions

2. **Effects are less pronounced after exposure to DEP suspensions stabilized with BSA protein**
   - Might be due to BSA covering the skin tissue



Images by Dr. M. Spuch-Calvar

Outlook

1. **Determine effects on (on an longer term)**
   - Oxidative stress markers
   - Activation of Aryl Hydrocarbon Receptor
   - Penetration of particles into tissue
2. **Further characterization of compromised barrier model**
   - Barrier function properties
3. **Fractions of DEP suspensions**
   - Soluble organic and particulate phase
4. **Synergistic effects with solar radiation**
5. **Improving DEP exposure model**
   - Aeorosolization of dry diesel particles
   - Exposure to whole diesel engine exhaust

Acknowledgements

CITYCARE consortium

Group of BioNanomaterials

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DOW

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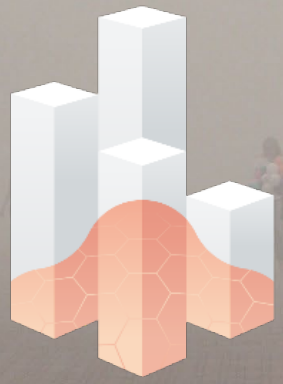

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