


# Short-term ex vivo tissue culture models help study human lung infections

## A review

Jing-Yan Xia, MD<sup>a</sup>, Yi-Fei Zeng, MB<sup>b</sup>, Xue-Jie Wu, MD<sup>b</sup>, Feng Xu, MD<sup>b,c,\*</sup> 

### Abstract

Most studies on human lung infection have been performed using animal models, formalin or other fixed tissues, and in vitro cultures of established cell lines. However, the experimental data and results obtained from these studies may not completely represent the complicated molecular events that take place in intact human lung tissue in vivo. The newly developed ex vivo short-term tissue culture model can mimic the in vivo microenvironment of humans and allow investigations of different cell types that closely interact with each other in intact human lung tissues. Therefore, this kind of model may be a promising tool for future studies of different human lung infections, owing to its special advantages in providing more realistic events that occur in vivo. In this review, we have summarized the preliminary applications of this novel short-term ex vivo tissue culture model, with a particular emphasis on its applications in some common human lung infections.

**Abbreviations:** AEC = alveolar epithelial cell, AM = alveolar macrophage, *C. pneumoniae* = *Chlamydia pneumoniae*, CD163 = cluster of differentiation 163, COPD = chronic obstructive pulmonary disease, COVID-19 = coronavirus disease 2019, CXCL = C-X-C motif chemokine ligand, EVLP = ex vivo lung perfusions, *H. influenzae* = *Haemophilus influenzae*, IFNs = interferons, IL = interleukin, *L. pneumophila* = *Legionella pneumophila*, MAPK = mitogen-activated protein kinase, NTHi = nontypeable *Haemophilus influenzae*, pHp = pulmonary haptoglobin, *S. pneumoniae* = *Streptococcus pneumoniae*, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, TLR2 = Toll-like receptor 2, TLR4 = Toll-like receptor 4, TNF- $\alpha$  = tumor necrosis factor- $\alpha$ .

**Keywords:** ex vivo tissue culture model, human lung, lung infection

### 1. Introduction

To date, most results of human lung infections have been obtained from in vitro experiments using animal models, conserved tissues, and established cell lines. Owing to their non-human background, animal experiments may not fully reflect the actual situation of the human body.<sup>[1]</sup> Thus, significant differences between animal and human immune systems may hinder the transmission of results obtained in animals to human pathophysiology.<sup>[2-4]</sup> In addition, due to the price and ethical limitations, some susceptible species do not fully represent human infection, or sometimes do not develop the expected symptoms.<sup>[5,6]</sup> Carefully conserved tissues pretreated by different tissue fixation techniques, such as formalin and Hepes-Glutamic acid buffer mediated Organic solvent Protection Effect based techniques, have been widely applied for immunohistochemical studies to characterize the immunological profiles of lung infection.<sup>[7,8]</sup> These data provide limited information on the molecular events involved in human lung infection in vivo, owing to the unexpected effects of fixatives on tissues. Results acquired

from established cell lines, which comprise only a singular cell type, cannot represent the complex interaction of different components in intact human lung tissues in vivo and reproduce the complex structures and immunological responses in the human respiratory tract completely.<sup>[9,10]</sup>

The establishment of an ex vivo tissue culture model using fresh and intact specimens may be a possible option for conserving the tissue architecture ex vivo. However, the premise to establish a tissue culture model is capable of producing sufficiently thin tissue sections because the lack of diffusion of oxygen and nutrients in the center of thick tissue cubes may lead to cell death when they are cultured ex vivo. Introducing a tissue microtome solved this problem and enabled the preparation of thin tissue slices.<sup>[11,12]</sup> Thereafter, a short-term ex vivo tissue culture model was established directly from fresh human lung tissues, which showed some advantages.<sup>[13,14]</sup> Figure 1 Shows the process of the establishment of an ex vivo tissue culture model.

Although ex vivo short-term tissue culture cannot be propagated, this model provides important information on the complex communication between different tissue cells and

This research was funded by the National Natural Science Foundation of China (82272222 and 82070006) and Binjiang Institute of Zhejiang University (ZY202205SMKY006).

The authors have no conflicts of interest to disclose.

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

<sup>a</sup> Department of Radiation Oncology, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, PR China, <sup>b</sup> Department of Infectious Diseases, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, PR China, <sup>c</sup> Research Center for Life Science and Human Health, Binjiang Institute of Zhejiang University, Hangzhou, China.

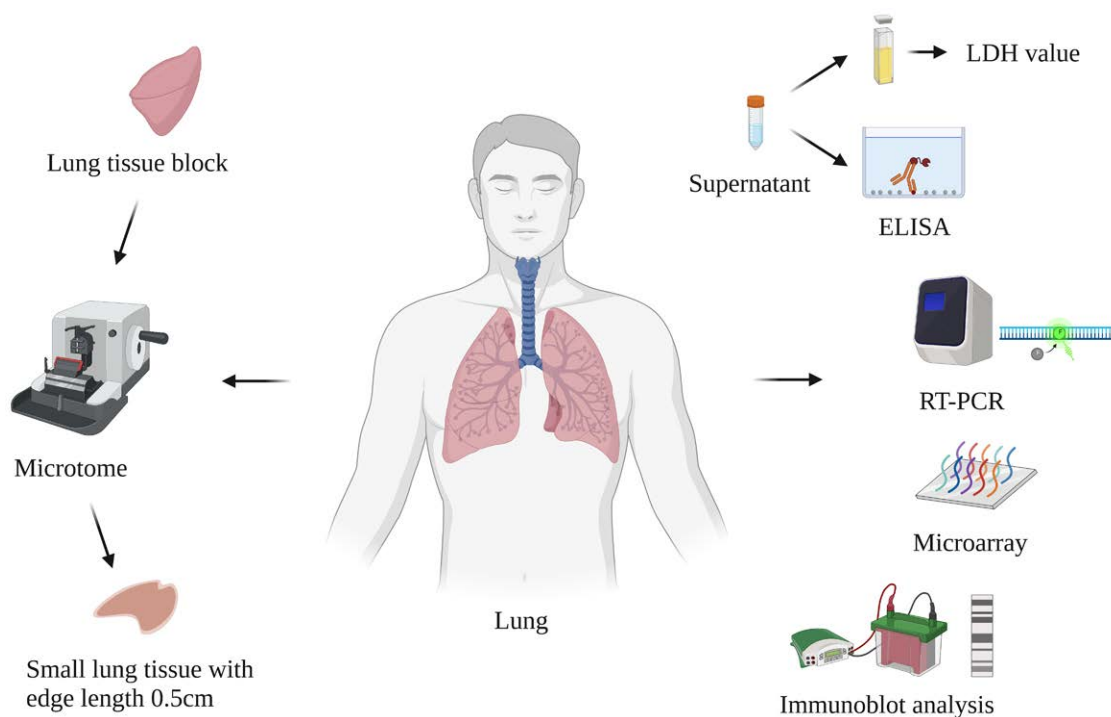
\* Correspondence: Feng Xu, Department of Infectious Diseases, The Second Affiliated Hospital of Zhejiang University School of Medicine, No. 88 Jiefang Road, Hangzhou, Zhejiang 310009, PR China (e-mail: xufeng99@zju.edu.cn).

Copyright © 2022 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the Creative Commons Attribution License 4.0 (CCBY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Xia J-Y, Zeng Y-F, Wu X-J, Xu F. Short-term ex vivo tissue culture models help study human lung infections: A review. *Medicine* 2023;102:1(e32589).

Received: 19 August 2022 / Received in final form: 14 December 2022 / Accepted: 15 December 2022

<http://dx.doi.org/10.1097/MD.0000000000032589>



**Figure 1.** The schematic diagram of ex vivo tissue culture model. Thin lung tissue blocks were prepared using a tissue microtome with edge length approximately 0.5cm and then were cultivated ex vivo in conditioned cell culture medium. After stimulation/infection with a variety of pathogens in tissue culture, molecular events can be detected ex vivo directly by appropriate techniques. Created with BioRender.com.

the extracellular matrix in intact tissues, and it also overcomes some of the differences in host–defense mechanisms between humans and other animal species.<sup>[15]</sup> For example, human lung organoid is a newly developed ex vivo tissue culture model. This model can present the in vivo environment and 3D lung structure, and simulate the function of human lung. Additionally, such ex vivo models can represent different part of respiratory tract and offer an opportunity for further studies of the whole respiratory system.<sup>[16]</sup> This kind of model derived from human pluripotent stem cells which can differentiate to airway and alveolar epithelial cells (AECs) in peripheral blood.<sup>[17,18]</sup> Human pluripotent stem cells-derived organoids can be induced to differentiate into specific cell types and form tissue-specific organoids.<sup>[19,20]</sup> Therefore, these cells can be co-cultured with mesenchymal and endothelial cells to build a 3D organoid which represents a part of structure and function of human lung. Although this kind of model may not fully recapitulate the whole functions of mature human lung, it remains an efficient way to study the mechanisms of lung infections because it replicates the human respiratory tract better than previous models.<sup>[21,22]</sup>

This mini-review summarizes the preliminary applications of the above-mentioned novel tissue culture model, with a particular emphasis on its applications in human lung infection.

## 2. Model applications in lung infection

### 2.1. Acute pulmonary *Chlamydia* infection

This ex vivo tissue culture model was first used to investigate acute pulmonary *Chlamydia* infection. Vital lung specimens were found to be infected with *Chlamydia pneumoniae* (*C. pneumoniae*) for at least 48 hours ex vivo. *C. pneumoniae* is predominantly found in alveolar macrophages (AMs). In contrast to acute *Chlamydia* infection, chronic obstructive pulmonary disease (COPD) patients with persistent *Chlamydia* infection showed a significantly higher infection rate in type

I AECs, which increased from  $2.3 \pm 0.9\%$  to  $18.2 \pm 3.5\%$ . However, only a few bronchial epithelial cells were found to be infected. *Chlamydial* viability and virulence were confirmed by detecting the expression of chlamydial heat shock protein 60 messenger ribonucleic acid in an acute *Chlamydia* infection model.<sup>[23]</sup> C-X-C motif chemokine ligand (CXCL)-8 expression, an important early innate response mediator, and Toll-like receptor 2 (TLR2), but not Toll-like receptor 4 (TLR4), were significantly increased in the acute *Chlamydia* infection model compared to COPD patients with persistent *Chlamydia* infection. Further studies showed that CXCL8 secretion was reduced dramatically when TLR2 signaling, but not TLR4, was blocked by a neutralizing antibody. Therefore, TLR2 signaling plays an important role in the early innate response caused by acute *Chlamydia* infection.<sup>[24]</sup>

### 2.2. *Streptococcus pneumoniae* (*S. pneumoniae*) infection

Later, application of this ex vivo tissue culture model was extended to explore acute *S. pneumoniae* infection, and similar to *Chlamydia* infection, *S. pneumoniae* was detected predominantly in AM with 80 to 90% positive rate for *S. pneumoniae* deoxyribonucleic acid after 24-hour infection. Depletion of AM with clodronate/liposomes revealed that AM was responsible for cytokine release from lung tissue. Furthermore, lung cell apoptosis has been shown to be associated with marked caspase-3 activation after *pneumococcal* infection in a time-dependent manner. In this study, although both TLR2 and TLR4 were upregulated in response to *pneumococcal* stimulation, they had negligible effects on the expression of interleukin (IL)-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and IL-6. However, inhibition of p38 mitogen-activated protein kinase (MAPK) signaling markedly reduced the production of inflammatory mediators, suggesting that p38 MAPK signaling may play a crucial role during *S. pneumoniae* infection.<sup>[25]</sup> Moreover, cyclooxygenase-2 was confirmed to be involved in *S. pneumoniae* infection in this model. Cyclooxygenase-2 was upregulated in AM,

vascular endothelium, and alveolar type III epithelial cells, but not type I epithelial cells. Extracellular regulated protein kinase and p38 MAPK signaling were found to be correlated with COX-2 induced prostaglandin E2 formation after *S. pneumoniae* infection. Further functional analysis showed that COX-2 and prostaglandin E regulate pro-inflammatory and anti-inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , granulocyte-macrophage colony-stimulating factor, platelet-derived growth factor, IL-10, IL-1RA, IL-15, and IL-17 during acute *S. pneumoniae* infection.<sup>[26]</sup>

### 2.3. *Haemophilus influenzae* (*H. influenzae*) infection

Using this tissue culture model, Drömann and colleagues showed that non-typeable *H. influenzae* (NTHi) infection induced a much higher infection rate of AM after acute infection with NTHi-1 and NTHi-2 strains than that of infected COPD lungs. In addition, acute NTHi infection in vitro induced a strong pro-inflammatory response with increased expression of CXCL8, TNF- $\alpha$ , and p38 MAPK, while transforming growth factor- $\beta$  release decreased. This study also depicted an obvious upregulation of transforming growth factor-pseudoreceptor BMP and activin membrane-bound inhibitor with ubiquitous expression on AM and AEC after in vitro infection of COPD lung tissue, indicating that activin membrane-bound inhibitor might play an important role in the early immune response of pulmonary NTHi infection.<sup>[27]</sup> In a comparative study, this ex vivo tissue culture model was infected with different respiratory pathogens including *C. pneumoniae*, *S. pneumoniae*, and *H. influenzae*. PCR results showed a significant downregulation of cluster of differentiation 163 (CD163) due to *H. influenzae* and *C. pneumoniae* infections. Further immunohistochemistry confirmed that the expression of pulmonary haptoglobin (pHp) was elevated more frequently in AM than that in AEC II, whereas CD163 expression was downregulated after 24-hour infection with *H. influenzae*. The upregulation of pHp and CD163 caused by *S. pneumoniae* infection was found to be mainly located in AEC II and AM. These results suggested that pHp and CD163 may be functional immunoregulatory elements in human lung infection.<sup>[28]</sup>

### 2.4. *Legionella pneumophila* (*L. pneumophila*) infection

Recently, this ex vivo tissue culture model has been utilized to determine the early stages of *L. pneumophila* infection. It was shown that *L. pneumophila* adhered to the alveolar lining and primarily infected AM and could replicate within this tissue culture model. This study revealed for the first time that *L. pneumophila* outer membrane vesicles, which contain many virulence-related proteins bound predominantly to AM surfaces, could be detected in the cytoplasm. In addition, downregulation of the macrophage receptor with collagenous structure was identified by transcriptome analysis and was further confirmed at the sites of pathogen-infected tissue destruction using immunohistochemistry.<sup>[29]</sup>

### 2.5. *Influenza A virus* infection

Moreover, the ex vivo tissue culture model also assisted in the study of other types of lung infections, such as viral and protozoal infections. Some subtypes of *influenza A virus* can lead to acute severe respiratory disease, resulting in epidemics every year.<sup>[30]</sup> It has been reported that using this ex vivo culture model can help understand the mechanisms that support or restrict the growth of influenza viruses in the lower respiratory tract. The research found that after 24-hour infection, influenza virus-positive cells were predominantly AEC II. In addition, different kinds of viral antigens could also be detected in AM in infected lung specimens, but they constituted only 4 to 11%.

However, although different *influenza A viruses* shared the same cell tropism for AEC II, large differences existed between the strains with regard to replication and cytokine induction, which might explain why different subtypes showed different infectivity and virulence. For example, porcine and low-pathogenicity *avian influenza viruses* were observed to have growth restriction, while the seasonal and pandemic *H1N1 viruses* propagated efficiently. At the same time, *avian influenza viruses* were found to induce a considerably stronger cytokine response in the alveolar tissue than other human-adapted viruses, such as pandemic *H1N1-2009 virus* and the seasonal *H3N2* and *H1N1 viruses*, even in the lack of recruited immune cells. The results showed differential induction of cytokines and chemokines in human lung tissues by human and animal *influenza A viruses*. This might explain why different subtypes grow differently in the human lungs.

These results indicated that differences in the pathogenicity of *influenza A viruses* in the human lung cannot be attributed to different cellular tropisms.<sup>[10]</sup> Instead, it depended on virus's inherent replicative properties in AEC II.<sup>[31]</sup> This research also stressed the value of ex vivo human lung cultures models in pathophysiological processes study.<sup>[10]</sup>

### 2.6. *Cryptosporidium* infection

Generally, *Cryptosporidium* infection causes self-limiting diarrhea in immunocompetent individuals,<sup>[32]</sup> but it can also target the respiratory tract, causing respiratory cryptosporidiosis in both immunocompetent and immune-deficient individuals.<sup>[33]</sup> However, the specific pathophysiology of respiratory infections is still unclear. Thus, ex vivo human lung culture models can be used to examine whether the parasite can infect the lungs and finish its life cycle. Within 24 hours after oocyst injection, quantification of 18S ribosomal ribonucleic acid showed that the parasite dramatically increased in the lung model. By performing immunofluorescence assays using a zoite-specific antibody, this model verified the development of asexual (meront I) and (macrogamont) stages. Finally, newly formed oocysts were observed in sporozoite-infected organoids at 6th day post-infection. Therefore, ex vivo human lung culture models allowed *C. parvum* to propagate and complete the full life cycle, which is a potential tool for further research.<sup>[34]</sup>

### 2.7. *Coronavirus disease 2019* (COVID-19)

In recent years, the ongoing COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has created an immense global health crisis.<sup>[35]</sup> The widely used cell culture infection models that typically use monolayers of a single cell type can support SARS-CoV2 replication and have been successfully applied to SARS-CoV-2 study.<sup>[36]</sup> For example, these models can help explore cell-autonomous defense mechanisms,<sup>[37]</sup> host cell interactions,<sup>[38]</sup> virus replication kinetics<sup>[39]</sup> and assess the effect of different kinds of medicines.<sup>[40,41]</sup> However, these simple cell culture models cannot explain the complex pathophysiological processes in organ level, due to the lack of cell-type diversity. Besides, the use of susceptible animal models is also limited because it may lead to problems such as high cost, moral and ethical controversies.<sup>[42]</sup>

An ex vivo human lung culture model has been used to study the mechanisms of SARS-CoV-2 infection.<sup>[43-45]</sup> The research showed that SARS-CoV-2 replicated in human lung tissues very efficiently within a 48-hour interval. SARS-CoV-2 targeted AEC I, II, and AM primarily in cell tropism. Importantly, SARS-CoV-2 did not significantly lead to type I, II, or III interferons (IFNs) in infected human lung tissues, despite highly efficient virus replication. In addition, SARS-CoV-2 infection only upregulated 38.46% of key inflammatory mediators, including IL-6, monocyte chemoattractant protein-1, CXCL1, CXCL5, and CXCL10 (IP10). Therefore,



some untested patients with COVID-19 showed mild or even no symptoms, resulting in spreading the virus in communities and hospitals unknowingly.<sup>[45,46]</sup> Furthermore, the ex vivo lung culture model will also help understand the earliest stages of SARS-CoV-2 infection in the human respiratory system and reduce the late-phase morbidity and mortality of COVID-19. It was found that lung cells presented a limited innate immune response to SARS-CoV-2, with an obvious lack of type I and III IFNs, contrasting with the strong innate immune response to the influenza virus. This indicated that the early innate immune response resulted in restricted viral clearance once the virus got access to the lung. Moreover, because type III IFN has been proven to regulate critical immune activities,<sup>[47]</sup> avoiding excessive local inflammation, the obvious lack of type I and III IFNs upregulation in early SARS-CoV-2-infected lung tissues could explain the uncontrolled SARS-CoV-2 replication in late-phase and imbalanced hyperinflammatory response of severe COVID-19. Therefore, studies using this ex vivo tissue culture model can be further employed to assess the impact of viral evolutionary changes and evaluate new therapies against SARS-CoV-2.<sup>[43]</sup>

## 2.8. New techniques in ex vivo lung model conservation

The ex vivo lung model is an efficient tool for human lung infection study and can be implied in other fields as well. However, one challenging problem is to keep the cell viability in this kind of model for a relatively long time. In recent years, the emergence of a new technique named ex vivo lung perfusions (EVLV) can be a solution to this problem. EVLV is regarded as the most effective technique for estimating marginal donor lungs and may help to prolong the normothermic preservation time of ex vivo lung tissues.<sup>[48,49]</sup> Most importantly, it can potentially recondition impaired lung tissues and restore good state of them.<sup>[50]</sup> The basic mechanism of EVLV is using a pump to perfuse pulmonary artery with high oncotic pressure preservation fluid which is pure or mixed with red blood cells and recollecting the liquid in a reservoir.<sup>[51]</sup> At present, there are 3 main EVLV techniques: the Lund, the Organ Care System and the Toronto protocols.<sup>[50]</sup> Nevertheless, the high cost of EVLV limit its application in science research and clinic.<sup>[49]</sup> Therefore, it's necessary to improve current EVLV technology to have longer running time and lower running cost. EVLV can be an effective way to retain viability of the ex vivo lung model in the future.

## 3. Conclusion

In summary, the short-term ex vivo tissue culture model mimics the in vivo tissue microenvironment with a complete tissue architecture and has been applied in different kinds of lung infections (Table 1). This model represents a promising tool for exploring molecular events during acute lung, as it allows the investigation of the early innate immune response in the human lung. However, it also has some inherent limitations: a short period of cell viability; inability of cells to propagate; lack of recruitment of cells and mediators from blood; incomplete compensation by conditioned cell culture medium; bypassing the natural route of bacterial infection; availability of such human lung tissue samples is limited; cannot represent the effect of host systemic inflammatory response and the adaptive immune response; and limited complex interactions with the host.<sup>[25,26,34,45]</sup> In addition to its application in lung infections, it may also be an important tool for identifying new potential molecular targets for immunotherapy and chemotherapy in lung cancer.<sup>[14,32]</sup> We believe that this model, in particular, combined with other techniques, will be widely applied in further research beyond human lung infections.<sup>[53]</sup>

## Author contributions

J-YX conceived and participated in drafting the manuscript and gave the final approval of the version to be submitted and of any

**Table 1**

### Different applications of the ex vivo tissue culture model.

Application	Major roles
Pulmonary <i>Chlamydia</i> infection	Help confirm viability and virulence of <i>Chlamydia</i> .
<i>Streptococcus pneumoniae</i> infection	Help confirm p38 MAPK signaling and COX2 are involved in <i>S. pneumoniae</i> infection.
<i>Haemophilus influenzae</i> infection	Help demonstrate NTHi infection induce a much higher infection rate of AM after acute infection with strains NTHi-1 and NTHi-2 than that of infected COPD lungs.
<i>Legionella pneumophila</i> infection	Help prove <i>L. pneumophila</i> infects AM primarily.
Influenza A virus infection	Help understand the mechanisms that support or restrict the growth of influenza viruses in the lower respiratory tract.
<i>Cryptosporidium</i> infection	Help examine whether the parasite can infect the human lung and finish its life cycle.
Coronavirus disease 2019	Help study the mechanisms of SARS-CoV-2 infection.

AM = alveolar macrophage, COPD = chronic obstructive pulmonary disease, COX2 = cyclooxygenase-2, *L. pneumophila* = *Legionella pneumophila*, MAPK = mitogen-activated protein kinase, NTHi = nontypeable *Haemophilus influenzae*, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

revised version. Y-FZ was responsible for writing the first draft, and was involved in the final approval of the version to be published. X-JW was responsible for collecting the documents. FX critically revised the manuscript for important intellectual content.

**Supervision:** Xue-Jie Wu, Feng Xu.

**Writing – original draft:** Jing-Yan Xia, Yi-Fei Zeng, Feng Xu.

**Writing – review & editing:** Yi-Fei Zeng, Xue-Jie Wu.

## References

- [1] Kukavica-Ibrulj I, Levesque RC. Animal models of chronic lung infection with *Pseudomonas aeruginosa*: useful tools for cystic fibrosis studies. *Lab Anim*. 2008;42:389–412.
- [2] Berg J, Weber Z, Fechner-Bitteti M, et al. Bioprinted multi-cell type lung model for the study of viral inhibitors. *Viruses*. 2021;13:1590.
- [3] Radigan KA, Misharin AV, Chi M, et al. Modeling human influenza infection in the laboratory. *Infect Drug Resist*. 2015;8:311–20.
- [4] Zscheppang K, Berg J, Hedtrich S, et al. Human pulmonary 3D models for translational research. *Biotechnol J*. 2018;13:1700341.
- [5] Bouvier NM, Lowen AC. Animal models for influenza virus pathogenesis and transmission. *Viruses*. 2010;2:1530–63.
- [6] Campos CL, Gomes LR, Covarrubias AE, et al. A three-dimensional lung cell model to *Leptospira* virulence investigations. *Curr Microbiol*. 2022;79:57.
- [7] Kothmaier H, Rohrer D, Stacher E, et al. Comparison of formalin-free tissue fixatives: a proteomic study testing their application for routine pathology and research. *Arch Pathol Lab Med*. 2011;135:744–52.
- [8] Braun M, Menon R, Nikolov P, et al. The HOPE fixation technique—a promising alternative to common prostate cancer biobanking approaches. *BMC Cancer*. 2011;11:511.
- [9] Rudd BD, Burstein E, Duckett CS, et al. Differential role for TLR3 in respiratory syncytial virus-induced chemokine expression. *J Virol*. 2005;79:3350–7.
- [10] Weinheimer VK, Becher A, Tönnies M, et al. Influenza A viruses target type II pneumocytes in the human lung. *J Infect Dis*. 2012;206:1685–94.
- [11] Nissen E, Tanneberger S, Weiss H, et al. In vitro cultivation of vital tissue slices: a new variation of organ culture technics. *Biomed Biochim Acta*. 1983;42:907–16.
- [12] Hood CJ, Parham DM. A simple method of tumour culture. *Pathol Res Pract*. 1998;194:177–81.
- [13] Goldmann T, Wiedorn KH, Kühl H, et al. Assessment of transcriptional gene activity in situ by application of HOPE-fixed, paraffin-embedded tissues. *Pathol Res Pract*. 2002;198:91–5.

- [14] Droemann D, Albrecht D, Gerdes J, et al. Human lung cancer cells express functionally active Toll-like receptor 9. *Respir Res.* 2005;6:1.
- [15] Nicholas B, Staples KJ, Moese S, et al. A novel lung explant model for the ex vivo study of efficacy and mechanisms of anti-influenza drugs. *J Immunol.* 2015;194:6144–54.
- [16] Edwards CE, Tata A, Baric RS. Human lung organoids as a model for respiratory virus replication and countermeasure performance in human hosts. *Transl Res.* 2022;250:36–45.
- [17] Konishi S, Gotoh S, Tateishi K, et al. Directed induction of functional multi-ciliated cells in proximal airway epithelial spheroids from human pluripotent stem cells. *Stem Cell Rep.* 2016;6:18–25.
- [18] Gotoh S, Ito I, Nagasaki T, et al. Generation of alveolar epithelial spheroids via isolated progenitor cells from human pluripotent stem cells. *Stem Cell Rep.* 2014;3:394–403.
- [19] Tian L, Gao J, Garcia IM, et al. Human pluripotent stem cell-derived lung organoids: potential applications in development and disease modeling. *Wiley Interdiscip Rev Dev Biol.* 2021;10:e399.
- [20] Porotto M, Ferren M, Chen YW, et al. Authentic modeling of human respiratory virus infection in human pluripotent stem cell-derived lung organoids. *mBio.* 2019;10:e00723–19.
- [21] Hsia GSP, Esposito J, da Rocha LA, et al. Clinical application of human induced pluripotent stem cell-derived organoids as an alternative to organ transplantation. *Stem Cells Int.* 2021;2021:6632160.
- [22] Li Y, Wu Q, Sun X, et al. Organoids as a powerful model for respiratory diseases. *Stem Cells Int.* 2020;2020:5847876.
- [23] Rupp J, Droemann D, Goldmann T, et al. Alveolar epithelial cells type II are major target cells for *C. pneumoniae* in chronic but not in acute respiratory infection. *FEMS Immunol Med Microbiol.* 2004;41:197–203.
- [24] Droemann D, Rupp J, Goldmann T, et al. Disparate innate immune responses to persistent and acute *Chlamydia pneumoniae* infection in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2007;175:791–7.
- [25] Xu F, Droemann D, Rupp J, et al. Modulation of the inflammatory response to *Streptococcus pneumoniae* in a model of acute lung tissue infection. *Am J Respir Cell Mol Biol.* 2008;39:522–9.
- [26] Szymanski KV, Toennies M, Becher A, et al. *Streptococcus pneumoniae*-induced regulation of cyclooxygenase-2 in human lung tissue. *Eur Respir J.* 2012;40:1458–67.
- [27] Drömann D, Rupp J, Rohmann K, et al. The TGF- $\beta$ -pseudoreceptor BAMBI is strongly expressed in COPD lungs and regulated by non-typeable haemophilus influenzae. *Respir Res.* 2010;11:67.
- [28] Abdullah M, Kähler D, Vock C, et al. Pulmonary haptoglobin and CD163 are functional immunoregulatory elements in the human lung. *Respiration.* 2012;83:61–73.
- [29] Jäger J, Marwitz S, Tiefenau J, et al. Human lung tissue explants reveal novel interactions during *Legionella pneumophila* infections. *Infect Immun.* 2014;82:275–85.
- [30] Thompson MG, Shay DK, Zhou H, et al. Centers for disease control and prevention. *Morb Mortal Wkly Rep.* 2010;59:1057–62.
- [31] Wang J, Nikrad MP, Phang T, et al. Innate immune response to influenza A virus in differentiated human alveolar type II cells. *Am J Respir Cell Mol Biol.* 2011;45:582–91.
- [32] Checkley W, White AC Jr, Jaganath D, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. *Lancet Infect Dis.* 2015;15:85–94.
- [33] Mor SM, Ascolillo LR, Nakato R, et al. Expectorator of cryptosporidium parasites in sputum of human immunodeficiency virus-positive and -negative adults. *Am J Trop Med Hyg.* 2018;98:1086–90.
- [34] Heo I, Dutta D, Schaefer DA, et al. Modelling cryptosporidium infection in human small intestinal and lung organoids. *Nat Microbiol.* 2018;3:814–23.
- [35] Cheng VC, Lau SK, Woo PC, et al. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clin Microbiol Rev.* 2007;20:660–94.
- [36] Banerjee A, Nasir JA, Budyłowski P, et al. Isolation, sequence, infectivity, and replication kinetics of severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis.* 2020;26:2054–63.
- [37] Huang J, Hume AJ, Abo KM, et al. SARS-CoV-2 infection of pluripotent stem cell-derived human lung alveolar type 2 cells elicits a rapid epithelial-intrinsic inflammatory response. *Cell Stem Cell.* 2020;27:962–973.e7.
- [38] Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun.* 2020;11:1620.
- [39] Chu H, Chan JF, Yuen TT, et al. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. *Lancet Microbe.* 2020;1:e14–23.
- [40] Bojkova D, Klann K, Koch B, et al. Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nature.* 2020;583:469–72.
- [41] Sheahan TP, Sims AC, Zhou S, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci Transl Med.* 2020;12:eabb5883.
- [42] Schloer S, Treuherz D, Faist A, et al. 3D ex vivo tissue platforms to investigate the early phases of influenza A virus- and SARS-CoV-2-induced respiratory diseases. *Emerg Microbes Infect.* 2022;11:2160–75.
- [43] Alfí O, Yakirevitch A, Wald O, et al. Human nasal and lung tissues infected ex vivo with SARS-CoV-2 provide insights into differential tissue-specific and virus-specific innate immune responses in the upper and lower respiratory tract. *J Virol.* 2021;95:e0013021.
- [44] Hui KPY, Cheung MC, Perera RAPM, et al. Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *Lancet Respir Med.* 2020;8:687–95.
- [45] Chu H, Chan JF, Wang Y, et al. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. *Clin Infect Dis.* 2020;71:1400–9.
- [46] Zhang W, Wu X, Zhou H, et al. Clinical characteristics and infectivity of asymptomatic carriers of SARS-CoV-2 (Review). *Exp Ther Med.* 2021;21:115.
- [47] Galani IE, Triantafyllia V, Eleminiadou EE, et al. Interferon- $\lambda$  mediates non-redundant front-line antiviral protection against influenza virus infection without compromising host fitness. *Immunity.* 2017;46:875–890.e6.
- [48] Ceulemans LJ, Inci I, Van Raemdonck D. Lung donation after circulatory death. *Curr Opin Organ Transplant.* 2019;24:288–96.
- [49] Wei D, Gao F, Yang Z, et al. Ex vivo lung perfusion with perfusate purification for human donor lungs following prolonged cold storage. *Ann Transl Med.* 2020;8:38–38.
- [50] Moreno Garijo J, Roscoe A. Ex-vivo lung perfusion. *Curr Opin Anaesthesiol.* 2020;33:50–4.
- [51] Erasmus ME, van Raemdonck D, Akhtar MZ, et al. DCD lung donation: donor criteria, procedural criteria, pulmonary graft function validation, and preservation. *Transpl Int.* 2016;29:790–7.
- [52] Lang DS, Droemann D, Schultz H, et al. A novel human ex vivo model for the analysis of molecular events during lung cancer chemotherapy. *Respir Res.* 2007;8:43.
- [53] Lang DS, Marwitz S, Heilenkötter U, et al. Transforming growth factor- $\beta$  signaling leads to uPA/PAI-1 activation and metastasis: a study on human breast cancer tissues. *Pathol Oncol Res.* 2014;20:727–32.