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Review Article

Cell culture models for SARS-CoV-2 infectivity and systemic complications

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ABSTRACT

COVID-19 was declared by WHO as a pandemic since March 2020. The vaccination program has been implemented worldwide. Specific antiviral drugs such as remdesivir, molnupiravir and ritonavir-based nirmatrelvir were effective against SARS-CoV-2 infection. However, the new SARS-CoV-2 variants have been elevated due to viral mutation causing vaccine resistance and rapid spreading. Long-term COVID-19 complications have been life-threatening in some recovery cases. To overcome viral adaptation, cell culture model is essential to comprehend SARS-CoV-2 infection, pathophysiology, complications, and drug target alterations. The classical 2D culture cell was frequency used for viral propagation and high-throughput screening. Modern 3D culture has recapitulated key cellular and molecular events of tissue physiology. Here, we reviewed the cell lines, 3D culture, organoid and relevant models for the aforementioned applications.

Keywords:
SARS-CoV-2, Cell culture model, COVID-19, 3D culture, Organoid

1. INTRODUCTION

Infectious diseases have evolutionally challenged human with annual mortality over 10 million individuals worldwide. They eluded all developed prevention and control strategies. New pathogens (e.g., H7N9 influenza virus in 2013, H5N1, H1N1, Zika virus in 2016 and Chikungunya in 2019 were continuously added into the endemic/epidemic list[1]. Recently, we have been threatened with a pandemic of SARS-CoV-2, a new strain of Coronavirus that was sequenced in early 2020 in Wuhan, Hubei, China. The first case was identified in December 2019. Since then, the number of patients and deaths due to the disease, termed Coronavirus Disease 2019 (COVID-19) have been increasing and spreading across the globe rapidly[2]. In January 2020, the World Health Organization (WHO) had declared the SARS-CoV-2 epidemic a global health emergency. In March 2020, the WHO had officially declared the outbreak a pandemic. The pandemic of COVID-19 has been an emergent global health crisis that requires urgent solutions. The total confirmed cases have approached 600 million with more than six million deaths globally by the end of May 2022. The SARS-CoV-2 pandemic created various issues on healthcare systems worldwide, overwhelming hospital intensive care unit (ICUs) with COVID-19 patients lacking mechanical ventilation to handle acute lung injury (ALI) in acute respiratory distress syndrome (ARDS)[3-4]. Clinical manifestations of COVID-19 were partly understood. The clinical outcomes ranged from asymptomatic, mild, moderate, severe, to critically severe. Common symptoms included cough, fever, dyspnea, muscle pain, sputum production and sore throat. Even though most patients exhibited mild symptoms, some COVID-19 patients suffered from organ damage, e.g., acute kidney, cardiac and liver dysfunction [5-6]. Patients with cardiovascular diseases, hypertension and respiratory tract diseases were more frequently associated with ICU admission with poor outcomes[5]. The mortality rate per number of diagnosed cases was roughly 3.4% depending on age and health conditions[7].

Recently, WHO has listed omicron (B.1.1.529) as a variant of concern (VOC) since November 2021. This

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variant contained many mutations that resulted in increasing transmissibility and vaccination resistance[8]. Besides, the long-term complications to major organs (e.g., lung, heart, kidney and brain) persisted[9]. To overcome these associated morbidities, the detailed pathobiological study of the infection was essential. This would require a robust infection and pathogenesis model[10]. Classical 2D culture could supported SARS-CoV-2 propagation and infection. The 2D culture based on Vero E6, Calu-3 cell and other cell lines were applied for anti-SARS-CoV-2 drug screening[11-12]. Vero E6 cell were isolated from kidney epithelial cell of African green monkey and Calu-3 cell were isolated from human non-small cell lung cancer[13]. In addition, the modern 3D cultures, e.g., air-liquid interface (ALI), and lung organoids were employed to investigate viral kinetics and lung integrity. ACE2 receptor and TMPRESS have been identified as SARS-CoV-2 entry receptor[14]. Long-term SARS-CoV-2 infection with systemic complications was found in cells highly expressing ACE2 and TMPRESS2. These cells were found in respiratory, gastrointestinal, cardiovascular, renal, ocular and hepatic systems[15]. The primary explants or stem cell derived organoids have provided evidence for prevention and treatment of long-term COVID-19 complications[16-17].

Base on 2D culture, Vero E6 and Calu-3 have been recommended for hosting SAR-CoV-2 and the screening for anti-viral compounds. The 3D lung epithelial culture is essential for the study of viral kinetics and airway pathology. The organoids derived from pluripotent stem cell (PSC) are suitable for the study of systemic complications and long-term effects of Covid-19. The advance of cell culture models would deepen our knowledge of COVID-19 pathogenesis and provide a platform for exploring prophylactic and therapeutic strategies. In this review, we summarized and discussed the currently available cell culture models for studying SARS-CoV-2 infection and pathogenesis. We also discussed the cell culture models derived from the major organs affected by SARS-CoV-2 infection.

2. SARS-CoV-2 LIFE CYCLE AND HOST CELL ENTRY

The entry of the virion to the host cell was initiated by the interaction between the spike glycoprotein (S) and the host receptor. This interaction indicated the diversity of host species and tissue tropism. SARS-CoV-2 used the S protein to bind the receptor to mediate host cell entry. The first identified receptor was angiotensin-converting enzyme 2 (ACE2)[18], a membrane-localized aminopeptidase highly expressed in the lungs, kidneys, blood vessels and heart. ACE2 has a crucial role in the cardiovascular and immune systems[19]. The spike protein was cleaved by furin at the S1/S2 cleavage site before attachment and binding to ACE2 receptor on cell surface that cooperated with host factors cell surface-associated transmembrane protein protease serine 2 (TMPRSS2). During the binding, the S protein was cleaved by TMPRESS2, a step recognized as S-protein initializing. TMPRSS2 cleavages the trimer protein on receptor-binding domain together with cathepsin promoting viral entry through endosomal transport. This mechanism was supposed to be the main route of SARS-CoV-2 infection via respiratory tract epithelial cells (Figure 1).

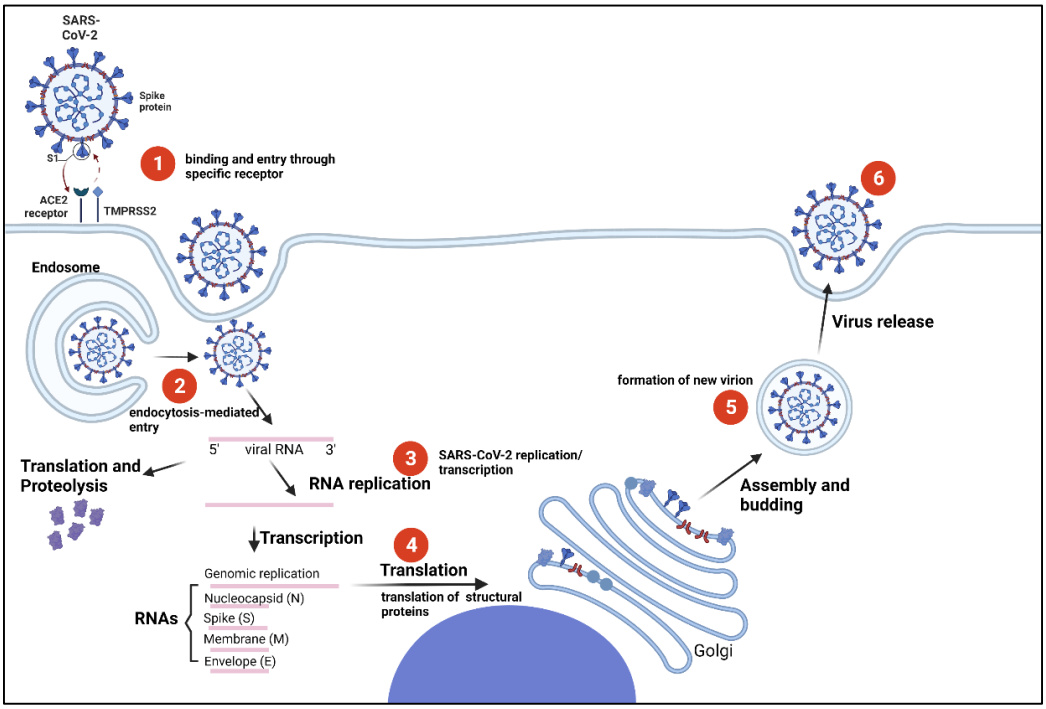

Figure 1. The SARS-CoV-2 life cycle. Viral particle bound and entered cells through an ACE2 receptor (1) and was internalized via endosome transport (2). Viral genomic RNA was released and acted as a template for negative (-) stand RNA (3) and translated into viral proteins (4). Viral genome was assembled, encapsulated with structural proteins (5) and released (6). This figure was created with BioRender.com.

After viral entry, the viral particle uncovered genomic RNA. ORF1a and ORF1ab genes were translated into pp1a and pp1ab polyproteins cleavable by viral protease (main protease: M^{pro}). Full-length negative (-) stand RNA would be synthesized and served as a template for producing positive (+) stand RNA (the viral genome). During viral replication, a nested set of sub-genomic RNAs (sgRNAs) were translated to viral structural proteins on endoplasmic reticulum (ER) membrane. The viral genome was assembled and encapsulated with structural proteins on endoplasmic reticulum-Golgi intermediate compartment (ERGIC), transferred to Golgi vesicle, and released outside the cells as a complete virion for the next round of infection[20].

3. PROPAGATION AND CELL CULTURE MODELS FOR SARS-CoV-2

The 2D or 3D culture had its strengths and limitations for SARS-CoV-2 applications (Table 1 and Figure 2). Cell culture models bypassed the use of animal, yet still provided sufficient data for SARS-CoV-2 infectivity and antiviral screening. These models would reproducibly identify cellular targets that could not be achieved in animal model. However, cell models could not entirely imitate the physiology of whole organism[21].

3.1. Classical 2D culture of continuous cell lines

Monolayer culture of continuous cell lines has been commonly used for isolating SARS-CoV-2 from clinical specimen.

Vero cell was the most widely used host for vaccine production and SARS-CoV-2 isolation owing to its natively high ACE2 expression. The cell line was established in 1962 from African green monkeys. Vero E6 or Vero C1008 cell line was superior to the original Vero cells due to better conserved primary cell characteristics including contact inhibition. Several research groups used Vero and Vero E6 cells for isolation, infection, pathogenesis and viral susceptibility[14,22]. Vero and Vero E6 cells were employed in the investigations on immune response, antibody screening, vaccine production, the reversal of virus-induced gene expression. Hydroxychloroquine, nelfinavir, lopinavir, emetine and remdesivir exhibited efficacy against SARS-CoV-2 in Vero and Vero E6 cells. Beside, Vero and Vero E6 express TMPRESS2 that aided in the isolation of SARS-CoV-2[22]. However, the anti-SARS-CoV-2 activity based on Vero and Vero E6 should be confirmed in human or other animal models due to the inter-species variations.

Huh-7, a hepatocellular carcinoma cell line, came from a liver tumor in a 57-year-old male patient in 1982.

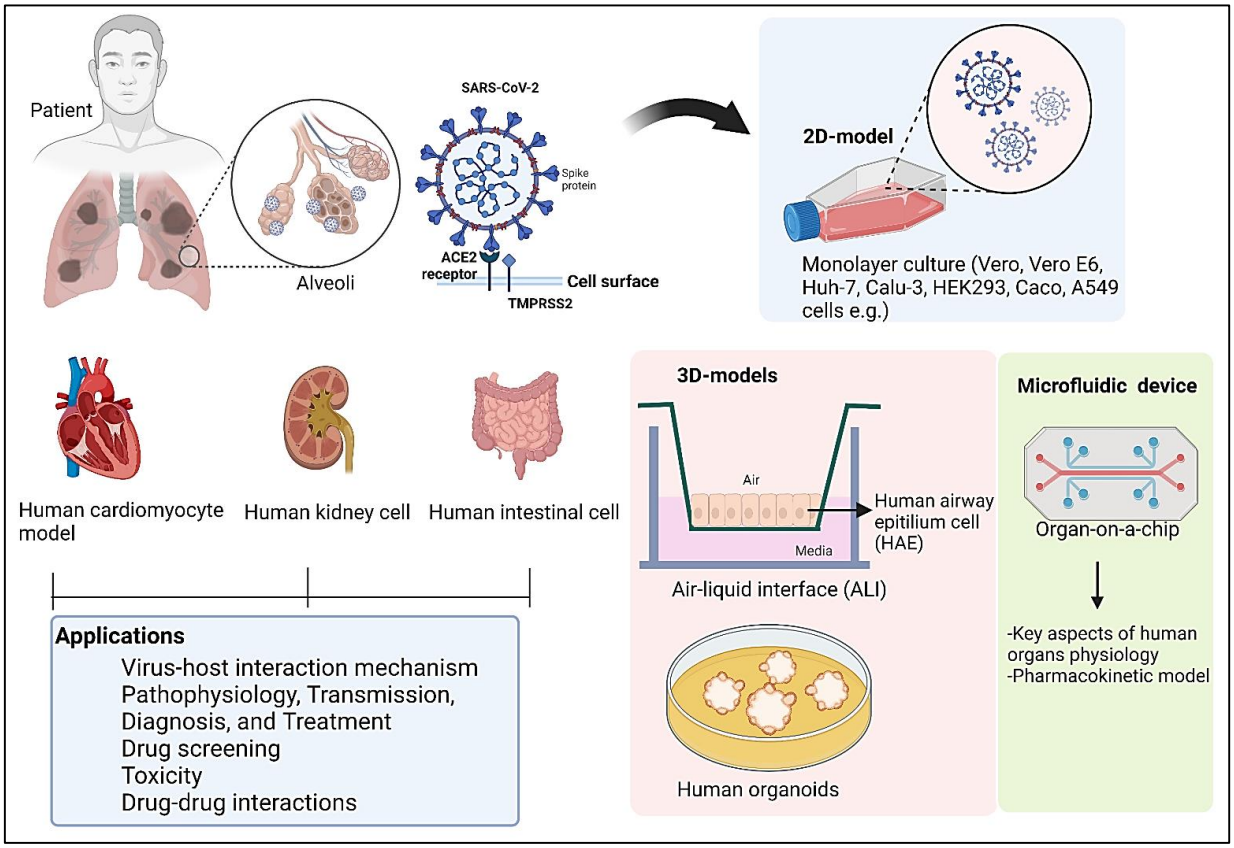

Figure 2. The models for SARS-CoV-2 study. Classical 2D culture was performed using Vero derivative and Calu-3 for viral propagation, infectivity, and anti-viral screening. 3D cultures, human organoids and organ-on-chip were applied for cellular pathophysiology and viral kinetic studies. The systemic complications were investigated in 3 major organs, i.e., heart, kidney, and GI. *In vitro*, *ex vivo* and *in vivo* models previously reported were organized based on their complexity. Advantages and disadvantages of each model were summarized in the table below. This figure was created with BioRender.com.

Table 1. Cell culture models for severe acute respiratory syndrome and systemic complications of SARS-CoV-2.

Cellular models	Cell origins	Key points	SARS-CoV-2 strains	Disease models /applications	References
2D-models					
Vero WT and Vero E6	Monkey kidney	Widely used for reproducing viral replication and viral isolation, high viral copies in culture medium, SARS-CoV-2 progeny from Vero cell. Spike deletion affect transmission and pathogenicity, lack of type I interferon cytokines response	BetaCoV/Korea/SNU01/2020; 2019-nCoV BetaCoV/Wuhan/WIV04/2019; SARS-CoV-2/USA-WA1/2020; Australia/VIC01/2020 C-Tan-nCoV Wuhan strain 01 SARS-CoV-2/01/human/ Jan2020/Thailand	Virus isolation of different SARS-CoV-2 strains, propagation, Vaccine production, Antiviral screening, SARS-CoV-2-host response	11,33, 72-80
Calu-3	Human lung	Use as airway epithelial models to observe cytopathic effect and antiviral screening, SARS-CoV-2 progeny. Produce the authentic human virion, high copies of SARS-CoV-2 pseudovirions.	SARS-CoV-2/USA-WA1/2020 SARS-CoV-2/01/human/ Jan2020/Thailand	Antiviral screening, Toxicity tests	12,77, 80-81
Caco-2	Human intestine	Use as infected intestine models for investigating extrapulmonary symptoms. Low viral copies in cell and culture medium, no clear cytopathic effect	BetaCoV/Hong Kong/VM20001061/2020	Antiviral screening, Toxicity tests	12,82-83
Huh-7	Human liver	Model for study of SARS-CoV-2 against type-I interferon signatures	2019-nCoV BetaCoV/Wuhan/ WIV04/2019; SARS-CoV-2/USA-WA1/2020	Antiviral screening, Toxicity tests	12,24-25, 77
HEK293, 293T cells, and other HEK293 derivatives	Human kidney	Rapidly grow, wildly used in cell-base assay for screening targeted drug		Viral isolation, Transmission, Vaccine production, Antiviral screening	13,84
A549 cell	Human alveolar basal epithelial carcinoma tissue	Susceptible to respiratory viral infection		Transmission, Vaccine production, Screening of anti SARS-CoV-2 targets	85
Human nasal epithelial cells (HNE), Large airway respiratory Cells (LAE), Small airway respiratory Cells (SAE), AT1/AT2 alveoli epithelial cells and human ciliated airway epithelial (HAE) cells	Human airway cells	Use as infected human respiratory model due to susceptible to respiratory viral infection	SARS-CoV-2/USA-WA1/2020	SARS-CoV-2-host response	24,33,36, 86
hPSC-derived cardiomyocytes (CMs)	Human cardiomyocyte	Use as human cardiomyocytes models	SARS-CoV-2/USA-WA1/2020	SARS-CoV-2-host response	87-88
3D-models					
Human lung organoids	Human lung		SARS-CoV-2/USA-WA1/2020	Identification of potential	71,89

Table 1. Cell culture models for severe acute respiratory syndrome and systemic complications of SARS-CoV-2. (cont.)

Cellular models	Cell origins	Key points	SARS-CoV-2 strains	Disease models /applications	References
3D-models Human lung organoids	Human lung		SARS-CoV-2/USA-WA1/2020	COVID-19 therapeutics	71,89
Human kidney organoids	Human kidney		SARS-CoV2/human/SWE/01/ 2020	Identification of potential COVID-19 therapeutics	90
Human liver organoids	Human liver		SARS-CoV-2/USA-WA1/2020	SARS-CoV-2-host response	91
Human intestinal organoids	Human Intestine		SARS-CoV-2/USA-WA1/2020	Identification of potential COVID-19 therapeutics	71
Blood vessels organoids	Cardiovascular		SARS-CoV2/human/SWE/01/ 2020	Identification of potential COVID-19 therapeutics	90

Huh-7 was well-differentiated and capable of hosting hepatitis C virus[23]. Huh-7 cells were used to isolate the virus from the first Wuhan patients diagnosed with COVID-19[24]. Infected cells exhibited cytopathic effect after infection by 6 days (dpi). The effect of SARS-CoV-2 on type I interferon (IFN-I) signaling in this cell was investigated. The virus could inhibit RIG-I mediated IFN-β production[25].

The important kidney-specific cell lines that have been used for SARS-CoV-2 studies include HEK293. This cell has been used for vaccine production, the studies for pathogenesis and transmission. 293T, a derived HEK293 cell line has been used for various studies (e.g., antiviral CRISPR, virus isolation, viral pathogenesis/ transmission, vaccine production, etc.). 293FT, another HEK293 derivative, has been use for screening dihydro-orotate dehydrogenase (DHODH) and mAbs production [26]. 293T-ACE2 cell line has been used for vaccine production, antibody response and viral pathogenesis. Other kidney cell lines used for SARS-CoV-2 study included: BHK-21 cell line for mAb screening; COS-7 cell used for antibody production; LLC-MK-2 for viral susceptibility; MDCKII cell for screening DHODH Inhibitors toward viral susceptibility, pathogenesis and transmission[14]; PaKi cell for methylenetetrahydrofolate dehydrogenase 1 (MTHFD1); HK-2/NRK-49F cells for remdesivir screening.

Calu-3, a lung adenocarcinoma epithelial cell, could host SARS-CoV-2. Calu-3 2B4, a Calu-3 derived cell line, carried higher ACE2 expression than did Calu-3. The 2B4 clone has been used for investigating viral pathogenesis, transmission and drug screening. IB3-1 and its derivative S9 cells were used for screening azithromycin and ciprofloxacin. KMB17 cell was used for vaccine production. Hep-2 cells carried ACE2 and could host CoVs.

Caco-2, a colorectal adenocarcinoma cell, was used for isolating SARS-CoV-2 from infected patient in Germany. The SARS-CoV-2 infectivity and cytokine production were evaluated in Caco-2 cell infected with SARS-CoV, SARS-CoV-2, MERS-CoV, H1N1pdm or H5N1 viruses. Caco-2 could host all these viruses. The pro-inflammatory response to SARS-CoV-2 in this cell was low and therefore unsuitable for anti-viral immunologic study. During the search for therapeutic targets, the proteomic analysis revealed that Caco-2 cell changed their metabolism during viral replication[27].

Human lung epithelial carcinoma cells e.g., A549, NCI-H1299 and BEAS-2B expressed ACE2, a prerequisite for SARS-CoV-2 entry that required for pathogenesis, transmission, and vaccine production. A549 cells lacked TMPRSS2, a prerequisite for S protein processing and viral spread and exhibited low expression levels of ACE2 in conventional submerged culture. However, ACE2 and TMPRSS2 levels in A549 could increase in air-liquid interface (ALI) culture. A549 cell was used as a model to screen for host factors that inhibited viral replication. The CRISPR screening for host genes that could suppress viral replication led to the discovery of antiviral targets[22].

The primary target sites of SARS-CoV-2 infection were cells in the respiratory tract, i.e., nasal and lung epithelial cells. Cellular models are essential to clarify the infection process, growth kinetics and tropism. Despite primary human lung epithelium was considered the gold standard for SARS-CoV-2 infection, classical cell lines have typically been employed for viral isolation, propagation and the screening for anti-SARS-CoV-2 agents. The enlisted classical cell lines included Vero E6, Calu-3 and A549. Vero E6 was used in high-content screening of 122 Thai medicinal plants that resulted in the discovery of *Boesenbergia rotunda* extract and its component

panduratin A contained anti-SARS-CoV-2 activity[11]. Although immortalized and cancerous cell lines had advantages over primary cell due to their reproducibility and simplicity, they still had some limitations. The results based on Vero E6 must be further validated in human lung or primary human airway cells. For instance, the screening for anti-SARS-CoV-2 agents from FDA-approved drugs using Vero E6 found that chloroquine and hydroxychloroquine (HCQ) could inhibit SARS-CoV-2. Numerous subsequent studies to explore the antiviral efficacy of HCQ were initiated in animal models and patients. Unfortunately, HCQ had no anti-SARS-CoV-2 activity in human airway epithelial cell, no prophylactic or therapeutic effects in hamsters and non-human primates[28]. In a double-blinded placebo-controlled trial, the participants receiving HCQ as a post-exposure prophylaxis demonstrated no advantage[29]. In another randomized, open-label, three-group controlled trial, patients received either HCQ alone or in combination with azithromycin had no clinical improvement[30]. Taken together, these trials verified that results from *in vitro* models should be confirmed in relevant humanized models.

3.2. Modern 3D cultures and organoids

Organoids are categorized as 3D culture, recapitulating native organs to a complex structure. These tissues are derived from various types of stem cells using self-organization mimicking organ development. Organoids have advantages over conventional 2D culture or animal models because they resemble human organs while providing *in vitro* platform for studying viral pathophysiology[31].

Similar to MERS-CoV, the primary target of SARS-CoV-2 is human airway epithelium (HAE) (Figure 2). These airway cells compose of nasal epithelial cell, large airway epithelial cell, small airway epithelial cell and AT1/AT2 alveoli epithelial cells. HAE culture simulated the human bronchial environment. After isolation, the tissues were maintained and differentiated on a porous membrane at air-liquid interface (ALI) where the apical part exposed to the air. HAE culture recapitulated a complex tissue with well-differentiated architecture such as ciliated, goblet and basal cells in physiological conditions[32]. The infectivity and replication of SARS-CoV-2 have been studied in HAE cell. ACE2 levels and viral titers were varied between individual cells[33-34]. The SARS-CoV-2 entry was mediated through ACE2 together with TMPRSS2 as a co-receptor. In contrary, MERS-CoV entry was mediated through dipeptidyl 4 (DPP4), not ACE2. HAEs from proximal airway were more susceptible to infection than were those from distal airway. Other airway epithelial cells (nasal goblet secretory cell, lung type II pneumocytes and enterocytes) highly expressing both ACE2 and TMPRSS2 were susceptible to SARS-CoV-2 infection. A parallel study also revealed that human nasal epithelial, large airway epithelium (bronchi), lower airway epithelium (bronchiolar and type I, type II pneumocytes) were susceptible to SARS-CoV-2[35]. However, these cells exhibited either low viral titers, or no viral infection[36]. In addition, two types of HAE cells (ciliated and secretory cells) were permissive to SARS-CoV-2. Both SARS-CoV and SARS-CoV-2 could infect HAE cell, tracheobronchial epithelial cell, and type II pneumocyte. MERS-CoV, SARS-CoV and SARS-CoV-2 shared common host cells (i.e., type II pneumocyte, and human airway epithelial cells).

The pluripotent stem cell derived-type II alveolar epithelial cell (iAT2) based on ALI platform was developed to identify cellular response to SARS-CoV-2 infection. The expressions of ACE2 and TMPRESS2, the essential molecules for SARS-CoV-2 entry[37], in iAT2 implied its viral susceptibility[38]. Using modern 3D culture, the cells could develop into lung organoid. Human lung organoid is an exciting alternative model for SARS-CoV-2 infection. Viral pathogenesis and pulmonary fibrosis have been investigated using this model. Although long-term expansion of pseudostratified airway organoids has been attempted, the presence of the inner lumen (facing inwards) made stimulation and sample collection difficult. As an alternative to this system, the air-liquid interface (ALI) model has been considered. The media could be removed from the apical side of the filter in ALI models because iPSC or lung epithelial cells were expanded and merged into the filter. The ALI model contained the apical and the basal chambers that were versatile for different treatments as well as for sample collection. The epithelial integrity, mucociliary clearance, and cilia beat frequency could be investigated with ALI model. The proinflammatory response of epithelial cells toward SARS-CoV-2 as well as the remdesivir efficacy were investigated.

The 3D-cell culture models in SARS-CoV-2 were increasingly applied to mimic physiological condition. Primary explants from tissue or organoids derived from pluripotent cell were considered a promising model for respiratory viral infections[39]. Data acquired through the 3D models have been essential for screening novel therapeutic targets. Patient derived primary explants had more advantages over organoids because they recapitulated tissue architecture and complexity. However, organoids, rather than patient-derived explants, were relatively easy to reproduce and standardize.

4. CELLULAR MODELS FOR SYSTEMIC COMPLICATIONS OF SARS-CoV-2

Systemic complications have been intimidating menaces in COVID-19 infection. The complications could involve several organ systems, e.g., respiratory, gastro-

intestinal, cardiovascular, kidney, ocular, and hepatic[40]. The prediction of possible SARS-CoV-2 systemic complications in candidate organs was alluded to the level ACE2 expression. At least 14 cell types, including alveolar type 2 cell, cholangiocyte, colonocyte, esophageal keratinocyte, ileum and rectum endothelial cell, stomach epithelial cell, renal proximal tubules and cardiomyocyte highly expressed ACE2[41]. These cells were susceptible to infection and complications. These studies with renal cell, cardiomyocyte and gastrointestinal cell as complication models were summarized below.

4.1. Human renal cell models

Kidney illness has been associated with a poorer COVID-19 infection outcome, which was attributed to several factors including acute respiratory distress syndrome and direct viral invasion[42]. A study from March 10 to July 31 of 2020 showed 78% of kidney impairment during ICU hospitalization for COVID-19[43]. This data indicated that the kidney is a primary target of COVID-19. The kidney contained the highest level of both ACE2 and TMPRSS2 expressions in the body and therefore served as a major viral target. Tubular epithelial cells and podocytes in the kidney were enriched with ACE2 and TMPRSS2. During the infection, the internalization of ACE2 led to enhancing Ang II signaling pathway followed by pro-inflammatory and profibrotic processes in the kidney. The severe multiple organ failure as a result of SARS-CoV-2 causes has been partially elucidated. Acute kidney injury (AKI) was the second leading organ damage in COVID-19 patients after the respiratory failure[44]. About 3-10% of COVID-19 patients were inflicted with AKI. This was consistent with the detection of viral RNA in urine samples of COVID-19 cases[45]. AKI could be a result of either virus-induced cytopathic effect or immunopathogenic damage. Evidences from single-cell RNA sequencing revealed that ACE2 expressed in proximal convoluted tubules in kidney[46]. These evidences indicated that renal tubule cells could be infected by SARS-CoV-2. The proximal tubule epithelial cell played key roles in various functions of human kidney such as filtering blood and resorption of crucial end-products from metabolism including sodium and proteins. These cells were recommended for investigating the pathophysiological complications induced by the infection. Nevertheless, primary kidney epithelial cell line had limited lifespan and therefore carried batch to batch variation. To overcome this limitation, the immortalized of kidney cells were developed using viral oncogenes HPV16 E6/E7, SV40 or hTERT[47]. However, these immortalized kidney epithelial cell lines lost differentiation capacity and could not mimic normal physiological function. Recently, human epithelial cells were developed using conditional reprogramming technique (CR) without introducing ectopic genes. CR cells sustained self-renewal and differentiation capability[48]. Long-term human kidney proximal tubule epithelial cells (KPTECs) were developed using condition reprogramming and 3D organoids. These cells expressed ACE2 and specific transporter SLC34A3 that allowed SARS-CoV-2 infection while retaining kidney functions[49].

4.2. Human cardiomyocyte models

The systemic complication of the cardiovascular system has been reported[50]. The symptoms including minor chest pain, palpitation, arrhythmia, cardiogenic shock, and fulminant heart failure. Approximately 20-40% of hospitalized patients suffered with a wide range of symptoms associated with heart damage. The abnormal host immune response mediated by natural killer cells, macrophages, and T-lymphocytes could be triggered by the systemic hyper-inflammatory state. SARS-CoV-2 could be detected in vascular endothelial cells of endotheliitis patients. The virus was detectable in cardiac macrophages in those with cardiogenic shock and myocarditis. Viral RNA could be isolated from virtually the entire heart of COVID-19 individuals, independent of cardiac phenotype[51]. ACE2 was strongly expressed by numerous cardiac cell types, including pericytes, cardiomyocytes, and endothelial cells. COVID-19 patients who developed cardiovascular complications have associated with age, gender and obesity. The severity depended on pre-existing diseases, e.g., cancer, chronic pulmonary disease, diabetes mellitus, hypertension, and cardiovascular disease. While the major pathologic target was the respiratory system, COVID-19 also inflicted cardiovascular complications, e.g., acute coronary syndrome, myocardial injury, and arrhythmia. About 20-30% of COVID-19 patients developed severe cardiovascular damage that resulted in poor prognosis[3]. Troponin I expression, a biomarker of myocardial infarction, has been widely used to predict myocardial infarction in COVID-19 patients.

To simulate the cardiovascular complication, an ideal human heart cell model would be investigated for the interaction with the virus and the immune system. This would solve the following questions: (1) which cell types in the heart was infectable and how these cells responded to the virus; (2) how the nature of inflammatory response was; and (3) how the infection could lead to myocardial infarctions. The primary heart tissues have remained a gold standard for drug discovery. The handling of the primary heart tissues was limited by the availability of cell sources and batch-to-batch variation. Immortalized human cell lines or human pluripotent stem cell (hPSCs)-derived cardiomyocytes could serve as alternative models for these studies. Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) were differentiated into different cardiac-derived cell types, e.g., endothelial cells (ECs), cardio-

myocytes (CMs), smooth muscle cells (SMCs), cardiac fibroblasts (CFs), and pericytes[52]. Currently established protocols provided the cell sources for various applications, ranging from cardiovascular development to disease modulations in new drug development[53]. These models would serve as platforms to investigate virus-host interactions that led to long-term cardiovascular complications. Cardiotoxicity could be examined using 2D and 3D cultures of cardiomyocyte derived from hPSCs (hPSC-CMs), heart-on-chip models, engineered heart tissues, and heart organoid[54].

Although ACE2 was highly expressed in human CMs and pericytes, transmembrane serine protease (TMPRESS2), which facilitated the entry of SARS-CoV-2, was undetectable in hPSC-CMs. This finding raised the possibility that other mechanisms could facilitate viral entry of SARS-CoV-2 into human heart. A potential alternative mechanism for viral entry in hPSC-CMs was through cathepsin-L-mediated endolysosome[55]. This alternative route of SARS-CoV-2 entry into CMs warrants further investigation. The detailed mechanism would aid in novel therapeutic strategies that would prevent or cure cardiovascular complications. Several reports indicated that SARS-CoV-2 infection of hPSC-CMs initiated morphological and functional aberrations to infected cell. Infected CMs with a high MOI of virus resulted in loss of beating and increasing apoptosis. The arrhythmic contraction was demonstrated in 2D hPSC-CMs monolayers[56]. Besides, the infection of hPSC-CMs with low MOI triggered nuclear DNA loss and sarcomeric fragmentation. Neuropilin-1, a transmembrane protein expressed in the cardiovascular system, was identified as a co-receptor for SARS-CoV-2. Neuropilin-1 involved in angiogenesis, cell survival, migration and invasion. The transcriptomic data revealed that this gene was expressed in hiPSC-derived cardiac ECs and CFs[57]. Neuropilin-1 expression could be investigated for viral interactions and cellular responses.

Clinical studies confirmed that SARS-CoV-2 could damage endothelial cells[58]. However, endothelial cell derived from hiPSC (hiPSC-ECs), although expressing ACE2, was not susceptible to SARS-CoV-2 infection[55]. In contrary, hiPSC-derived blood vessel organoids were infectable. Since ECs could not susceptible to SARS-CoV-2 infection, endothelial cell injury in COVID-19 patients might not be directly damaged by the virus, but indirectly by immunological reaction[59]. Cardiac fibroblasts (CFs) representing 15% in heart involved in cardiac injury. The hiPSC-ECs cannot host SARS-CoV2 infection because ACE2 was not expressed in this cell. However, the toxicity was detected in ECs after viral exposure and raised the possibility that heterogeneous cell culture was required for viral pathologic study. Mural cells, pericytes and smooth muscle cells expressed ACE2. However, the effect of SARS-CoV-2 for these cell types is under investigation. Therefore, hPSC-derived cardiovascular cell is a promising *in vitro* platform to investigate cardiovascular damage caused by SARS-CoV-2 infection. As heart failure led to 20-30% of COVID-19 patients developed severe cardiovascular damage, these models could unveil viral pathology associated with cardiovascular complications.

4.3. Gastrointestinal cell models

Gastrointestinal symptoms were manifested in 20-50% of COVID-19 patients. These include diarrhea, abdominal pain, hematochezia and intestinal perforation [60]. Clinical evidence revealed that the intestine was a major organ for SARS-CoV-2 infection besides lung, kidney and heart. Clinical manifestations could appear before the onset of respiratory signs. SARS-CoV-2 could lead to acute hemorrhagic colitis. ACE2 and TMPRSS2 were co-expressed in esophageal, upper epithelial, and gland cells, absorptive enterocytes from the ileum and colon. Viral entry into these cells would give rise to GI symptoms early in COVID-19 infection. Viral pathogenesis has been studied extensively using intestinal organoids derived from both mouse and human tissues. Adult tissue stem cells and induced pluripotent stem cells were used to create human organoids. The presence of high ACE2 level and viral RNA in anal swabs, stool, and sewers suggested that the intestinal epithelium was susceptible to significant COVID-19 infection. The most common targeted cell of the intestine was the enterocyte [61]. The study using human multipotent adult tissue stem cell-derived intestinal organoids, implied that the intestinal epithelium was one of the highest infection locations for SARS-CoV-2. Intestinal tissue composed of both dormant and active stem cells[62]. To develop a potential therapeutic target, the ACE2 expression level in these two types of stem cells, as well as their susceptibility to COVID-19 infection, would be investigated. Since beta-catenin signaling was involved in viral propagation inhibition, the role of Wnt/beta-catenin signaling in response to COVID-19 infection in intestinal stem cells warranted further investigation. The histopathological examination of biopsy specimens displayed intestinal edema, many plasma cells and lymphocytes infiltrated into the lamina propria of duodenum, stomach and rectum. The gastrointestinal tract was one of several routes for SARS-CoV-2 infection[63]. The viral RNA detected in the stool samples and the virions found in the biopsy of rectal tissue using electron microscope indicated that SARS-CoV-2 was feasibly transmitted by the fecal to oral route[64]. Clinical data indicated that the intestine was another target organ for SARS-CoV-2 infection apart from the lung, kidney, and heart, but the pathogenesis of the intestinal infection in COVID-19 was not elucidate. The cell culture model that precisely imitates human intestine responding to the virus is required. Human intestine comprised intricate multi-

cellular components and host-virus interaction in a physiological microenvironment. SARS-CoV-2 infection in the intestine has been examined based on 2D cultures of intestinal epithelial cells[65] and 3D culture as human organoids. The *ex vivo* human intestinal tissues isolated from patients were infected with coronaviruses. The results revealed that SARS-CoV-2, replicated less efficiently than SARS-CoV, induced less cytopathology, and induced more innate immune response by activating both type I and type III interferons than did SARS-CoV in human intestinal tissues[66]. A transiently transfected Caco-2 sub-clone (C2BBe1) with hACE2 or hTMPRSS2 expressing plasmids increased SARS-CoV-2 infection and propagation. During enterocytic differentiation, ACE2 expression was significantly increased, but not TMPRSS2. This intestinal brush border cells were applicable for investigating early SARS-CoV-2 infection[67]. Organ-on-chip culture were developed to reproduce the complex architectures and physiological functions of human organs in an engineered microfluidic device (Figure 2). The engineered gut-on-chip device was developed using ECM-coated porous polydimethylsiloxane (PDMS). The device composed of a human intestinal epithelial layer and an endothelial cell layer derived from human colon adenocarcinoma (Caco-2) cells, human colorectal adenocarcinoma grade II (HT-29) cell and human umbilical vein endothelial cell (HUVECs). Gut-on-chip system was susceptible to SARS-CoV-2 infection, produced obvious morphological alterations with injury of intestinal villi, increased mucus-secreting cells and decreased expression of tight junction (E-cadherin). The destruction of intestinal barrier integrity in 3D structure was observed[68].

Due to the 2D cultures lacked cell heterogeneity and tissue architecture, intestinal organoid or 3D culture could serve as alternative models for SARS-CoV-2 infection. Recent studies investigated SARS-CoV-2 infection and replication in epithelial cells of human colon-derived intestinal organoids and human and bat small intestinal organoids[69]. Organotypic cultures derived from pluripotent stem cell or adult tissue stem cells carried the possibility to mimic *in vivo* architecture and multipotent differentiation of mature cell lineages. Both organoids were derived from stem cell sources with unlimited self-renewal capability. Human small intestinal organoids (hSIOs) were established from primary gut epithelial stem cells. The intestinal organoids (HIO) could be expanded indefinitely in 3D culture and contained some differentiated cell types mimicking *in vivo* epithelium. hSIOs allowed *in vitro* propagation of norovirus. Differentiated enterocytes in hSIOs expressed ACE2 and supported SARS-CoV-2 entry and replication[61]. Human intestinal organoids can be derived from both adult gut stem cells and pluripotent stem cell. However, HIOs derived from gut stem cell contained only epithelial cell types[70]. Human intestinal organoid derived from pluripotent stem cell (PSC-HIOs) contained all endodermal and mesodermal lineages, imitating epithelium, fibroblast, and blood vessel of the gut. PSC-HIOs expressed both ACE2 and TMPRSS2 in gastrointestinal tract with highest levels in the intestine. These organoids were infectable with SARS-CoV-2. The virus spread across entire PSC-HIO structure, causing organoid deterioration. Remdesivir treatment in infected-PSC-HIO could rescued organoid morphology[70]. hPSC-derived colonic organoids (hPSC-COs) comprising multiple colonic cell types, particularly enterocyte, were permissive to SARS-CoV-2 infection. Applying hPSC-COs together with high-throughput screen of FDA-approved drugs could identify the SARS-CoV-2 entry inhibitor candidates such as imatinib, mycophenolic acid and quinacrine dihydrochloride[71]. These data indicated that human intestinal organoids derived from both hPSC or adult stem cells were robust models for drug screening.

5. CONCLUSION

The pandemic caused by SARS-CoV-2 affected human activity worldwide and its rapid spread has challenged the scientific community to develop an effective treatment to contain the virus. Key success for eradicating the COVID-19 and minimizing the long-term systemic effects relied on feasible cell culture model that recapitulated both infectivity and pathophysiology in such organs exhibiting systemic complications. Thus, this article reviewed the cell culture model for SARS-CoV-2 infection. Based on 2D, 3D and organoid culture models, remdesivir and molnupiravir had beneficial effects against SARS-CoV-2 in HAE cultures. The application of 2D and 3D cultures such as bronchial and lung organoids as an antiviral treatment for COVID-19 suggests that it could be useful tools for examining newly developed anti-SARS-CoV-2 agents. Furthermore, lung, renal, heart, kidney and GI organoids were primary targets for investigating COVID-19 complications based on the presence of both ACE2 and TMPRSS2.

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