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REVIEW ARTICLE

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Spheroids and organoids as humanized 3D scaffold-free engineered tissues for SARS-CoV-2 viral infection and drug screening

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Abstract

The new coronavirus (2019-nCoV) or the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was officially declared by the World Health Organization (WHO) as a pandemic in March 2020. To date, there are no specific antiviral drugs proven to be effective in treating SARS-CoV-2, requiring joint efforts from different research fronts to discover the best route of treatment. The first decisions in drug discovery are based on 2D cell culture using high-throughput screening. In this context, spheroids and organoids emerge as a reliable alternative. Both are scaffold-free 3D engineered constructs that recapitulate key cellular and molecular events of tissue physiology. Different studies have already shown their advantages as a model for different infectious diseases, including SARS-CoV-2 and for drug screening. The use of these 3D engineered tissues as an in vitro model can fill the gap between 2D cell culture and in vivo preclinical assays (animal models) as they could recapitulate the entire viral life cycle. The main objective of this review is to understand spheroid and organoid biology, highlighting their advantages and disadvantages, and how these scaffold-free engineered tissues can contribute to a better comprehension of viral infection by SARS-CoV-2 and to the development of in vitro high-throughput models for drug screening.

KEYWORDS

3D in vitro models, COVID-19, drug screening, high-throughput, infection model, organoids, SARS-CoV-2, scaffold-free tissue engineering, spheroids

1 | INTRODUCTION

For centuries, infectious diseases have been a major challenge to the existence of humanity.[1] Together, these diseases represent the annual mortality of more than 10 million people worldwide.[2] Even with all the progress in the prevention and control of infectious diseases, pathogens still represent a major threat to human health.[3] Every year, the world is somehow affected by a new disease and new infectious agents being inserted into our environment, representing a continuous challenge, especially those caused by viruses, such as the H7N9 influenza virus in 2013,[4] Zika virus outbreak in 2016,[5] and Chikungunya outbreak in 2019.[6]

Currently, we are experiencing a pandemic caused by the SARS-CoV-2 virus, a new type of coronavirus, which was reported earlier this year in Wuhan, Hubei Province, China.

The first case occurred on December 12, 2019, and since then, the numbers of notifications of contagion and deaths attributed to the disease, called COVID-19, have been growing and spreading across the world rapidly.[7] Epidemiological investigations have suggested that the outbreak was associated with a seafood market in Wuhan.[8] With the exponential growth of cases, as of February 14, 2020, the WHO declared it a state of a public health emergency, and then, moved on to a pandemic state. COVID-19 had already spread all over the world, killing more than 700,000 people from different countries. To achieve all these outbreaks, it is mandatory to understand the mechanisms of infection by viral agents, leading to the production of vaccines and antivirals that are increasingly effective and specific to respond to these diseases.

Pharmaceutical industry in vitro methods for drug screening, metabolism, and toxicity are based on bidimensional (2D) cell culture of animal lineages. However, 2D cell cultures and cell lineages from animal sources are not able to mimic the tissue microenvironment found in vivo, mainly due to the limitations of cell-cell and cell-extracellular matrix interactions. In this context, three-dimensional (3D) cell culture models emerge as a relevant support tool, as they can provide reproducible results and adaptation of experiments to high throughput performance. Humanized 3D cell culture may fill the gap between 2D cell cultures and animal models, contributing to an understanding of cell and molecular mechanisms closer to human tissue physiology. Tissue engineering is essentially a 3D cell culture approach, and its recent advances have contributed to the development of complex and highly organized 3D cell culture models mimicking tissue microenvironments at the protein and gene levels.

The majority of tissue engineering approaches are based on the use of scaffolds as a substitute for extracellular matrices. However, scaffold-free approaches are increasingly being recognized as humanized 3D cell culture models capable of recapitulating cell-cell and cell-extracellular matrix interactions mimicking cell differentiation and physiology. The main objective of this review is to understand spheroid and organoid biology, highlighting their advantages and disadvantages, and how these scaffold-free engineered tissues can contribute to a better comprehension of viral infection by SARS-CoV-2 and to in vitro high-throughput models of drug screening.

2 | SARS-COV-2

SARS-CoV-2 belongs to the Coronaviridae family, order Nidovirales. They have the largest genome of all RNA viruses, usually ranging from 27 to 32 kb. The single-stranded positive-sense RNA is packaged inside a helical capsid formed by the nucleocapsid protein (N) surrounded by an envelope. Associated with the viral envelope are at least three structural proteins: the membrane protein (M) and the envelope protein (E) that are involved in the assembly of the virus, while the glycoprotein spike (S) is involved in the entry of the virus into cell hosts. These structural proteins form spikes on the surface of the virus, giving the appearance of having crowns, hence, its name. In addition to mediating the entry of the virus, protein S is determinant in reaching the viral host, tissue tropism and an important inducer of the host immune responses.[9] The protein spike (S) is divided into two domains: S1 and S2. The first is responsible for the interaction with the ACE2 receptor (angiotensin-converting enzyme 2), initiating the infection of the host cell by SARS-CoV-2.

Since coronavirus was discovered as the causative agent of COVID-19, scientists have been attempting to better understand the genetic makeup of the virus and to discover how to effectively treat the infection. Until now, no anti-SARS-CoV-2 drug or vaccine has been officially approved due to the absence of adequate evidence, and medical experts can only treat the symptoms of the disease. The individual, when infected, must remain isolated to prevent the disease from spreading for 14 days.[8] One strategy that has been applied is convalescent plasma transfusion, which may be beneficial in the treatment of critically ill patients with severe infection.[10] Many drugs, such as ivermectin,[11] hydroxychloroquine,[12] azithromycin,[12] and dexamethasone[13] are being speculated to treat the infection.

Drug screening based on in silico analysis has been used to predict the pathogenic mechanisms and some drug targets of SARS-CoV-2. The structure determination by cryo-electron microscopy (cryo-EM) and X-ray crystallography of the viral proteins is essential for this kind of approach, where drug therapies can be useful affecting the interaction with the host cells or blocking virus replication. After in silico analysis, the potential drugs can be tested using 3D cell culture models analyzing the virus replication and drug candidates to inhibit the SARS-CoV-2 infection.[14]

Another recent approach to combat SARS-CoV-2 is the therapeutic use of extracellular vesicles derived from adult stem cells, due to their effect on the modulation of anti-inflammatory and antiapoptotic pathways. The extracellular vesicles were already tested in acute respiratory distress syndrome and promise a novel treatment for the patients with COVID-19.[15] Furthermore, the efficacy of extracellular vesicles could be tested using 3D cell culture models.

The long-term strategy to combat COVID-19 is the development of a vaccine. Currently, approximately 250 candidate vaccines against SARS-CoV-2 are in development worldwide,[16] including mRNA vaccines, replicating or nonreplicating viral vectored vaccines, DNA vaccines, autologous dendritic cell-based vaccines, and inactive virus vaccines.[17] To date, at least 27 of these vaccine candidates are under evaluation in clinical trials. Two replicating or nonreplicating viral vectored vaccines are being tested in phase 3, namely, ChAdOx1 nCoV-19 (Oxford University, UK),[18]

Ad5-vectored COVID-19 (CanSino Biologics, China),[16] and one mRNA vaccine called mRNA-1273 (Moderna, USA).[19] These vaccines have been described as capable of producing neutralizing antibodies and humoral and cellular responses.

The race for rapid detection of the disease has caused many laboratories and companies work on rapid, sensitive, and low-cost tests, in addition to vaccines and treatments. The rapid tests can detect antigen or antibodies. Rapid antigen tests detect the presence of viral proteins and return positive results during infection. Antigen tests provide results in less than 30 minutes, do not need to be processed in a laboratory and are inexpensive to produce, but this speed comes at a cost in sensitivity. If a person has a low amount of virus in their body, the test can give a false negative result.[20] The antibody test detects the body's immune system response to the virus but is not effective in the early stages of infection. Some companies that produce this type of test are: Panbio Abbott test with sensitivity of 93.3% and specificity of 99.4%, this test can detect antigens and antibodies in swab and blood samples; One Step COVID-19 test from Celer with sensitivity of 86.43% and specificity of 99.57%, this test uses only a blood sample and gives the result in 15 minutes, for example.

However, to combat COVID-19 in a specific way, a greater understanding of the molecular mechanisms of infection is necessary, emphasizing the importance of the 3D cell culture models developed by the tissue engineering field. Based on these studies, more effective treatment and diagnoses can be produced.

3 | UNDERSTANDING HUMAN VIRUS INFECTION FROM IN VITRO MODELS

To understand human virus infections, different methodologies are used to study the entry of viruses into animal and human cells. For example, scanning electron microscopy was recently used by Caldas and collaborators[21] to identify for the first time the exact moment when SARS-CoV-2 infects a cell and was used in the past to study the morphological structure of SARS-CoV.[22] Other microscopy analyses, such as confocal and epifluorescence analyses, can also be used to track the cytoskeletal movements of viruses during cell infection. This fluorescence technique was already used for HIV, dengue, and hepatitis C virus.[23]

In some cases, viral nucleic acids or proteins are abundant and can be easily detected by in situ hybridization or immunohistochemistry. Currently, the most common analysis performed to identify SARS-CoV-2 infection and diagnosis[24] is polymerase chain reaction (PCR),[25] which amplifies nucleic acids to detect the virus at a molecular level. In addition, high-throughput proteomic analysis was also performed to identify the set of proteins modulated in Chikungunya infection. Among the 1047 proteins expressed in infective cells, 209 proteins were related to transcription, translation, apoptosis and stress response of the Chikungunya virus.[26] Functional analysis performed by supernatant harvesting as ELISA, multiplex and proteomics might be useful to quantify inflammatory mediators directly related to human virus infections. This approach is particularly interesting for infections caused by SARS-CoV-2, as it has already been shown that the virus is capable of establishing an inflammatory condition known as a "cytokine storm".[27]

Proteome analysis has been applied to better understand the changes in the total protein repertoire suffered by the host cell during viral infection. Using two-dimensional difference in gel electrophoresis (2-D DIGE), eight significant changes in host proteins have already been identified (MDCK and A549 cells) in H1N1 infection.[28] A proteomic approach was also used to detect virion-associated viral and cellular proteins during HCV infection, and because of that, new host factors, including a nuclear pore complex (NPC) protein that participates in HCV infection, were characterized.[29]

The measurement of the release of infectious particles per cell is important because they are related to applications ranging from the manufacture of vaccines to serum neutralization tests for clinical effectiveness. The most commonly accepted methods include endpoint dilution (TCID50) and plate assays.[30,31] However, depending on the type of virus, the result can take more than two weeks, in addition to requiring intensive work of handling cell culture. The interpretation of the results of these tests is subjective and highly variable, requiring many replicates to obtain a reliable statistical result.

In conclusion, different analyses to identify human virus infections in animal and human cells are available, and many of them are helpful for clinical diagnosis. Furthermore, the use of high-throughput methods is considered promising for drug screening tests in in vitro models of virus infection.

4 | SCAFFOLD-FREE 3D ENGINEERED TISSUES AS INFECTION MODELS

2D cell culture is commonly used in research laboratories and pharmaceutical industries. It is considered cost-effective and easy, and most of the analysis protocols are already well established and validated. The overwhelming majority of our knowledge of how viruses cause infection is based upon studies with 2D culture using animal cell lines. However, 2D cell cultures do not resemble the tissue microenvironment found in vivo (Table 1). Engineered scaffold-free approaches as 3D cell cultures, mostly represented by spheroids and organoids, have gained attention in the last few years due the capacity to closely mimic cell and tissue physiology. In 3D cell cultures, cells are architecturally organized in a compact structure,

where a dynamic equilibrium is reached over time in culture of gene expression and protein production, mimicking the performance of tissues in vivo. Besides, the extracellular matrix found in spheroids are produced by their own cells only, so there is no interference of a hydrogel or scaffold or the plastic of culture flasks.[35,40]

Since the 2000s, the number of scientific studies using spheroids and organoids to understand the host cell's response to viral infections has been increasing (Figure 1).[23,41,42] The use of these scaffold-free 3D models can provide information about host-pathogen interactions, which are necessary for the formulation of more effective antivirals and vaccines. Recently, Takayama reviewed cell culture models in the literature that can faithfully reproduce the viral life cycle and pathology of SARS-Cov-2. The work presents relevant published studies with cell lines, organoids and animal models that were performed this year to better understand COVID-19 infection.[1,43]

4.1 | 3D scaffold-free engineered spheroids

Spheroids can be formed from differentiated and undifferentiated cells, including adult and multipotent stem cells.[35] Cells are architecturally organized in a compact structure, where a dynamic equilibrium of gene expression and protein production is reached over culture, mimicking the physiology of tissues in vivo.[44] More importantly, the extracellular matrix found in spheroids is produced by their own cells once there is no interference of a hydrogel or scaffold. Cells inside spheroids are responsive to external stimuli, including differentiation events at the molecular level.[45,46]

TABLE 1 Main differences between 2D and 3D cell culture models

	2D cell culture	3D cell culture	References
Cell-to-cell contact	+	+++	32
Extracellular matrix production	+	+++	33
Higher cell density in vitro	++	+++	34
Production of pro angiogenic factors	+	++	35
Capacity of large-scale tissue production	+	++	36
Use for high-throughput systems and drug screening tests	++	+++	37
Mimicry in vivo tissue microenvironments	+	+++	38
Mimicry of embryogenesis and organogenesis processes	+	+++	39

In the past few years, many studies have been performed using spheroids for tissue engineering approaches. To engineer these 3D models, spheroids were produced from different cell sources, such as human bone marrow mesenchymal stromal/stem cells (MSCs) and human adipose-derived stem cells (ASCs), to engineer bone[46-50] and cartilage[44,50,51] microtissues. In addition, human chondrocytes were also successfully used to engineer cartilage in vitro.[52,53] Spheroids were also produced from induced pluripotent stem cells (iPSCs), mostly to engineer the liver,[54] neural[55] and cardiovascular tissues.[56] However, compared with the number of studies performed with MSCs or ASCs, studies with iPSCs to produce spheroids remain limited. Human umbilical vein endothelial cells (HUVECs) were also already used to produce spheroids for endothelial tissue engineering approaches, especially in coculture with MSCs.[57,58] In addition, a high number of studies with cancer lineages were also published to produce reproducible tumor spheroid models for drug screening tests in vitro.[59,60]

Spheroids can be produced by different 3D cell culture techniques.[35,40] Different studies have already been published showing high-throughput production of spheroids from different cell types by using the hanging-drop technique.[61-65] Other techniques already describe high-throughput spheroid production consisting of using nonadhesive agarose microwell systems,[66] cell culture plates with a higher number of wells, as described by Grinner et al,[67] which used a 1536-well plate format to engineer tumor spheroids for drug screening tests. Other studies were already performed using well plates for high-throughput applications, as performed by Liao and collaborators[68]; however, these authors used a 96-well plate to cast agarose microwells. Alginate hydrogels produced from casted PDMS molds were also used to produce a large number of MSC spheroids with homogeneous size and shape.[69] Another technique consists of using magnetic nanoparticles to assemble spheroids, as performed by Kim and colleagues.[70] The authors used a magnetic pin-array system to concentrate magnetic nanoparticle-incorporated cells and form spheroids. The main advantage is the possibility of assembling 96 spheroids at once with homogeneous size and shape.

4.2 | 3D scaffold-free engineered organoids

Prior to 2005, the term organoid was referred to as small tissue fragments taken from organs. Most studies were performed with epithelial tissues, separated from stroma by mechanical and/or enzymatic digestion and cultured in different types of

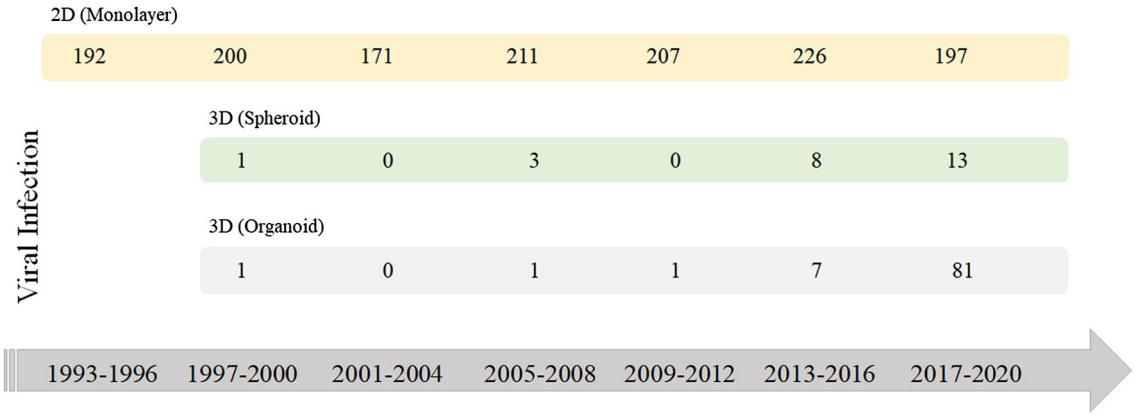

FIGURE 1 Timeline of numbers of articles published related to the use of 2D and 3D cell culture techniques as viral infection models. The search was performed on the PubMed database, by entering the combination of the following words: "virus infection" or "viral infection," "spheroid," and "virus infection" or "viral infection" and "organoid." Review articles were not included. The search was conducted on November 6, 2020 [Color figure can be viewed at wileyonlinelibrary.com]

3D gels to produce organ-like structures in vitro.[71] Currently, an organoid is defined as "a 3D structure grown from stem cells and consisting of organ-specific cell types that self-organizes through cell sorting and spatially restricted lineage commitment".[72] Organoids must be formed from embryonic stem cells (ESCs), iPSCs, and adult stem cells that spatially organize into a restricted lineage functional structure.[72-74] Organoids must develop functions of that specific organ to be considered organoids, and for that reason, they are commonly used to recapitulate morphogenesis in vitro.

During the past few years, different organoid models have been successfully developed in vitro, such as the brain,[75,76] intestine,[77-79] lungs,[80] pancreas,[81] stomach,[82] and liver.[83] Recent successful models of the intestine[84,85] derived from adult stem cells and brain derived from iPSC[86] organoids showed that the constructs were able to mimic morphological and functional properties of both native tissues. To allow organoid formation in vitro, several growth factors are needed to control the self-renewal, self-organization and differentiation of stem cells.[74] The use of Matrigel, a gelatinous protein mixture secreted by mouse sarcoma cells, is required, providing key biomechanical cues to organoid formation.[74,87] However, their inherent lot-to-lot variability and tumor- derived nature impair the use of organoids as humanized platforms for drug screening.[88]

4.3 | Spheroids and organoids for viral infection

Spheroids and organoids have already been explored as a model of viral cycle infection by human viruses (Figure 1).[23,41,42] A scalable model of spheroids derived from human hepatocytes to study hepatitis C virus (HCV) infection and replication was developed by Ananthanarayanan and collaborators.[41] The main results showed that spheroids presented liver-specific functions and a higher level of HCV infection compared with 2D cell culture. Hepatocyte spheroids infected with HCV were produced embedded in Matrigel and showed functional liver-like structures in addition to releasing infectious virions from HCV.[89] The life cycle of blood-borne HCV was also investigated on a 3D nonadherent gel showing high levels of the enzyme thromboxane A2 synthase (TXAS). This enzyme is required for the production of infectious HCV. The result was corroborated in a preclinical assay. Using a TXAS inhibitor (prostaglandin I2 receptor agonist), in an animal model, the levels of HCV infection were reduced.[90]

To explore the viral tropism of SARS-CoV-2, Yang and collaborators have used a human pluripotent stem cells (hPSCs) platform to generate multiple different cells and organoids. To determine the permissiveness of hPSC-derived cells, different MOI of SARS-CoV-2 was used for infection and the results showed the liver organoids, cardiomyocytes, pancreatic alpha and beta cells and dopaminergic neurons are permissive to SARS-CoV-2 infections.[91] Lamers and collaborators[92] infected gut enterocyte organoids with SARS-CoV and SARS-CoV-2, revealing upregulation of typical genes related to SARS-CoV-2 infection. Another recent study published by Monteil and collaborators[93] investigated the impact of human recombinant soluble angiotensin-converting enzyme 2 (hrsACE2) on SARS-CoV-2 growth. In summary, the authors used blood vessels and kidney organoids as a 3D model of SARS-CoV-2 infection, and hrsACE2 had a positive effect in blocking the infection in both organoid models. However, although hrsACE2 could block the early stages of SARS-Cov-2 infection, new studies with lung organoids must be performed to better evaluate its effects in vitro. Recently, Susuki and colleagues[94] cultivated normal human bronchial epithelial cells in Matrigel drops as a 3D scaffold-free model for SARS-CoV-2 infection. The 3D culture system was

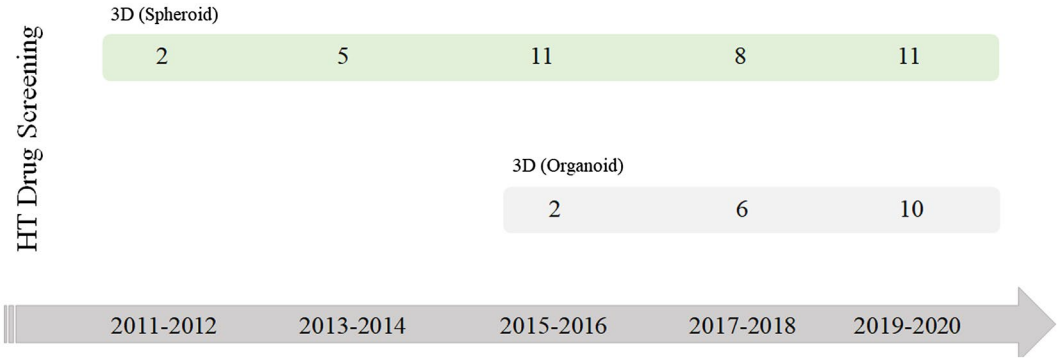

FIGURE 2 Timeline of numbers of articles published related to the established protocols of spheroids and organoids for high-throughput drug screening. The search was performed on the PubMed database, by entering the combination of the following words: "drug screening," "high-throughput" and "organoid," and "drug screening," "high- throughput" and "spheroid." Review articles were not included. The search was conducted on November 6, 2020. HT: High-throughput [Color figure can be viewed at wileyonlinelibrary.com]

responsive to infection, showing fibrose areas. The authors named the strategy lung organoids; however, the culture was not derived from stem cells.

All over the world, there is a concern with diseases transmitted by viruses transmitted by arthropods, such as the Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) viruses, but there is still no treatment for this type of infection. Garcez and collaborators[95] investigated the effects of Zika virus (ZIKV) infection in human neural stem cells growing as neurospheres and brain organoids. The results showed that the virus was able to infect the cells and, in the organoids, reduced the rate of growth after 11 days in culture. The use of a 3D model was able to show more reliable evidence of ZIKV behavior in vivo, contributing to a better comprehension of microcephaly cases in newborn children during ZIKV epidemics in Brazil.

5 | SCAFFOLD-FREE 3D ENGINEERED TISSUE PLATFORM FOR DRUG SCREENING

High-throughput systems have the potential to contribute to reducing the number of preclinical studies because they can test a larger set of compounds faster. An efficient scaffold-free 3D engineered tissue platform for drug screening must show scalability and have well-established end-points. More importantly, the platform must be amenable to the high-throughput system needed for the fast screening of large numbers of drugs. The data generated by high-throughput systems need to be interpreted by adequately designed software, making bioinformatics an essential tool. An important technological bottleneck in the adaptation of these systems is the size and shape of the 3D scaffold-free tissues, because the readout is commonly based on fluorescence emission.

5.1 | High-throughput systems for spheroids and organoids

Currently, the protocols used for spheroid production are more amenable to high-throughput drug screening compared with organoids (Figure 2). Ivanov and Grabowska[96] presented a device that allowed the formation and arrangement of 66 spheroids in a unique platform for high-throughput biomarker analysis of spheroids. Recently, Li and collaborators[97] developed a micro-scaffold array showing advantages related to the reduction of cell number, assay time, culture media, and drug consumption that can be applied for different cell types, tissue engineering studies, and new drug discoveries.

Cytotoxicity test results showed that the higher drug resistance of the 3D spheroids from cancer cells was independent of cell density. In this line, the spheroid model for high-throughput hepatotoxic studies discussed that drug resistance can be explained by the higher amount of extracellular matrix production, proving its functionality and sensitivity to drug response.[98] Hepatocyte spheroids are sensitive to inducers and inhibitors of liver metabolizing enzymes and are maintained for up to 20 days of culture.[99] An innovative method suitable for high-throughput drug screening was developed by Knowlton and collaborators.[100] The method relies on the bioprinting of encapsulated hepatic spheroids into a microfluidic device, showing the maintenance of spheroid morphology after bioprinting.

Interestingly, metabolism can be measured in hepatocyte spheroids using a microsensor platform integrated into a 96-well plate.[101] The main results showed alterations in lactate production under exposure to different drug concentrations. The perspective is that the platform can be used for complex organ-on-chip studies. Another method was developed by Abe-Fukasawa and collaborators[102] and consists of the use of a low-molecular-weight agar, which allows cells to grow as

dispersed spheroids suitable for drug assessment. The main advantage of the method is the possibility to create numbers of spheroid clones and image them with quality through the period of culture. Lim and Park[103] developed a microfluidic device to produce spheroids of cancer lineage and tested the responsiveness of the spheroids to anticancer drugs. The spheroids were formed even with a few cells, showing remarkable changes in their morphology in the presence of these drugs.

Recent studies have begun to test drugs in 3D culture models infected with SARS-CoV-2 (Figure 3). Katsura and collaborators reported an alveolosphere culture system for the propagation and differentiation of human alveolar type 2 cells derived from primary lung tissue. The pneumocytes expressed the SARS-CoV-2 receptor ACE2 and the infection with the virus was confirmed by transcriptome and histological analysis. The authors showed that the treatment with low doses of interferons (IFNs) promoted a reduction in viral replication.[104] Han and collaborators produced human pluripotent stem cells derived colonic organoids as a model of SARS-CoV-2 infection. The authors performed a high throughput screen of FDA-approved drugs and identified entry inhibitors of SARS-CoV-2 in the colonic organoids.[105]

The number of studies dedicated to high-throughput systems using organoids has increased in the last decade; however, the study of these systems are still low compared with spheroids (Figure 2). The majority of studies use organoids derived from tumor biopsies as a model for cancer,[106-109] and recent studies are investigating the development of organ-on-chip devices with organoids for drug screening.[110,111] The tumor organoids usually have a diameter of 200 micrometers, making them appropriate for high-throughput systems. The other types of organoids, including the brain and intestine, can reach up to 1 millimeter in diameter, in addition to having various shapes, because they can recapitulate tissue morphogenesis, resulting in tissue microenvironments similar to those in vivo.

Driehuis and collaborators[106] used a biobank of 30 pancreatic tumor organoids from patients. Phan and collaborators[109] developed a high-throughput approach to establish and perform drug screening of tumor organoids. A total of 240 tumor inhibitors were used, and organoids were sensitive

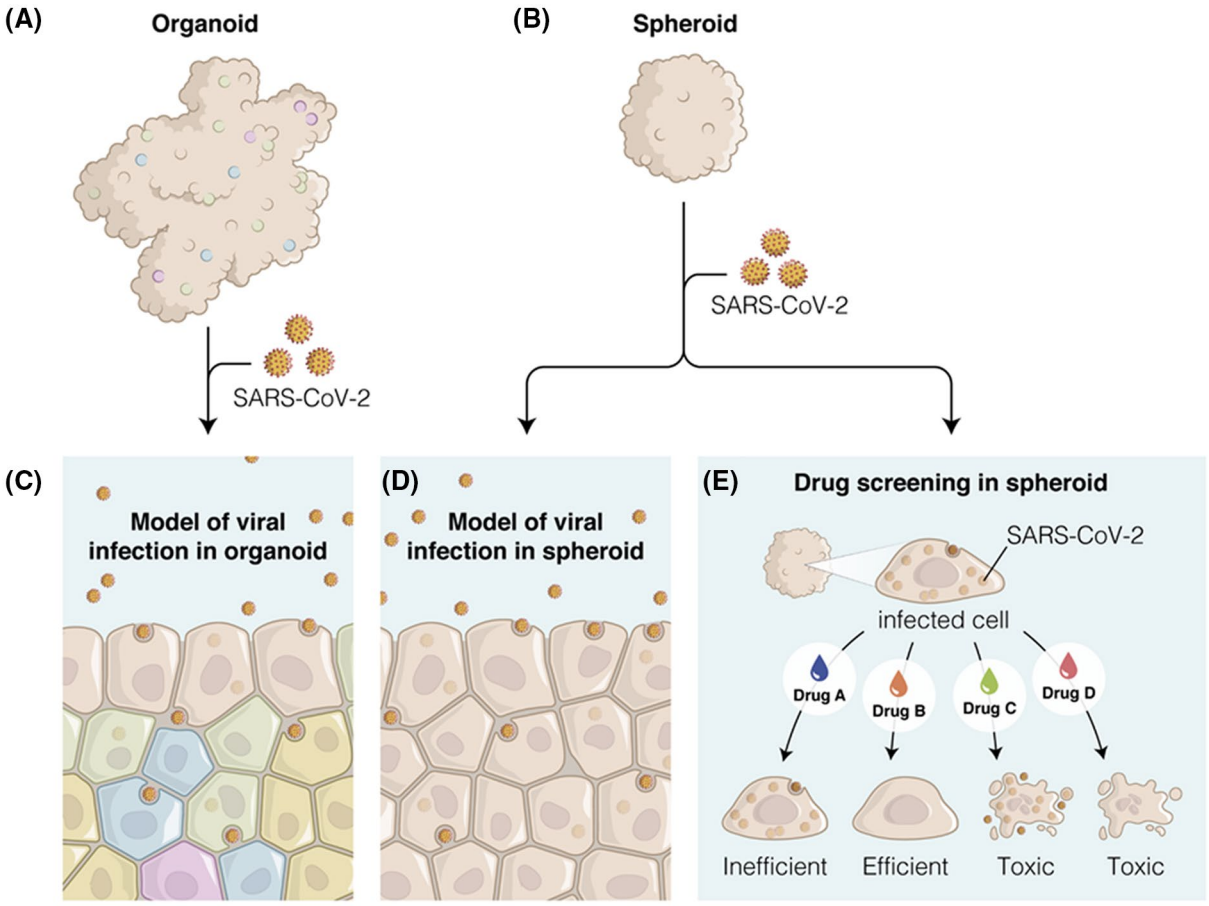

FIGURE 3 Spheroid and organoid can be used as a model of SARS-Cov-2 infection (A, B) to understand the mechanism of virus entry into cells (C, D). (E) An efficient drug candidate should eliminate the virus while maintaining cell viability inside spheroids. Toxic drug candidates show no antiviral effect or even toxic effects in both cells and virus [Color figure can be viewed at wileyonlinelibrary.com]

to changes in size and morphology. Recently, a method to produce liver organoids by using Matrigel suspension instead of drops was developed by Garnier et al.[112] The use of Matrigel suspension allows automation and high-throughput applications, in addition to showing better stability in the expression of typical liver genes, a usual challenge to be addressed in the development of liver models. This study may represent an important step toward the change of organoid protocols for high-throughput systems. Qian and colleagues also developed a scalable brain organoid platform to model ZIKV exposure.[113]

The complexity of organoids also results in a lack of reproducibility and different shapes and sizes, and the same batch impairing image assays are usually used in high-throughput systems for drug screening.[114] As an in vitro viral infection model, the organoid becomes appealing for understanding the impact of infection on cell differentiation and in subpopulations of a given tissue. For example, SARS-CoV-2 seems to infect a transient population of bronchial secretory cells.[115]

6 | CONCLUSION AND PERSPECTIVES

The pandemic caused by SARS-CoV-2 and other human viruses, such as Zika, Dengue and Chikungunya, present worldwide could be better understood through the development of reproducible 3D tissue models. The use of human cells in complex 3D scaffold-free culture systems that reproduce tissue microenvironments and cellular interactions is the future for a better understanding of the mechanisms of viral infection, the discovery of therapeutic targets and high-throughput platforms for drug screening. In this context, high-throughput platforms have already been developed using spheroids and some types of organoids, especially tumor organoids. Organoids represent an interesting humanized 3D model for a deeper understanding of human virus infection due to their complexity of cell subpopulations. The use of CRISPR, transduction and other technologies of gene editing can expand the application of these 3D scaffold-free models for drug screening and discovery. In the near future high-throughput platforms using spheroids or organoids should add microphysiological systems (organ-on-a-chip) to reach a more accurate cell physiological response, under dynamic conditions and real-time monitoring.

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CONFLICT OF INTEREST

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