Microplastic in the lung

In vitro model of exposing primary human nasal epithelial cells to plastic particles at the air-liquid interface

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1. Abstract

Microplastic is continuously released in the environment due to the steady fragmentation of plastic waste. Through air pollution the pieces enter the human body by inhalation and may interfere with lung cells. The aim of this thesis was to expose nasal epithelial cells to plastic particles when the focus was the localisation of the particles within the cell culture. In order to do this, the cells were cultured at the air-liquid interface (ALI), which is a commonly used method for culturing respiratory cells. For the aerosol exposure the "Cloud Alpha12" from Vitrocell© was used and the exposed cultures were labelled employing immunofluorescence. Finally, the images were acquired using a confocal microscope. The results obtained from the initial two approaches were suboptimal due to the lack of deposited particles. The third approach was utilized to test different methods of labelling and ultimately optimized the protocol for the last exposure. In all attempts the particles were not detected entering the epithelial cells, but mostly they were located inside the mucus layer, which would indicate the clearance *in vivo*.

2. Introduction

The previous state in the laboratory was an already established standard operating procedure (SOP) for the cell culture method with the ALI. The "Cloud Alpha12" device from Vitrocell was in contrast recently installed and the SOP was developed within the project. As for the work with microplastic particle suspensions, there were no experiments done so far in this laboratory nor on the Vitrocell© device.

Air-liquid interface

The respiratory cells, collected with nasal brushing, are seeded on plastic vessels like a multi-well-plate and later on transferred to a culture flask. When the cell layer reached a confluence of 80% they are seeded onto the porous membrane of a culture insert and in the first phase submerged in the medium until full confluence is reached, visible as a monolayer. Finally, the cells are exposed to air at the apical side, which allows them to develop cilia and produce mucus, therefore generating a pseudostratified epithelium as shown in figure 1. [1], [2]

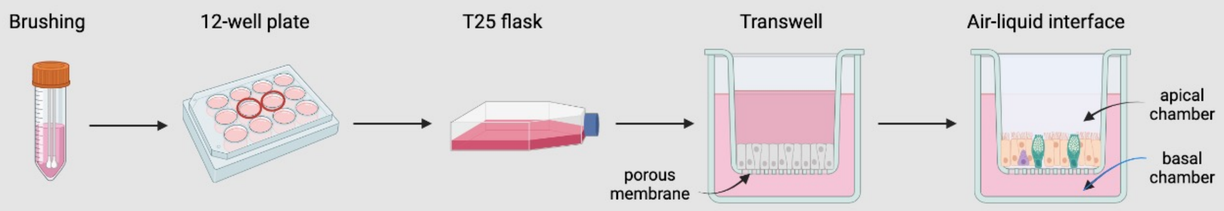

Fig. 1 – Overview of the culture process for nasal epithelial cells to get a pseudostratified epithelium at the ALI. (Reichen, 2023)

Vitrocell Cloud System

The main part of the device are the two nebulizers on top, which are able to transfer a liquid solution into a gas cloud. The particles in the aerosol spread within the chamber lid and finally deposit on the culture inserts at the bottom. In the control chamber are three places for culture inserts and in the sample chamber are eight places. One part of the insert holders is occupied by the hypersensitive scale called quartz cristal microbalance (QCM), which is used to measure the final deposition of the particles in ng/cm^2. [3]

3. Aims & Questions

The aim of the thesis was to expose the cell cultures and primary to **localise the plastic particles** within the epithelium.

- Where inside the cell culture can the plastic particles be localised after a single exposure with aerosol at the ALI?

Further aims:

- A new culture membrane with 1µm pore sizes was used

- The particle suspension should generate reproduceable depositions

4. Methods & Material

The experimental setup and workflow can be seen in figure 2.

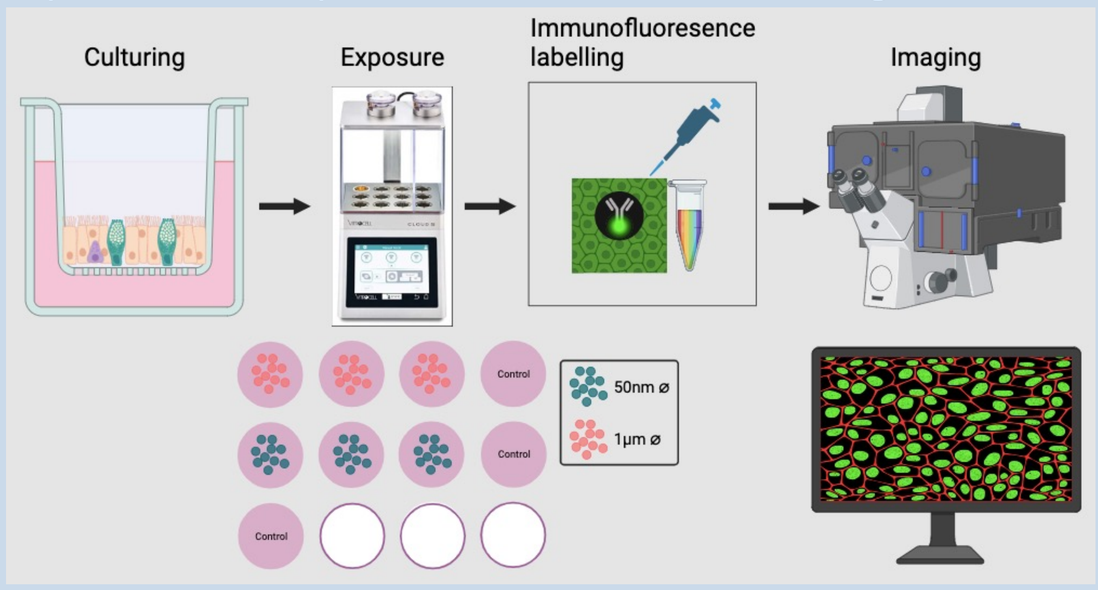

Fig. 2 – Overview of the experimental setup. (Reichen, 2023)

5. Results

The deposited particles for the first (figure 3) and second exposure were around four particles per field of view. For the third approach (figure 4) the labelling was changed and the deposition was higher. In the fourth exposure (figure 5) the deposition was overall higher again and the particles were mostly captured in the mucus layer.

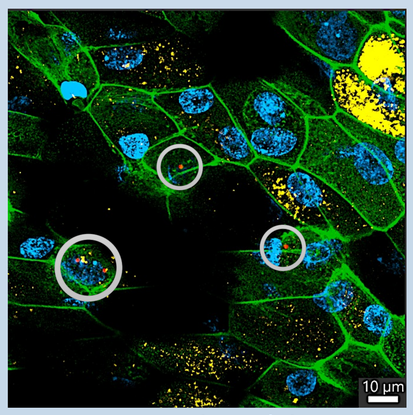

Fig. 3 – Exposure 1, **1`000µg/ml** 1µm particles, shown in red. Labelled: mucus, cytoskeleton and nuclei. (Reichen, 2023) - adapted

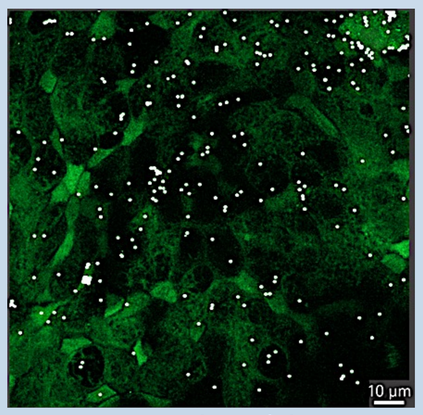

Fig. 4 – Exposure 3, **5`000µg/ml** 1µm particles, shown in white. Labelled: cell membranes. (Reichen, 2023)

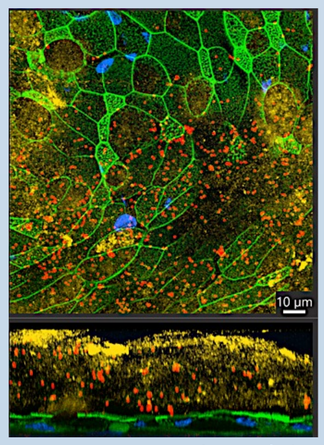

Fig. 5 – Exposure 4, **5`000µg/ml** 1µm particles, shown in red. Labelled: mucus, cytoskeleton and nuclei. (Reichen, 2023)

6. Discussion

The localisation of the particles could not be completely evaluated yet, since the protocols had to be changed between the approaches. The tendency seems to be that the particles settled in the mucus layer or were transported there through the cilia. In both cases it would indicate an *in vivo* clearance. The conclusion of all experiments was, that the final deposition depended on the labelling protocol, which was further adapted to the exposures in the course of the project. Further on it could be a possibility to test different materials of plastic which may vary in size and shape or to test a chronic model with several exposures.

Figures

Fig. 1 – Reichen, S. (2023). *Overview of the culture process for nasal epithelial cells to get a pseudostratified epithelium at the ALI*. medi.

Fig. 2 – Reichen, S. (2023). *Overview of the experimental setup*. medi

Fig. 3 – Reichen, S. (2023). *Exposure 1, 1`000µg/ml 1µm particles, shown in red. Labelled: mucus (yellow), cytoskeleton (green) and nuclei (blue).* medi.

Fig. 4 – Reichen, S. (2023). *Exposure 3, 5`000µg/ml 1µm particles, shown in white. Labelled: cell membranes (green).* medi.

Fig. 5 – Reichen, S. (2023). *Exposure 4, 5`000µg/ml 1µm particles, shown in red. Labelled: mucus (yellow), cytoskeleton (green) and nuclei (blue).* medi.

References

[1] – Müller, L., Brighton, L. E., Carson, J. L., Fischer, W. A., & Jaspers, I. (2013). Culturing of Human Nasal Epithelial Cells at the Air Liquid Interface. *Journal of Visualized Experiments: JoVE, 80,* 50646. https://doi.org/10.3791/50646

[2] – PromoCell. (2021). *Air-liquid interface culture: Getting a closer look into our airways* [Blog]. Retrieved on 24.01.2023 from: https://promocell.com/blog/air-liquid-interface-culture-getting-a-closer-look-into-our-airways/

[3] – Vitrocell. (n.d). *Vitrocell© Cloud alpha12 device*. Website. Retrieved on 11.09.2023 from: https://www.vitrocell.com/inhalation-toxicology/exposure-systems/vitrocell-cloud-system/vitrocell-cloud-alpha-12/