# Evaluation of fiber and debris release of protective COVID-19 facemask textiles and possible *in vitro* acute cytotoxicity effects

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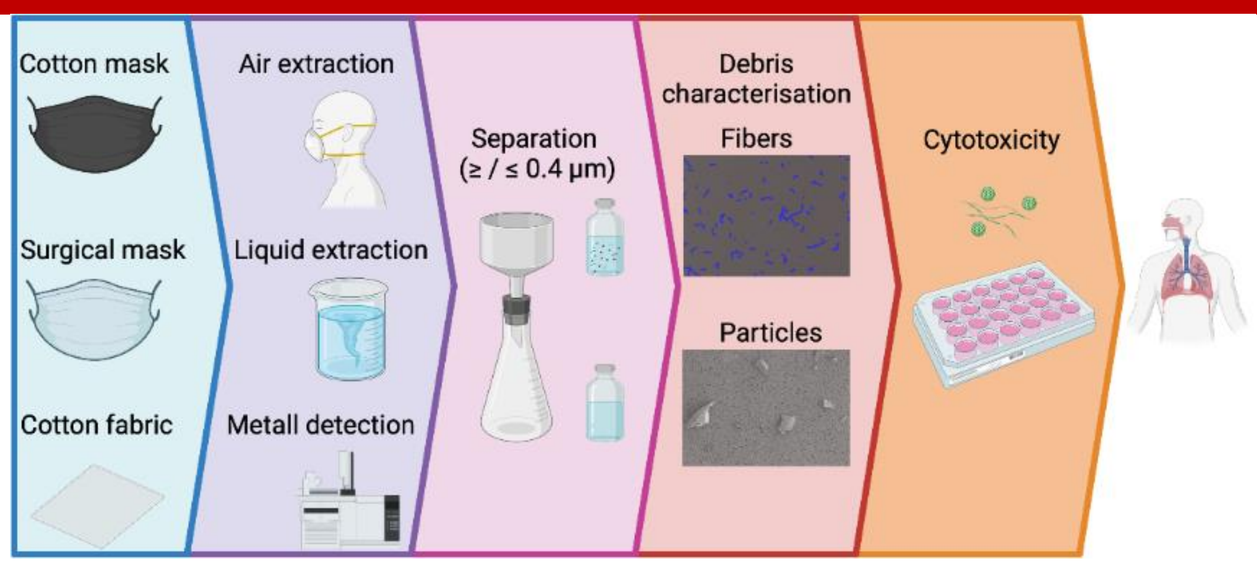
## 1. Introduction and aim of the study

The new normal is characterized by non-pharmaceutical measures. Despite vaccines and further medication, face masks remain an important tool in fighting against COVID-19, and other diseases transmitted by droplet infection. The collapse of the delivery chain of single use masks initiated a boom and innovation progress in textile based reusable face masks [1, 2]. With the broad use of face masks consumer concerns on safety and innocuity raised. However, little is known about the release from face masks and particularly cotton based textile masks [3]. Therefore, the aim of this study was to investigate the type (fibers, particles), the amount of released debris, the metal composition as well as its *in vitro* acute cytotoxicity from cotton based masks compared to a surgical mask and a reference cotton tissue (Figure 1).




## 2. Material & Method


**Figure 2**: Graphic summary of the applied methodology. EMPA designed filter holder (A1) and Sheffield head design according to DIN EN 149 (A2). A respiratory ventilation air flow intensity of 14.2 L min$^{-1}$ was applied for 1 h for fiber recovery onto filter membranes (B). Laser cutting of textile sample pieces with a dimension of 4 x 10 cm (C). Liquid fiber extraction of textile sample pieces in Gyrowash instrument for 45 min at 25°C (D). The light-white fibers on dark-black background pictures (E1) were analyzed (amount of fibers & length distribution ) with FiberApp software 1.51. Analyzed fibers are indicated in blue (E2). The amount of particles / the size distribution of the particles present in the SDE ≤ 0.4 µm were analyzed with a Malvern NanoSight LM20 (F). Nitric acid digested mask textile samples for quantification of the total textile metal content fresh from the packaging without any pretreatment by washing (G). A549 cells were seeded on the apical site of Thincert™ Tissue culture inserts in 12-well plates and treated under air-liquid interface cultivation conditions (H1). The sterile collected fibers were resuspended in 1 mL sterile ultrapure deionized water which equals the debris high fraction (DH) before further dilution took place (H2). 70 µL of fiber and particle extract suspended in 10-fold RPMI medium concentrate plus additives was added apically to the cell monolayer (I1). Metabolic activity of viable cells (viable cells – brownish-red, non-viable – yellow color) was quantified colorimetrically at 490 nm absorbance using a standard *in vitro* viability MTS assay (I2).

## 3. Fibre and debris characterization & quantification

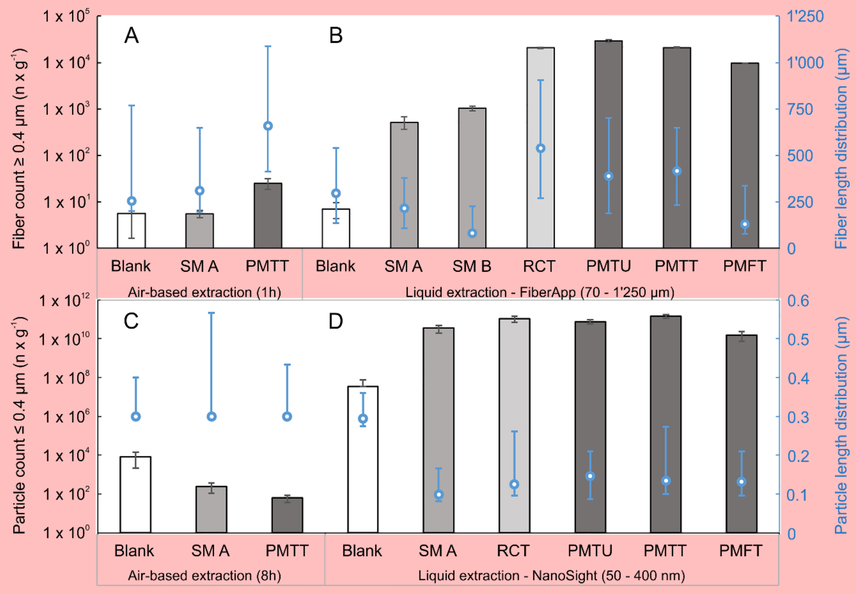



**Figure 3**: Fiber-/ particle release and length distribution of air-based (A&C) and liquid extracts (B&D) from masks and mask textiles.

- **Only a fraction (0.12-1.07%)** of fiber-/ particles was released with **air-based** Sheffield head scenarios compared to **liquid** extraction (Fig. 3)
- **Cotton textiles** (PMTU: 29'452±1'996; PMTT: 20'941±622 fibers g$^{-1}$ textile) show a **higher fiber release** compared to **synthetic textiles** (SM A: 519±153; SM B: 1'030±115 fibers g$^{-1}$ textile, Fig. 3)

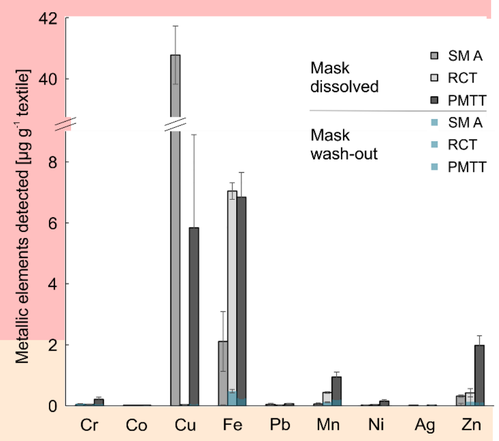



**Figure 4**: Metal element analysis of dissolved vs. aqueous mask text. extracts.

- With ICP-MS the elements Cr, Co, Cu, Fe, Pb, Mn, Ni, Ag and Zn were measured within the **dissolved-** & **aqueous wash-out** extracts (Fig. 4)
- The highest metal content was measured within the dissolved surgical mask (SM A) textile containing a **copper** content of **40.78 ± 0.95 µg g$^{-1}$** and **iron** up to **7.04 ± 0.27 µg g$^{-1}$** was present within reference cotton textile (Fig. 4)

## 4. *in vitro* cytotoxicity

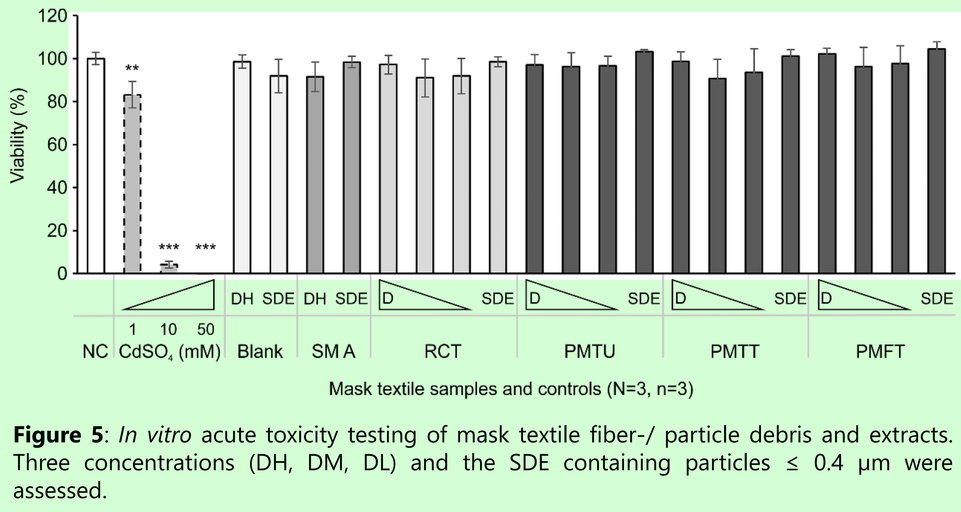



**Figure 5**: *In vitro* acute toxicity testing of mask textile fiber-/ particle debris and extracts. Three concentrations (DH, DM, DL) and the SDE containing particles ≤ 0.4 µm were assessed.

- The cell monolayer was kept under pseudo air-liquid interface cultivation condition to assure **direct contact** of the **textile debris with the cells**
- **Positive controls** ($CdSO_4$) caused a significant cell viability reduction (Fig. 5)
- **No significant reduction in viability** was observed when fiber-/ particle fractions were exposed on *in vitro* alveolar human lung epithelial cells (A549, Fig. 5)

## Acknowledgment

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## 5. Conclusion

- Fibers & particles were released from all synthetic (SM A, SM B, PMFT) and cotton-based (RCT, PMTU, PMTT) mask textiles
- Metals were detected in a low ppm concentration range in acid dissolved textiles whereas with water only a very small extent was washed out
- During the acute cytotoxicity assay at least $10^5$ times more fibers where applied compared to the more realistic Sheffield head scenario
- No significant reduction in viability was observed when fiber- & particles (> 5 µm - not reaching the deeper airways of the lungs; ≤ 5 µm potentially respirable particles; [5]) were exposed on *in vitro* alveolar human lung epithelial cells (A549).
- No acute cytotoxicity was observed against A549 human epithelial lung cells testing different layers of Livinguard's Masks. Prediction of possible long-term exposure effects was not in the scheme of this study.

## References

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