Safety of Antimicrobial nanoparticles on 2D and 3D *in vitro* models of the Skin

Rossella Bengalli[@], Alessandra Colantuoni, Paride Mantecca, Luisa Fiandra. POLARIS Research Centre, Dept. of Earth and Environmental Sciences, University of Milan-Bicocca, Piazza della Scienza 1, 20126, Milano, Italy. [@]mail to: rossella.bengalli@unimib.it

Introduction

Nanoparticles (NPs), especially metal oxide nanoparticles (MeO-NPs) have a great potential in the prevention of nosocomial infection thanks to antibacterial properties [1]. Since the resistances to conventional antibiotics increased in the last years, the problem of bacterial infections determined strong need for new antimicrobial agents. Copper (CuO) and zinc (ZnO) oxide NPs, silica/titania (SiO_2/TiO_2) core/shell NPs and polymeric NPs, such as Polypyrrole (PPy), are among the best candidate nanomaterials (NMs) for the coating of functionalized textiles such as gowns, bead sheets and recently also face masks[2] that can help in reducing the spread of infections. However, the evaluation of the safety of these NMs is pivotal in order to develop sustainable and safe NPs for human health, for example through the modification of their physicochemical properties. The present work, which is included in the EU-funded H2020 project "PROTECT", is aimed to evaluate the skin toxicity of textiles coated with different antibacterial NPs.

Experimental design

Skin exposure to NPs and extracts from textiles coated with NPs in Artificial Sweat (AS) at pH 4.7 and 6.5

1. NPs properties

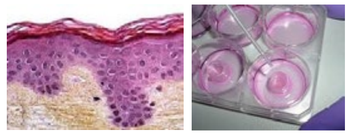
- ✓ NPS: **CuO, ZnO, SiO2/TiO2 and PPy**
- ✓ Transmission Electron Microscopy (TEM): primary size and shape
- ✓ Dynamic Light Scattering (DLS): hydrodynamic diameter and PdI
- ✓ Surface charge: z-potential (ZetaSizer Malvern)

2. Textiles characterization

- ✓ Analysis of the release of ions from textiles during extraction procedure by ICP-EOS
- ✓ PPy release evaluated with DataColour Spectraflash
- ✓ Coating of polyester cotton fabrics performed by **roll-to-roll sonochemical process** (CuO, ZnO)[3] or by **spray coating process with US nozzles** (SiO2/TiO2, PPy)[4]

3. In vitro reconstructed human epidermis (RhE)

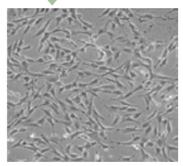
- ✓ **Corrosion Skin test** (OECD TG. 431): NP suspensions
- ✓ **Irritation Skin test** (OECD TG. 439) and IL-8 release: Textile extracts (ISO 10993-12)
- ✓ Morphological analysis



3D model: EpiDerm™ (MatTek)

4. …and in case of wounded skin and permeation??

- ✓ MTT viability test
- ✓ Morphological analysis
- ✓ Cell migration assay: wound healing test



2D model: Balb/3T3 fibroblasts

NPs and coated textiles characterization

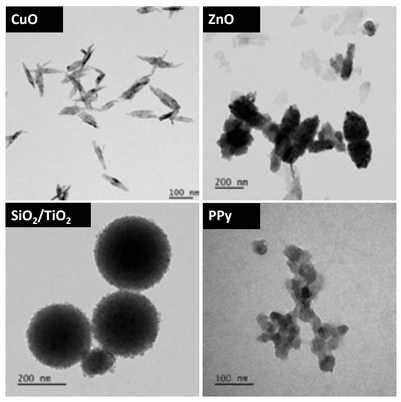


Table 1. Hidrodynamic NPs diameter, polidispersity index (PdI) and surface charge (z-potential) were evaluated for NPs dispersed in mQ water (100 ppm).

NPs	Z-average (nm)	PdI	z-potential (mV)
CuO	244 ± 64	0.275	11
ZnO	600 ± 110	0.548	28
SiO_2/TiO_2	297 ± 4.5	0.068	-39
PPy	331 ± 0.1	0.226	-55

NPs tend to agglomerate in solution. CuO and ZnO have positive charges, while SiO_2/TiO_2 and PPy are negative.

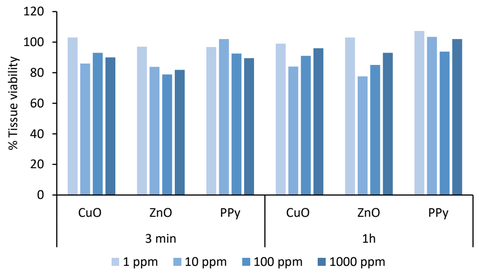
Fig. 1 – TEM images of CuO, ZnO, SiO2/TiO2 and PPy nanoparticles. CuO NPs have leaf-like shape, while ZnO NPs are irregular and form bigger agglomerates. SiO_2/TiO_2 are spherical NPs, with a core of silica and a shell of TiO_2. PPy NPs form agglomerates, single PPy NPs are spherical and below 50 nm.

Table 2 - ICP-OES data from textile extracts. No particles, but free metal ions are released during the extraction in AS at pH 4.7 and 6.5

	Amount of free ions in extraction AS (mg)		Total amount in extraction AS (mg)		Total amount on textile (mg/0.1 g textile)
	pH 4,7	*pH 6,5*	*pH 4,7*	*pH 6,5*	
Cu^{2+}	0,65 ± 0,005	0,04 ± 0,001	0,61 ± 0,017	0,02 ± 0,010	3,38 ± 1,420
Zn^{2+}	1,60 ± 0,044	0,07 ± 0,001	1,78 ± 0,047	0,07 ± 0,004	9,58 ± 1,190
Ti^{4+}	2,47 ± 0,17	0,21 ± 0,02	4,65 ± 0,18*	0,32 ± 0,09	38,1± 0,7

- **~17% of total Cu and Zn ions are released at pH 4.7 from textiles, < 1% of ions are released at pH 6.5.**
- **For SiO_2/TiO_2 coated textiles: at pH 4.7, almost 12% of total Titanium loaded on the textiles is released, at pH 6.5, < 1%.**

Skin corrosion test (OECD TG. 431)



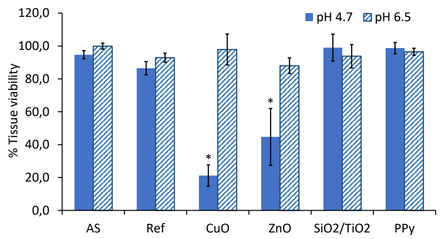
MeO and PPy NPs are non-corrosive on EpiDerm up to 1000 ppm in water!

Mean Tissue Viability (% of NC)	Classification*
3 min < 50%	Corrosive
3 min ≥ 50% and 1 h < 15%	Corrosive
3 min ≥ 50% and 1 h ≥ 15%	Non-Corrosive

*Classification of skin corrosion hazard according to the GHS System adopted by the OECD

Fig. 2 – Corrosion skin test. NPs suspensions were added directly to the EpiDerm™ model at different concentration for 3 min and 1h and then MTT was performed to assess the % of tissue viability.

Skin Irritation test (OECD TG. 439)



Exposure to CuO and ZnO textile extracts in AS at pH 4.7 strongly impacts on tissue viability → irritant compound

Exposure to textile extracts in AS at pH 6.5 does not affect tissue viability… …but CuO affects interleukin-8 release

Fig. 3 – Irritation skin test. Tissue viability (MTT assay) of EpiDerm™ model exposed for 18h to extracts. NC: negative control; AS: artificial sweat; Ref: reference textile. *p<0.05

Mean Tissue Viability (% of NC)	*Classification, in vivo prediction
< 50%	Irritant (R38 or GHS category 2)
> 50%	Non-Irritant (NI)

*According to the EU and GHS classification

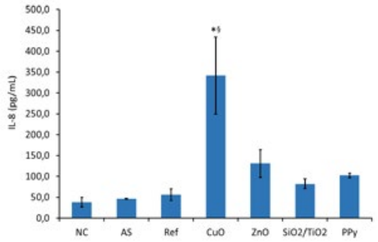
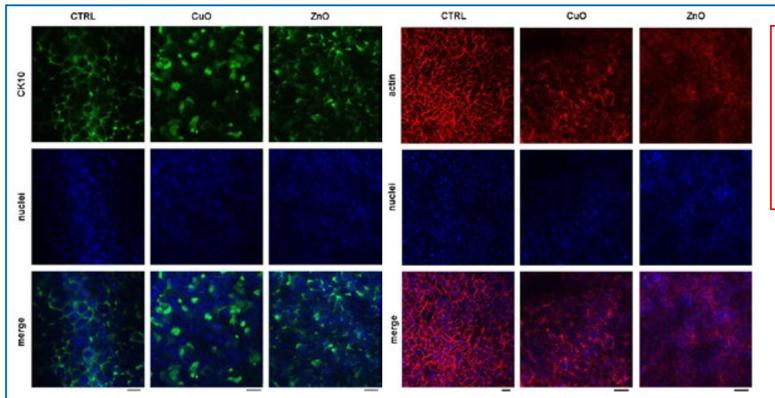


Fig. 4 – IL-8 release. ELISA test was performed on supernatants after 18h exposure to textile extracts (AS pH 6.5).

*p<0.05 respect to NC (negative control); §p<0.0 respect to Ref (reference textile)



MeO-NPs influence tissue morphology, causing cytokeratin condensation and actin disassembly

Fig. 5 – Morphological alteration of EpiDerm model. EpiDerm™ model exposed for 18h to CuO and ZnO textile extracts (AS 4.7). Cytokeratin CK10 (green), nuclei (blue) and actin filaments (red). Scale bar: 20 µm.

Toxicity of MeO-NPs on fibroblasts

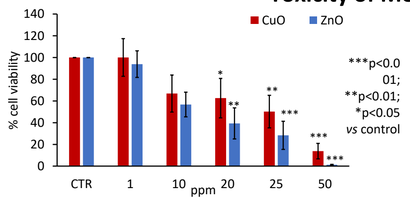


Fig. 6 – Cell viability. MTT test was performed on Balb/3T3 cells after 24h of exposure to CuO and ZnO NPs at different concentrations.

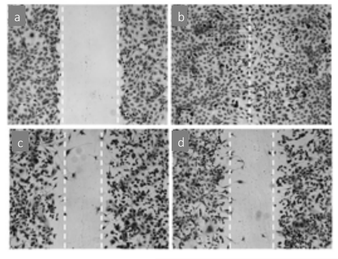


Fig. 8 – Wound healing assay. Scratch assay performed on Balb/3T3 at time 0 (a), and after 24h of exposure: b) control cells; c) CuO 20 ppm; d) ZnO 20 ppm.

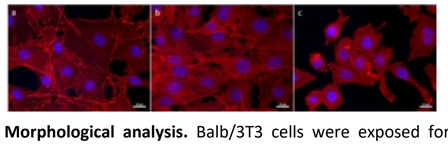


Fig. 7 – Morphological analysis. Balb/3T3 cells were exposed for 24h to CuO (b) and ZnO (c) NPs. a) Control (unexposed cells). Nuclei are stained in blue (DAPI) and actin filaments in red (Phalloidin-Rhodamine).

MeO-NPs (CuO and ZnO) decrease fibroblast cells viability from the dose of 20 ppm, induce morphological alteration and affect cell migration, inducing a delay in wound healing

Conclusions

✓ SiO_2/TiO_2 hybrid-NPs and Polypyrrole NPs and respective coated textiles are safe on intact epidermis, unrelatedly of ions or NPs release.

✓ Antibacterial MeO-NPs (CuO and ZnO) are safe on intact epidermis, unless ions dissolution from textiles occurs in acidic conditions. Antibacterial NMs may affect dermal cells, when eventually achieved through skin lesions or through skin permeation.

✓ **TAKE HOME MESSAGE→** NMs p-chem characterization should be performed thoroughly, as well as the evaluation of toxicity by mean of different exposure models, in order to develop safe, but at the same efficient, antibacterial NMs.

References: [1] Perelshtein I, et al. 2015. Ultrason Sonochem, 2015, 25: 82-8; [2] Chaitanya B. et al., 2018. Vacuum, 2018, 56: 475-482; [3] Perelshtein I, et al 2015. Nano Research, 2015, 8: 695–707; [4] Ortelli et al., 2020. NanoImpact 2020, 19: 100245.

Acknowledgement: This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nr. 720851 (http://protect-h2020.eu/; https://twitter.com/H2020Protect). We would like to thank in particular the partners from CNR_STIIMA, Bar-Ilan University and CeNTI which have provided NPs and coated textiles and helped in NMs characterization.