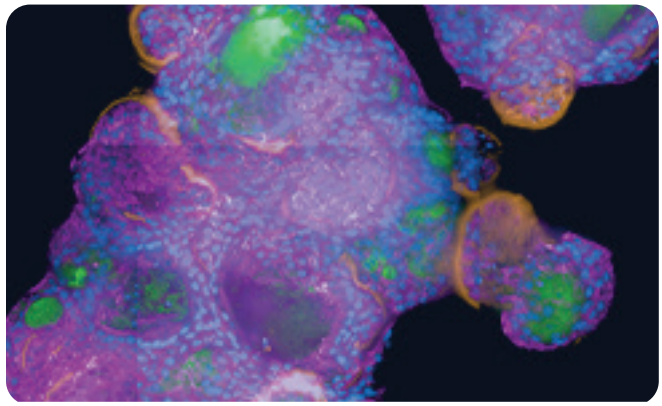
EXPERT INTERVIEW

Combining expertise in 3D airway cell models with SARS-CoV-2 research

Doris Wilflingseder, from the Institute for Hygiene and Medical Microbiology of the Medical University of Innsbruck (MUI) in Innsbruck, Austria, and colleagues are combining their expertise in 3D airway cell model development together with high-content analysis on the Operetta® CLS™ system to study the first interaction of SARS-CoV-2 with host respiratory tissues.

Here, Univ. Prof. Wilflingseder shares details of their model and discusses why an *in vitro* system has advantages over *in vivo*/small animal experiments.



Which complex model are you currently employing, and can you describe the key features and components unique to your model?

In 2012, we set up an *in vitro* 3D lung/immune cell model to study the first interactions of *Aspergillus fumigatus* with upper and lower respiratory tract epithelial and immune cells.[1] Over the last few years, we have worked to further optimize our system, which meant that when the SARS-CoV-2 pandemic started, our system was fully optimized. We are interested in the entry of pathogens via mucosal surfaces and have been using 3D models to work with HIV-1 and SARS-CoV-2.

A key feature of our models is that we are now completely animal free. For the extracellular matrix researchers often use rat tail collagen, or another collagen from animals. We exchanged this for a cellulose-based hydrogel called GrowDex. Interestingly, when we seeded primary respiratory cells in the cellulose-based hydrogel, they had a very fast differentiation, meaning they could be used earlier.

Another unique feature of our system is that we let the cells grow upside down. Normally, cells are seeded in collagen-based matrices to the inner field of transwell inserts. For imaging, you then have to cut out the whole filter and turn it upside down. To overcome these problems, we optimized the culturing conditions by putting the transwell upside down and seeding the cells on the bottom of the filter.[2] This meant we could simply lift the filter and image the same transwell over a period of time and we can easily add immune cells of interest to the upper chamber. This is also an advantage when, for example, you want to conduct multiple respiratory challenges.

We have had a lot of collaboration interest and research questions coming our way. Over the last month, a post-doc in my lab has cultured apical-out polarized lung organoids,[3] which are more amenable to high-content screening. This is fantastic to work on and we are looking to further establish this by also implementing the immune system.

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What key technologies are you employing in your work with the 3D airway model system and what technological challenges have you had to overcome?

Over the last few years, Operetta CLS has become increasingly important for our analysis and it provides a lot of insights from a single transwell filter of cells. Not only do you retrieve a lot of information, but you also get fantastic pictures. For example, we can visualize the interactions of SARS-CoV-2 with the multilayered epithelia (Figure 1). The tissue models also give information on whether the epithelial integrity is hampered by the virus by measuring TEER. In addition, the ability to analyze the supernatants of those transwell filters by multi-parameter cytokine analysis is a valuable tool.

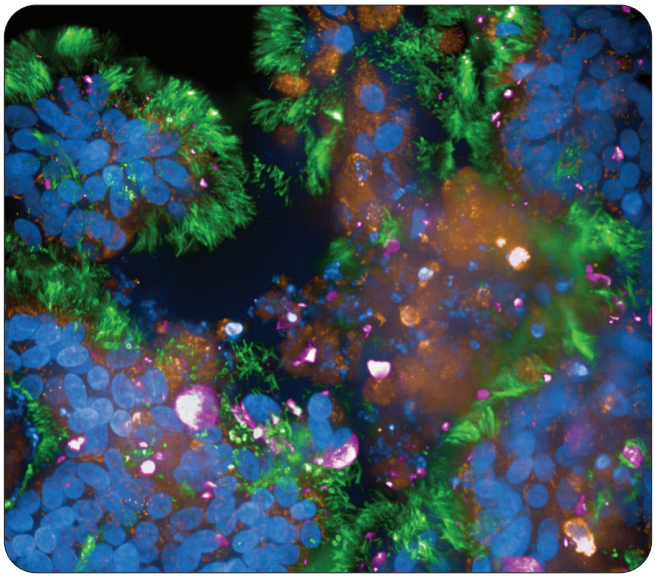

Figure 1: SARS-CoV-2-infected fully differentiated primary airway epithelia. Human airway epithelial cells were cultured in air-liquid interphase (ALI) for 80 days prior to infection with SARS-CoV-2. After two days, cells were fixed, permeabilized, and stained for SARS-CoV-2 nucleocapsid using a specific antibody (pink), acetylated tubulin for cilia (green), höchst for nuclei (blue), and MUC5AC for mucus (orange). Cells were analyzed using the Operetta CLS system.

The challenge is you cannot do high-throughput screens with the transwell filters. However, now that we have established the apical-out polarized lung organoids (Figure 2), we hope to pre-screen in medium to high throughput in the organoids and then move to the airway transwell system containing immune components. This ability to circumvent the challenges by using other techniques to complement the process is great.

Researchers from multiple research areas are turning towards more physiological relevant model systems that better mimic *in vivo* biology. Is this a trend that continues into infectious disease research, and what are the hopes associated with this?

Unfortunately, this is not the case. In infectious disease research, as well as basic research in general, mouse models are mostly used. This is a pity because we have seen huge progress in respect to cell culture models, induced pluripotent stem cells, microfluidic devices, etc., especially over the last 10 years.

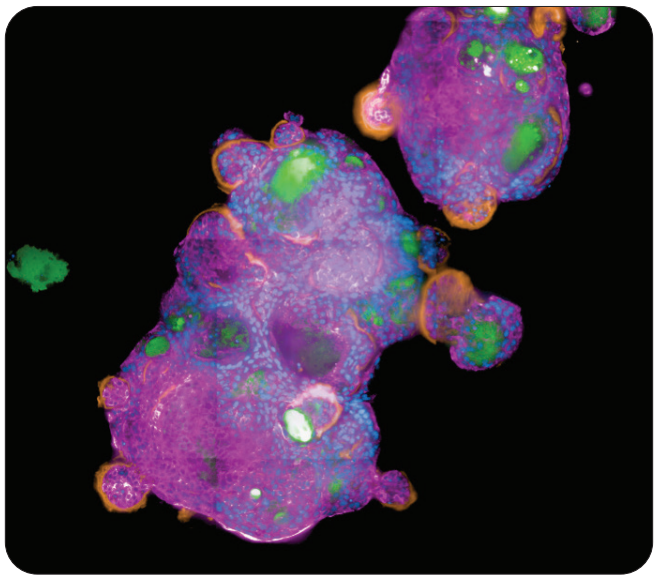

Figure 2: SARS-CoV-2-infected apical-out polarized lung organoids. Differentiated apical-out lung organoids were infected for three days using a SARS-CoV-2 patient isolate, which was expanded *in vitro* in Vero-TMPRSS2 cells (MRS AIDS Reagent). Thereafter, organoids were fixed, permeabilized, and stained using Höchst for nuclei (blue), MUC5AC for mucus (orange), phalloidin for tight junctions (pink), and an anti-SARS-CoV-2 antibody (green). Images were taken using the Operetta CLS system. ©D.Wilflingseder, A.Noureen.

I think it is important that people know there are validated alternatives that exist. For example, I work in immune diseases and my current interests are HIV-1 and SARS-CoV-2. For both viruses you need to make genetically engineered mice so that they can get infected. In my opinion, this doesn't make sense; why genetically modify a mouse so that it can be infected with a human pathogen? I am trying to optimize the cell systems so that we can get the most out of these. I want to spread the word so that we can collaborate with people who are working on alternatives to mouse models.

Here in Innsbruck, we have established the MUI animal Free Research Cluster, which progresses alternatives to animal experimentation with respect to models (organoids, stem cells, 3D cultures, etc.) or techniques. We also made a change within the curricula and are already educating young researchers within the masters and PhD programs here at the MUI on alternative methods to animal experiments. In my opinion, this is the only way to induce a paradigm shift in the direction of replacement.

What are the main advantages and disadvantages of more complex model systems for infectious disease research?

From an ethical stance, the main advantage is that you don't have to use animals for your experiments. Also, working within a human system means the cells express the desired receptors. For example, the ACE2 receptor is expressed in humans but not in mice. Of course, some people will say that you can never have the desired complexity within a cell culture system when compared with mice. At the moment, this is true. Nevertheless, people are working hard to enhance the complexity of these systems.

One disadvantage of complex model systems is that psychological questions cannot be modelled *in vitro*. However, you could also argue that psychological observations in mice may not reflect what would happen in humans.

What findings have surprised you the most during your research?

What really surprised me was that pseudostratified primary epithelial cultures produce immune components, such as complement, upon pathogen interactions. This surprised me as up to now it was believed that these components were only produced by immune cells and the liver. When we exposed our tissues to SARS-CoV-2, we found an overshooting production of innate immune components.

Another surprising observation was that when we co-cultured macrophages on the air interface of such co-cultured tissues, about 70% survived after four weeks, just because they could interact with other cells. Normally, on plastic dishes, they only survive for about 10 days. In all my 20 plus years of research, I'd never have expected this. It never gets boring.

The current COVID-19 pandemic has shown us that respiratory infections are still a major threat to human health. What are the ways your institute is working to help address these threats?

We are closely collaborating with the Internal Medicine Institute of the ´tirol klinken´ (Tyrol clinics) and receive patient swabs and samples from COVID-19-postive individuals. In the beginning, this was challenging as we had never worked with SARS-CoV-2 before, but after about two months everything was standardized and moving fast. Together with the working group of Assoc.- Prof. Wilfried Posch at the Institute of Hygiene and Medical Microbiology, we are now using our 3D respiratory model to conduct further research on SARS-CoV-2. Our focus here is on activation of innate immunity by SARS-CoV-2 and whether this activation could be involved in tissue damage and potentially linked to case severity. We also set up really sensitive ELISpot and neutralization assays, which allowed us to measure T- and B-cell responses in COVID-19-positive patients or those who had been vaccinated, which has been a valuable tool.

Can you share some insights into projects that you've published or have ongoing that use your group's airway model?

We've had four publications over the last few years: two on the optimization of the airway cell model system and two studying *A. fumigatus* interactions within the system. We also have two studies on SARS-CoV-2, which are currently in revision in high impact journals.

We have several grants relating to our systems and various infections and pathogens. The first COVID-19 project that was funded here used our system. For this, Wilfried received a COVID-19 emergency grant from the Austrian Science Fund. I also had, together with a collaboration partner at the Northwestern University in Chicago, an NIH pilot grant on HIV mucosal infection.

Where do you see your research moving to in the future?

I'd really like to set up a system where I can connect the epithelial/immune barrier with a lymph node and look at the very first dendritic cells-T cells interactions within the lymph nodes. In animals, this isn't possible because you can't follow all reactions immediately after the challenge. This presents a great opportunity for such systems.

Learn more about the group's work on 3D cell models here:

1. Chandorkar, P., Posch, W., Zaderer, V. et al. (2017) Fast-track development of an *in vitro* 3D lung/immune cell model to study Aspergillus infections. *Sci Rep*, 7, 11644. https://doi.org/10.1038/s41598-017-11271-4.

2. Zaderer, V., Hermann, M., Lass-Flörl, C., Posch, W., Wilflingseder, D. (2019) Turning the World Upside-Down in Cellulose for Improved Culturing and Imaging of Respiratory Challenges within a Human 3D Model. *Cells*, 8, 1292. https://doi.org/10.3390/cells8101292.

3. Salahudeen, A.A., Choi, S.S., Rustagi, A. et al. (2020) Progenitor identification and SARS-CoV-2 infection in human distal lung organoids. *Nature*, 588, 670–675. https://doi.org/10.1038/s41586-020-3014-1.

Doris Wilflingseder

Doris Wilflingseder studied zoology at the Leopold-Franzens-University in Innsbruck, Austria. During her thesis at the Division of Physiology, Medical University Innsbruck, and her first postdoc years at the Division of Hygiene and Medical Microbiology, Medical University Innsbruck, she focused on cell biology and immunology and worked on signal mechanisms – i.e., MAPK signaling – in human primary cell models upon stimulation with fungal or viral pathogens. Doris continued these analyses using dendritic cells (DC) and differentially opsonized HIV-1 during her stay in collaboration with Paul Kellam at the Division of Infection and Immunity, University College London.

Doris's research group at the Division of Hygiene and Medical Microbiology of the Medical University Innsbruck is interested in modulation of DC and macrophage function by the opsonization pattern of pathogens, i.e., HIV-1, HIV-2 or *Aspergillus fumigatus* in relevant three-dimensional cell culture microenvironments containing epithelial borders. To address these issues, they use molecular biologic, imaging and immunologic approaches, and primary cell models – where possible – or appropriate cell lines for e.g., gene editing approaches.

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