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Mini Review

In Vitro and *In Vivo* Models for SARS-CoV-2 Research

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Abstract

The COVID-19 pandemic, caused by the coronavirus SARS-CoV-2, has created an upheaval in the world. Scientists are working to find a cure amidst the risk and shelter-in-place restrictions to find a solution for the disease. Researchers are racing to find the right model system for addressing the relevant questions. Appropriate research models can be critical to a quicker solution for the prevention and intervention of SARS-CoV-2. A brief analysis of the *In Vitro* and *In Vivo* models currently used for SARS-CoV-2 research is presented.

Keywords: *In Vitro Model; Organoid; Air Liquid Interface Culture; Animal Model; SARS-CoV-2; COVID-19*

Abbreviations

WHO: The World Health Organization; COVID-19: 2019-New Coronavirus Disease; SARS-CoV-2: Severe Acute Coronavirus Syndrome 2; ACE2: Angiotensin-Converting Enzyme 2; S Protein: Spike Protein; HBEC: Human Bronchial Epithelial Cells; ALI: Air Liquid Interface

Coronavirus disease 2019 (COVID-19) is a respiratory illness that emerged from the Chinese city of Wuhan in December 2019. The World Health Organization (WHO) called the disease '2019-new coronavirus disease' (COVID-19), and the novel coronavirus was scientifically termed as the "Severe Acute Coronavirus Syndrome 2 (SARS-CoV-2)" by the International Committee for Virus Taxonomy [1]. SARS-CoV-2 consists of a positive-sense single-stranded RNA virus, encoding four vital proteins: spike, membrane, nucleocapsid, and envelope. The spike (S) protein enables viral entry into the target cell via the angiotensin-converting enzyme 2 (ACE2) protein [2]. SARS-CoV-2 causes acute respiratory syndrome as well as other clinical symptoms consistent with previous coronaviruses but have generated a more severe pandemic with a higher risk to human lives and the global economy [3]. The reported critical clinical symptoms in COVID-19 patients include fever, dry cough, fatigue, sputum production, dyspnea, myalgia, sore throat, and chills. A limited percentage of patients experience symptoms associated with a gastrointestinal infection. Though several clinical trials are underway, concurrent basic research work on SARS-CoV-2 is essential for a thorough understanding of the pathophysiology and drug development. Emphasis is laid down on *in-vitro* and *in vivo* models that can efficiently reproduce a COVID-19 pathology.

The lungs are the first and the most severely affected organ by COVID-19. The last decade has seen the emergence of several *in vitro* and *in vivo* model systems to study and model lung diseases [4,5]. COVID19 has ushered a renewed interest in using lung and other organ-specific model systems to study the disease pathophysiology, molecular mechanism, and drug discovery. A discussion about the relevant cell lines, 3D models, and animal models for COVID-19 research is the need of the hour [6]. The human epithelial cells of the airways are infected as they highly express ACE2 (the SARS-CoV-2 entry receptor) and TMPRSS2 (S protein priming) needed for the viral entry [2]. Working with primary cells, especially with airway cells, poses several problems including, difficulty to culture, varied efficiency of

infection, finite life span, limited proliferation, and arduous process of obtaining normal samples. Therefore, for conducting SARS-CoV-2 experiments, different human cell lines like Calu-3 (non-small cell lung cancer), Caco-2 (colon adenocarcinoma), HEK293T (embryonic kidney), and Huh7 (hepatoma) are used by scientists [7]. However, these cell lines do not correctly imitate human SARS-CoV-2 infection but have provided useful knowledge about the virus. Interesting to note that SARS-CoV-2 infection is not efficient in the commonly used A549 lung adenocarcinoma cells. Vero E6 (monkey kidney epithelial) cells can be very efficiently infected and produce a high titer of viral particles and can be used to replicate and isolate the SARS-CoV-2 virus for experiments [8]. Vero cells express low levels of TMPRSS2 and complementation can increase the infection rate by greater than 100 times [9]. Untransformed/transformed primary human airway epithelial cells are commercially available. Human bronchial epithelial cells (HBEC) and their variants (immortalized by CDK4 and hTERT) exhibit a range of ACE2 expression and can be useful in mimicking SARS-CoV-2 infection and studying virus-specific immune response.

The advantages of a 3D culture system over 2D cell culture systems have created a spur in the use of 3D culture models [4]. Lung-specific 3D tissue culture models are advancing *in vitro* respiratory research specific to SARS-CoV2. Organoids are composed of multiple cell types and mimics miniaturized organ-like systems and can provide a window to study the SARS-CoV-2 infection process *in vitro*. Mini lung organoids, by virtue of self-replication, can offer high hopes for SARS-CoV-2-specific large-scale drug discovery and basic research. SARS-CoV-2 not only affects the lungs but also affects several organs, including: kidney, liver, cardiovascular system and an organoid-based approach can be useful. Several researchers have used the organoid-based approach to address the critical question in different human organ systems including: bronchial organoids, lung organoids, kidney organoids, liver ductal organoids and blood vessel organoids [6,10]. Most of the organoids are permissive to SARS-CoV-2 infection and have provided pathophysiological information. Another physiologically relevant model system for SARS-CoV-2 viral infection is the Air Liquid Interface (ALI) culture. ALI cultures preserve essential growth and differentiation characteristics of the *in vivo* airway epithelium and are easy to infect with viruses and have been used widely to study viral infectivity [11-13]. Human organ-on-a-chip can also be very useful in conducting similar studies.

Though *in vitro* models can be useful but to better understand the complex pathophysiology of systemic infection, *in vivo* models may be used to study inter-tissue and intra-tissue specific interactions. Different animal models are being used to study SARS-CoV-2 infection, conduct tests for therapeutic candidates and strategies [14]. Small animals can reproduce faster, easy to handle and the availability of mutants make it a favorite to study COVID-19 pathology. Experiments suggest that when ACE2 from different animals were expressed in Hela cells, SARS-CoV-2 was unable to bind to the mouse ACE2 protein. Transgenic mice expressing human ACE2 exhibited viral replication and developed interstitial pneumonia [15]. Syrian hamsters might be a good model based on S protein-ACE2 binding efficiency and the evolution of pulmonary complications after infection.

Among the larger animal models, the ferret model develops SARS-CoV-2 associated acute bronchiolitis in the lungs and mimic human pulmonary complications [16]. Interestingly, ferrets and cats show high SARS-CoV-2 infection and replication, but viral replication was inefficient in pigs, chickens, and ducks [14]. Ferrets can be considered as an ideal model system for COVID-19 studies [6,17]. The primate cynomolgus macaques are used for other coronavirus studies. Upon SARS-CoV-2 infection, the primate developed lung infection similar to humans, including pulmonary edema and inflammation, and can be used as a useful model system for COVID-19 studies. The much-used rhesus macaques have also played an essential role in COVID-19 studies for vaccine evaluation to drug efficacy testing [14,18]. A quick analysis of relevant experimental models can save much time and paving the path for newer model systems that can mimic patients with underlying conditions.

Conclusion

Though COVID-19 has caused approximately 15,406,225 infections and 630,000 death worldwide till now, we still do not have any effective medicines, vaccines, or prevention approaches to stop the infection and associated fatalities. Studying COVID-19 infection using a 2D culture system does not mimic and phenocopy the patient conditions and therefore both *in vitro* 3D culture systems as well as *in vivo*

models are critical for SARS-CoV-2 research. Organoid cultures provide a unique platform for studying pathogenesis and drug screening. Several preprints are showing interesting data using the novel 3D *in vitro* culture systems. At the same time, animal models like ferrets, cynomolgus macaques and rhesus macaques are quite beneficial for SARS-CoV-2 research. Although our hopes are pinned for a quick vaccine, appropriate *in vitro* and *in vivo* research models can accelerate our knowledge base, thereby aid in planning better therapeutic strategies with appropriate safety considerations for the prevention and intervention of SARS-CoV-2.

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Conflict of Interest

No financial interest or any conflict of interest exists.

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