FRAUNHOFER-INSTITUT FÜR BIOMEDIZINISCHE TECHNIK IBMT

Exposure of cellulose nanocrystals on human lung cells at the air-liquid-interface

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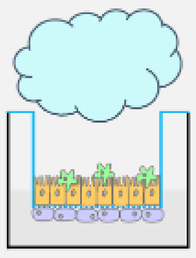
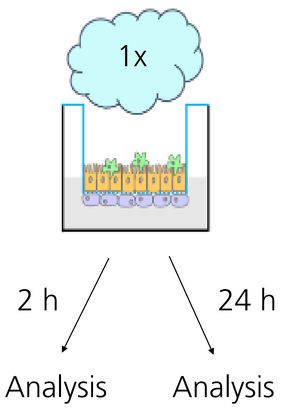
Motivation and study design

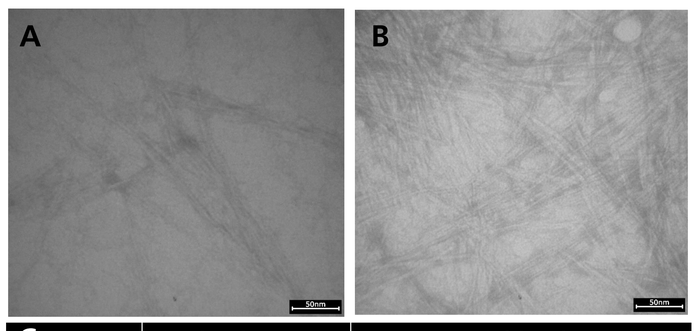
Cellulose is the most abundant organic substance on earth. **Cellulose nanocrystals (CNC)** are extracted from renewable resources such as wood or cotton and therefore are a **raw material** in the field of bioeconomy. Its unique **physical and structural properties** feature **low-cost, renewable and biodegradable products**. However, the high aspect ratio and fibrous structures of nanocellulose also raise **health concerns**, especially for workers in manufacturing industries (e.g. CNC inhalation). Therefore, reliable *in vitro* models that mimic the scenario of inhalation are needed to evaluate possibly harmful effects of CNCs to the lung.



- ***In vitro* aerosol exposure** studies were performed with an **air-liquid-interface (ALI)-lung model** consisting of A549 (epithelial cells), EA.hy926 (endothelial cells) and THP-1 (macrophages) cells.
- Two different types of **CNC** extracted from α-cellulose (CNC-W) and pulp (CNC-G) by sulfuric acid hydrolysis **were studied** in a **concentration of 100 µg/ml** applied as **aerosols** with VITROCELL® Cloud system.
- **Single and multiple exposure** with and without a 24 h **regeneration phase** were compared.
- **Endpoints** of the study: **Cell viability, ROS generation** and **DNA damage.**

Single application

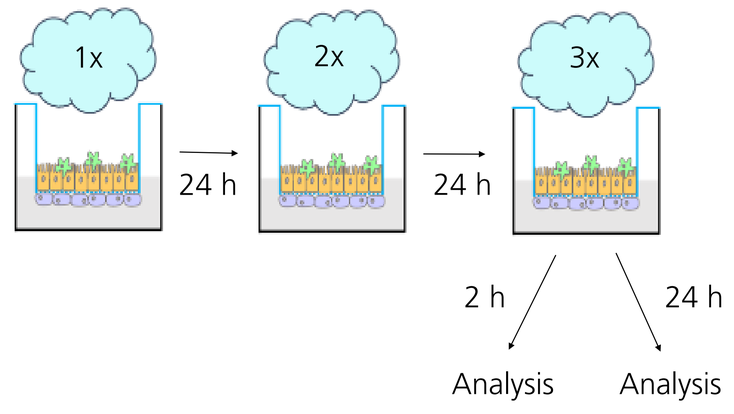




C	Size (nm)	Zeta-Potential (mV)
CNC-G	178	-59
CNC-W	242	-54

Fig.1: TEM images of 100 µg/ml CNC-G in cell culture medium aerosolized on culture insert membranes without cells with the VITROCELL® Cloud system to determine the distrubution of CNCs after application. A: single application. B: multiple application. C: average size and Zeta-Potential of CNCs.

Multiple application



Cell viability

2 h

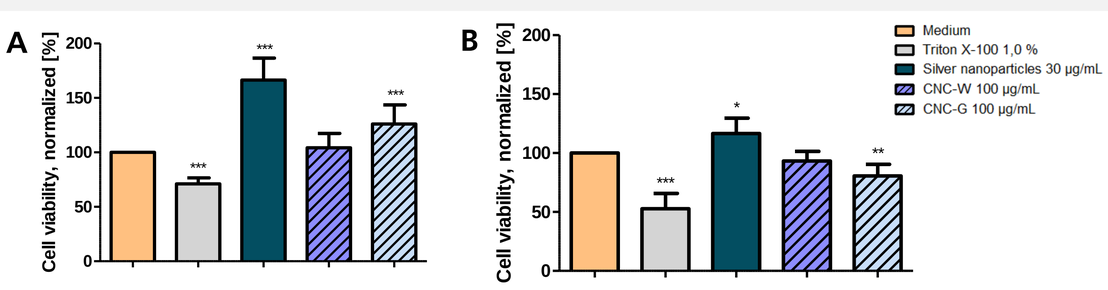


Fig.2: Cell viability after single application of 100 µg/ml CNC-W and CNC-G relative to culture medium treated cells. TritonX and silver nanoparticles were used as positive controls. A: Analysis 2 h after exposure. B: Analysis 24 h after exposure. *p< 0.05; **p<0.01; ***p<0.001.

2 h

24 h

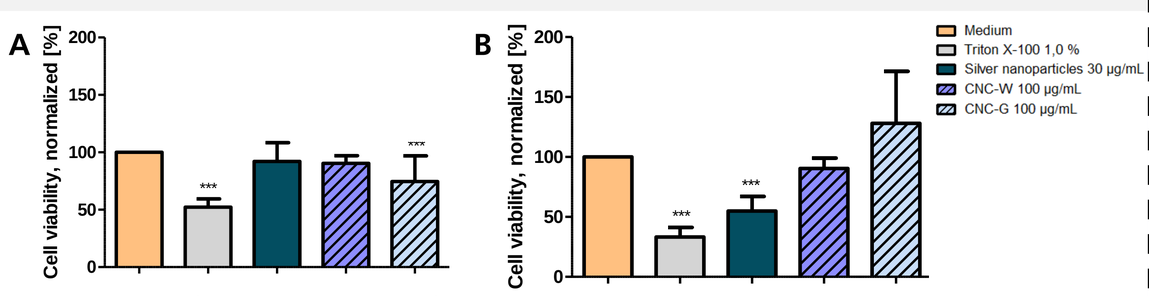


Fig.3: Cell viability after multiple application of 100 µg/ml CNC-W and CNC-G relative to culture medium treated cells. TritonX and silver nanoparticles were used as positive controls. A: Analysis 2 h after exposure. B: Analysis 24 h after exposure. ***p<0.001.

ROS generation

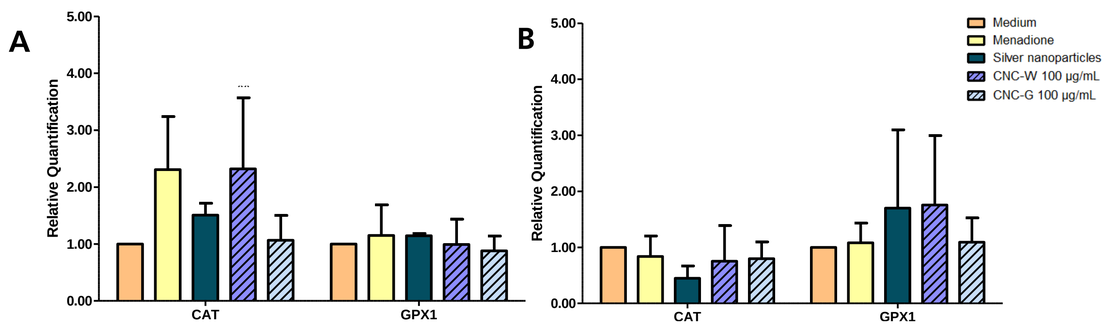


Fig.4: Quantitative RT-PCR for ROS associated genes (CAT, GPX1) after single application of 100 µg/ml CNC-W and CNC-G in comparison to culture medium treated cells. Menadione and silver nanoparticles were used as positive controls. Samples were normalized against house keeping genes GAPDH and HPRT1 expression of the same cDNA samples. A: Analysis 2 h after exposure. B: Analysis 24 h after exposure.

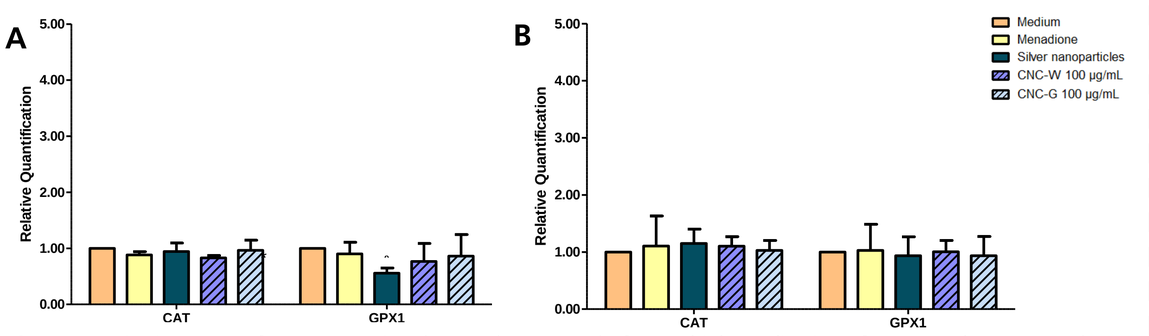


Fig.5: Quantitative RT-PCR for ROS associated genes (CAT, GPX1) after multiple application of 100 µg/ml CNC-W and CNC-G in comparison to culture medium treated cells. Menadione and silver nanoparticles were used as positive controls. Samples were normalized against house keeping genes GAPDH and HPRT1 expression of the same cDNA samples. A: Analysis 2 h after exposure. B: Analysis 24 h after exposure.

DNA damage

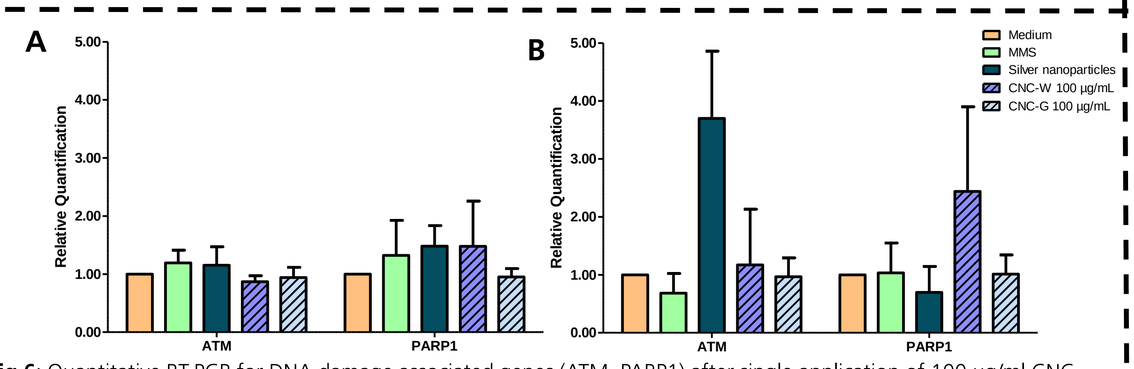


Fig.6: Quantitative RT-PCR for DNA damage associated genes (ATM, PARP1) after single application of 100 µg/ml CNC-W and CNC-G in comparison to culture medium treated cells. MMS and silver nanoparticles were used as positive controls. Samples were normalized against house keeping genes GAPDH and HPRT1 expression of the same cDNA samples. A: Analysis 2 h after exposure. B: Analysis 24 h after exposure.

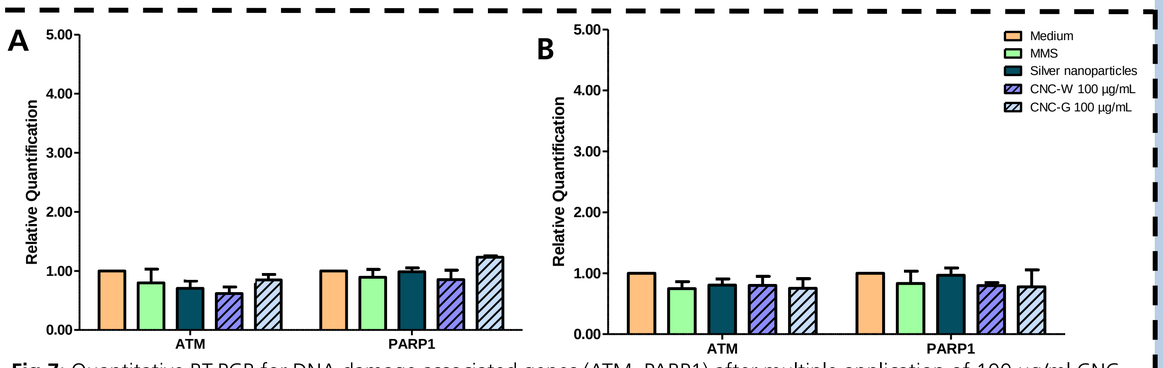


Fig.7: Quantitative RT-PCR for DNA damage associated genes (ATM, PARP1) after multiple application of 100 µg/ml CNC-W and CNC-G in comparison to culture medium treated cells. MMS and silver nanoparticles were used as positive controls. Samples were normalized against house keeping genes GAPDH and HPRT1 expression of the same cDNA samples. A: Analysis 2 h after exposure. B: Analysis 24 h after exposure.

Conclusion

The number of exposure cycles as well as the duration of the regeneration phase after CNC exposure at the air-liquid-interface seems to have an influence on the cellular response. Further studies are needed for a precise hazard assessment of aerosolized CNC on human lung cells.

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