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Review Article

Application of Cell Culture Models in Studying Viral Diseases (SARS, H1N1 Flu, MERS, COVID-19): A Review

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ABSTRACT

The emergence of recent viral outbreaks, especially the COVID-19 pandemic, and the resulting global mortality and damage has created an urgent need to accelerate the identification, prevention, and treatment of these viral diseases. Due to the limitations in the use of humans, and animal models in terms of time, costs, metabolism differences and ethical issues, *in vitro* models have become essential in virology research. In the present review, we collected the application of several used cell culture models in studies on four pathogenic viruses - severe acute respiratory syndrome coronavirus (SARS-CoV), influenza A virus (H1N1), middle east respiratory syndrome coronavirus (MERS-CoV), and 2019 novel coronavirus (SARS-CoV-2). These models included, 2D and 3D cell culture (organoids, microfluidic-chips, and bioprinted models). A collection of existing research on these viruses can help fight against the SARS-CoV-2 virus and speed it up against future emerging viruses. Moreover, it can show the shortcomings of *in vitro* models in virology studies that have been performed to date and provide researchers with new ideas for developing models that are more efficient to deal with similar viral outbreaks.

Introduction

Over the past two decades, four highly pathogenic and deadly human viruses have emerged. they were severe acute respiratory syndrome coronavirus (SARS-CoV), influenza A virus (H1N1), middle east respiratory syndrome coronavirus (MERS-CoV), and 2019 novel coronavirus (SARS-CoV-2) [1]. Currently, the coronavirus disease-2019 (COVID-19) pandemic caused by SARS-CoV-2 is spreading worldwide and causes deaths and injuries to a large number of people every day due to the lack of knowledge about COVID-19 pathogenesis and the absence of decisive treatment and vaccines available to the public. it is essential to learn more about pathophysiology, in order to prevent further spread and high mortality rate and to identify the best drug targets and vaccines. The use of study models can help to achieve this [2, 3]. Since these four viruses (SARS-CoV, MERS-CoV, H1N1, and SARS-CoV-2) are somewhat similar in some genomic characteristics, pathogenesis, and transmission, the researchers can rely on earlier experience about these viruses to speed up developing new relevant study models [1, 4].

There are generally three types of models for studying viral infections - humans, animals, and cell culture models. Restrictions on animal and human model testing, such as high costs, ethical issues, systemic impact, and animal stress on test results and virus species specificity, have led researchers to prioritize the use of cell culture models. As cell culture models can be designed differently depending on the study's requirements and the type of virus, they can work with a wide range of host-specific viruses. As a result, these models have various applications in experiments of viral infections, including studying virus pathophysiology, virus isolation, vaccine manufacturing, drug designing, effects of drugs and toxic compounds on cells, and monoclonal antibody production [2, 5]. Models that have been used for SARS-Cov, MERS-CoV, H1N1, and SARS-CoV-2 infections include 2D models (monolayer cultured cell lines), and 3D models (organoid, microfluidic [organ-on-chip], and bioprinted systems) (Figure 1). Earlier findings from cell culture models in similar pathogens (SARS-CoV, MERS-CoV, H1N1) can provide useful ideas to fight against SARS-CoV-2, and future pandemics.

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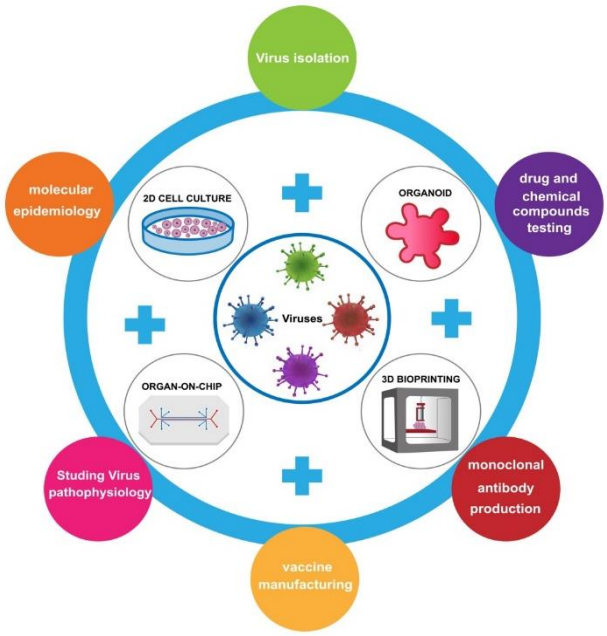

Figure 1: Cell culture models application for viral disease including 2D cell culture, organoid, organ-on-chip, 3D bioprinting.

2D Cell Culture Application for SARS-CoV, MERS-CoV, H1N1 and SARS-CoV-2

The oldest and most common type of cell culture is two-dimensional cell culture [6]. This system is used more than other models (3D models) because it is less costly and often requires fewer facilities, so this method is more accessible (Figure 2). To date, 2D cell cultures have had various applications in virology, so that their results have led to many remarkable advances in cognition, and control of viral diseases such as SARS, MERS, H1N1 and COVID-19 [5]. Therefore, the study of applications of two-dimensional cultures for studies on SARS-CoV-2 and similar viruses can be a great help for further research. One of the most common applications of 2D culture in virology is the effect of various antiviral drugs and compounds. For instance, Martin Spiegel and his teammates indicated that multiplication of SARS-CoV in Vero cell culture is prevented by pretreatment with interferon-beta [7]. Also, Birgit Morgenstern and her co-workers showed that the combination of ribavirin with interferon-beta inhibits SARS-CoV replication in lower concentrations compared to either single treatment [8].

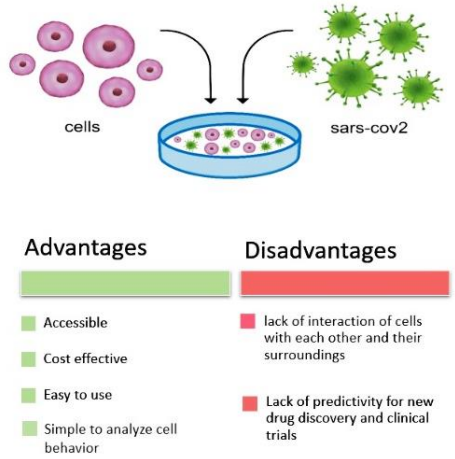

Figure 2: Advantages and disadvantages of 2D cell culture for virology.

In another research, Timothy P. Sheahan and his teammates, revealed that GS-5734 (remdesivir) prevents SARS-CoV and MERS-CoV replication in human airway epithelial cells [9]. Adriaan H. de Wilde and his fellow-worker showed that four compounds - chloroquine, chlorpromazine, loperamide, and lopinavir, inhibit MERS-CoV and SARS-CoV replication in the low micro molar range (50% effective concentrations [EC50s], 3 to 8 M) [10]. Meanwhile, antiviral properties of Chloroquine for SARS-CoV replication in cell culture was reported [11]. In another research, Yuanjiang Zhang demonstrated that siRNA might act as an inhibiting factor of gene expression of SARS-CoV's Spike protein in 2D cell culture [12]. Other research on the anti-influenza A virus showed three compounds with antiviral activity related to the efficacy of Flos Trollii and antiviral activities of compounds isolated from Pinus densiflora (pine tree) in cell culture [13, 14]. Recent research demonstrated the antiviral activity of ATR-002 and selenium supplementation against influenza A virus in the cell culture [15, 16]. Also, Yejin Jang and his teammates showed that lambda-carrageenan (λ-CGN) could be a promising and preventive agent for several respiratory infections, especially influenza A virus and SARS-CoV-2 [17]. In another research, both pyronaridine and artesunate inhibited the development of SARS-CoV-2 and seasonal influenza A virus in Calu-3 cells. Therefore artesunate and pyronaridine might be effective drugs for COVID-19 or influenza A sufferers [18].

Isolating and identifying viruses in clinical specimens, preparing viruses for vaccines, and studying the viral structure, multiplication cycles, genetics, and virus-host cell interaction are other essential and common applications of 2D cell cultures in virology [5]. Research based on these applications demonstrated that LoVo cells are useful for studying biology and persistent infection of SARS-CoV in *in vitro* models. They also showed that the expression of angiotensin-converting enzyme 2 (ACE2) is not probably the mere determinant for cells susceptibility to SARS-CoV infection [19]. In addition, Hin Chu and his teammate showed that MERS-CoV actively infected Mo-DCs while SARS-CoV infection was abortive. MERS-CoV induced IFN-γ, IP-10, IL-12, and RANTES in a higher rate than SARS-CoV. MERS-CoV induced more surface expression of MHC II and CD86 than SARS-CoV [20]. In the other research by Isabella Eckerle and her teammate, potential intermediate host species of MERS-CoV were identified, using *in vitro* testing [21]. Further studies by Kaveh Sadeghi and his colleagues demonstrated that the influenza virus is capable of infecting the pancreas and damaging the pancreas cell line [22].

Recently, with the advent of SARS-CoV-2 and the urgent need to identify the virus for treatment and prevention of the disease, two-dimensional cultures have been used in many studies for various applications like previous similar viral outbreaks. It was shown that the Vero-E6 cell line expresses TMPRSS2 and it is the most widely used cell line to replicate and isolate SARS-CoV-2, compared with other Vero cell clones [23, 24]. Vero-E6 cell line has also been used to evaluate the inhibitory effect of remdesivir and chloroquine and to study morphology and morphogenesis of SARS-CoV-2 in 2D cell culture [25, 26, 27]. Besides, to compare viral tropism, replication and innate immune responses of SARS-CoV-2 with SARS-CoV, MERS-CoV, and the influenza A virus (H1N1), researchers used 2D cell cultures of primary human alveolar epithelial cells and macrophages [28]. In another research, cytopathic effects were observed in human airway epithelial cells after SARS-CoV-2 infection. The isolation of SARS-CoV-2 was demonstrated, and mimic infected human lung cells was made possible

in human airway epithelial cells [29]. Another cell line used for research on the SARS-CoV-2 includes LLC-MK2 (Rhesus monkey kidney cells). One study using this cell line showed that aerosol produced by COVID-19 patients could be a source of transmission of the virus [30]. Also, Caco-2 cells (human colon adenocarcinoma), and CL14 detected the sufficiency of SARS-CoV-2 and SARS-CoV replication as shown by cytopathic effects (CPE). Also, it proved that SARS-CoV-2 is more sensitive to TMPRSS2 inhibitors than SARS-CoV and that the anti-SARS-CoV-2 activity of remdesivir and aprotinin can be increased by omeprazole in cell culture [31]. HEK293T (human embryonic kidney grown in tissue culture) can be an alternative cell line for SARS-CoV-2 *in vitro* infection [32].

3D Cell Culture Application for SARS-CoV, MERS-CoV, H1N1, and SARS-CoV-2

To date, animal and two-dimensional models have been used extensively for virological studies and research. Two-dimensional models have helped us to expand our knowledge of virology. However, they also have limitations and problems, such as not being able to model the inner body due to the growth of cells on smooth layers of glass or plastic, the lack of interaction of cells with each other and their surroundings in two-dimensional culture, not reproducing human disease pathology (Figure 2). Animal models have also restricted uses since they are both time-consuming and costly, not to mention stark differences between human and animal metabolisms. Difficulties mentioned above, led researchers to try other models, especially 3D cell culture models, which are more similar to the human organs [33, 34].

I Organoid

Human organoids, a type of three-dimensional culture, can largely meet the requirements of laboratory models in virological research. Organoids are miniaturized and simplified versions of an organ and are produced *in vitro*. They can recreate the structure and physiology of human organs to a large extent [35]. Organoids can be suitable models for studying viral diseases, developing antiviral drugs, and so on in virology research. Applying previous research and studies using organoids for viral diseases such as SARS, MERS, H1N1 can give new ideas to study and deal with the current coronavirus (SARS-CoV-2) and similar viruses that may appear in the future. for instance, Jie Zhou and colleagues could show three-dimensional cultured intestinal organoids, also called intestinoids or mini-gut, are very sensitive to MERS-CoV and maintain strong viral replication [36]. Further Kenrie P Y Hui's research in human airway organoids concluded that human airway organoid provides a related physiological *in vitro* model. It can be used to examine virus tropism and reproduction ability as well as assessing and comparing different epidemic of animal influenza viruses [37].

Recently, Vanessa Monteil and her teammate showed engineered human kidney and blood vessel organoids can be infected with SARS-CoV-2, and human recombinant soluble ACE2 (hrsACE2) could act as an inhibitor in the early stages of SARS-CoV-2 infections [38]. In another recent research, human bronchial organoids (hBO), which contain basal, club, ciliated, and goblet cells, were generated and used as a model for SARS-CoV-2 research and drug discovery. It was showed that camostat is an inhibitor of TMPRSS2 and can reduce the viral copy number [39]. In addition, human liver ductal organoids and human small intestinal organoids (hSIOs) are sensitive to SARS-CoV-2 and support strong virus replication. Therefore, these can be an *in vitro* model for SARS-CoV-2 research [40, 41]. In addition, researchers generated a lung organoid model using human pluripotent stem cells (hPSC-LOs) and hPSC-derived colonic organoids (hPSC-COs) that express ACE2 and can support SARS-CoV-2 infection. They found out that FDA-approved drugs such as imagine, mycophenolic acid and quinacrine dihydrochloride can inhibit SARS-CoV-2 entry and decrease the infection of both hPSC-LOs and hPSC-COs with SARS-CoV-2 [42, 43].

Anna Z. Mykytyn and colleagues reported that SARS-CoV-2 is more fusogenic than SARS-CoV, and camostat mesylate is an inhibiting factor for SARS-CoV-2 penetration and multiplication in differentiated organoid-derived human airway cells [44]. Mulay established an alveolar organoid model that enables researchers to evaluate host response to SARS-CoV-2 infection [45]. Moreover, clinical reports of neurological symptoms in COVID-19 patients necessitate research on the effects of SARS-CoV-2 on the central nervous system (CNS). For this purpose, researchers utilized human brain organoids as a useful model to elucidate the susceptibility of the brain to SARS-CoV-2 [46]. Results showed SARS-CoV-2 does not replicate but infect the cortical region of the human brain organoids [47]. In another study, to evaluate virus replication and the host response to infection, Makovoz and colleague infected eye organoids and adult human ocular cells with SARS-CoV-2. Although the degree of replication in the central cornea was not noticeable, the limbus showed the highest susceptibility to infection. In addition, they demonstrated that infections happen directly in the human eye [48].

II Organ-on-Chip

Organ-on-chip, another type of three-dimensional culture, is a microfluidic culture device that involves microchannels. They make it possible to simulate the main parameters (fluid flows, mechanical stimulation, tissue interfaces, etc.) in the physiological environment of tissues and living cells. Microfluidic models can overcome the inefficiencies of the previously mentioned models mostly with spatiotemporal controllability, fluid and gas flow control, physiological limitations of living tissue, and high-output analysis in smaller sample sizes. In recent years, organ-on-chips have been very useful applications in virological research, such as the study of virus-host interactions as well as drug and vaccine responses and development. Moreover, because such research is conducted in micro dimensions and fewer materials and supplies are used, these models could decrease the cost of antiviral drug development (Figure 3) [49-51]. Long Si and colleagues were able to build a human lung airway microfluidic chip that showed the influenza virus replication, host responses to infection, evolution by gene re-assortment or mutation, and effects of antiviral drugs. They successfully demonstrated influenza virus replication, their effect on host cells, and the clinical impact of antiviral drugs. The results showed that this influenza chip may be an alternative preclinical tool for producing antiviral drugs and vaccines [52]. In another recent study, Gard and his teammate designed a human primary airway epithelial cell-based model in a high-output platform. cells cultured at an air-liquid interface (PREDICT96-ALI) in this study could evaluate the therapeutic effect of various small molecules and antiviral agents (such as oseltamivir) against the influenza A and other respiratory viruses, especially coronaviruses [53].

To study recapitulated alveolar-capillary barrier injury and inflammatory responses of SARS-CoV-2 infection, Zhang and his co-worker created a micro-engineered alveolus chip model [54]. Similarly, to recapitulate the intestinal injury and immune response by SARS-CoV-2, Yaqiong Guo and colleagues engineered an intestine-on-chip device [55]. Besides, the microfluidic model of the human blood-brain barrier indicated that spike protein subunits of SARS-CoV-2 can affect the function of blood-brain barrier [56].

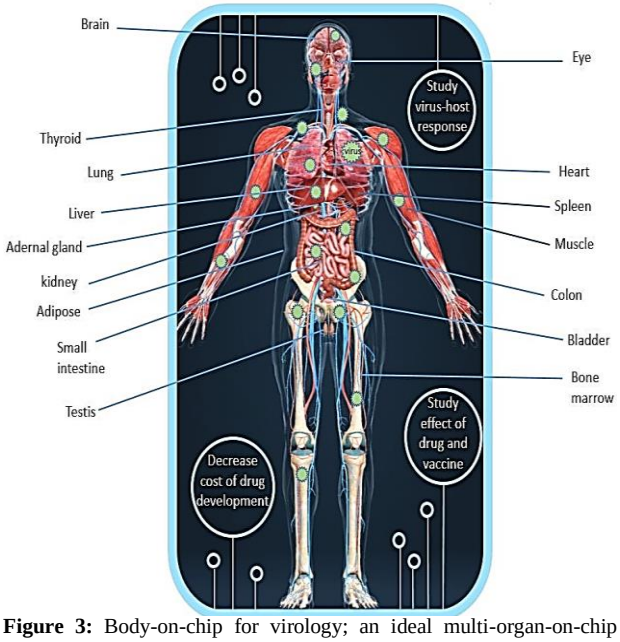

Figure 3: Body-on-chip for virology; an ideal multi-organ-on-chip model including SARS-CoV-2 target tissues.

Since ACE2 enzyme, as a specific target for SARS-CoV-2, expressed with different levels in the small intestine, testis, kidneys, heart, thyroid, adipose tissue, lungs, colon, liver, bladder, adrenal gland, blood, spleen, bone marrow, brain, blood vessels and muscle an ideal model should include these tissues interacted each other to mimic the physiological structure of internal organs accurately (Figure 3) [57]. Although the single organ-on-chip models have made great strides in the past decade, they are inadequate models to simulate the complexity, functionality, and integrity of human organs. To solve this problem, the multi-organ-chip models (also known as human-on-a-chip) are options that provides integrated cultured cells of different organs and tissues using microfluidic channels. They can also be a suitable model for virology studies, especially drug development. Therefore, this model can show the effects of viruses, drugs and even vaccines on cells more comprehensively than other models due to the simultaneous study of connective target tissues [6]. Although the multi-organ-chip face many challenges, significant improvements that to date has been made by researchers such as two-organs, three-organs, four-organs, and ten organs on the chip, increase the possibility of creating such a model in the future [34, 51, 58-62].

III 3D Bioprinting

Another 3D cell culture model that has recently drawn more attention in fighting infectious diseases due to its imitation of physiological conditions, complex architectures and structure of biological organs and tissues compared with other models is 3D bioprinting model. This model uses layered cells and biomaterial printing technology to build structures like native tissues using specific tissue cell types in bioink. Several methods are used in biofabrication; the most common of them are extrusion-based, inkjet-based, laser-assisted, stereolithography. However, the extrusion bioprinting method is the most commonly used, because of its convenience and low cost. In virology research, we often require modeling various conditions and certain cell lines that are specific to each type of virus. Due to the selectivity of the underlying factors such as multiple cell types, biomaterials, structure design, and biofabrication methods, bioprinting technology allows researchers to create a wide range of tissues that are accurate *in vitro* 3D models, based on the type of virus and the required application. As a result, 3D bioprinted structures provide *in vitro* models of various systems, and make it possible to understand pathogens and pathogen-host interactions, the production of effective vaccines and drug development. Therefore, it can be an ideal model of infectious diseases (Figure 4) [63-65].

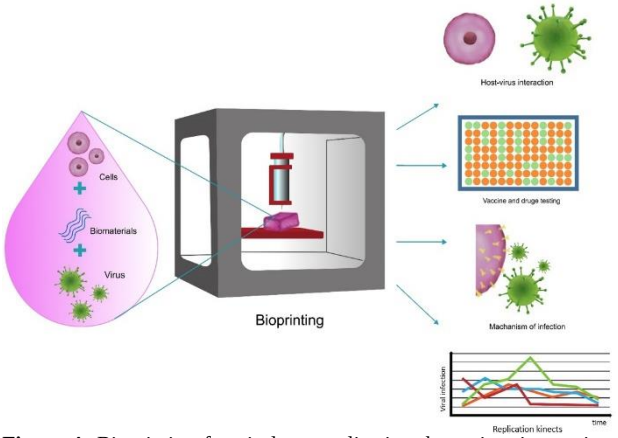

Figure 4: Bioprinting for virology application; host-virus interaction, vaccine and drug testing, mechanism of infection, replication kinetic.

Johanna Berg reported 3D bioprinted human models for infection studies on influenza A using a bioink which was made up of 2% alginate and 3% gelatin and fulfilled with 20% Matrigel. She succeeded in providing a 3D lung model with A549 cells by extrusion bioprinting technology. The results suggested that the IL-29 antiviral (interferon λ1) agent shows an immune response in bioprinted cells. The 3D bioprinted model also supported distributed infection with influenza A virus quite similar to the natural human lung [66]. There are various challenges in 3D bioprinting of models regarding the study of viral infections, such as developing suitable biomaterial, cell viability, controlling mechanical stress during bioprinting, ethical issues, and many more. With all those, they have shown to be appropriate *in vitro* models and have great potential and reliability in the successful construction of tissues, such as liver, kidney, lung, cardiac in various researches [63, 67]. Thus rapid advances in 3D bioprinting technology can develop *in vitro* platforms which explain the mechanisms of infection, replication kinetics, test vaccines and drugs in inhibiting SARS-CoV-2 infection [64]. Models that have been used for SARS-CoV, MERS-CoV, H1N1, and SARS-CoV-2 infections are summarized in (Table 1).

Table 1: Cell culture models to study viruses (SARS-CoV, H1N1, MERS-CoV, SARS-CoV-2).

		Influenza A (H1N1)	SARS-CoV	MERS-CoV	SARS-CoV-2
2D Cell Culture		• RAW264.7 cells [13,14] • human adenocarcinoma cells (A549) and Madin-Darby canine kidney (MDCK)cells [16, 17] • human lung epithelial (Calu-3) cells [18] • mouse pancreatic cell line and Mouse Insulinoma cell line (MIN6) [22]	• Vero E6 cells [11, 12, 19] • Vero cells caco2, pk-15, cl14 and HPEK [7, 8] • human airway epithelial cells [9, 68] • Human embryonic kidney cell line 293T [63] • human embryonic kidney cells (HEK-293) [69] • HeLa, HOS, C8166, BL41, Hep-2, Huh-7 and NIH3T3 cells [70] • LoVo Cells [19]	• Huh7 cells [10] • human airway epithelial cells [66, 68] • Monocyte-derived-dendritic cells (Mo-DCs) [20] • human adenocarcinoma cells (A549), ZN-R, ZLu-R, LGK-1-R, TT-R.B, PO, KN-R, KLu-R [21]	• Vero cells [32] • Vero E6 cells [23, 24, 26, 27, 32] • Human airway epithelial cells [29] • human adenocarcinoma cells (A549), human liver cells (HUH7.0), and human embryonic kidney cells (HEK-293T) [25] • human lung epithelial (Calu-3) cells [15] • primary human alveolar epithelial cells and macrophages [28] • Caco2, CL14, HT-29, and DLD-1 [31]
3D Cell Culture	Organoid	• human airway organoid [28] • intestinal organoid [71]	-	• intestinal organoid [36]	• blood vessel and kidney organoids [38] • bronchial organoid [39] • liver organoid [41] • lung organoid [43] • small intestinal organoids [40] • colonic organoid [42] • organoid-derived human airway cells [44] • alveolar organoid [45] • brain organoid [47] • eye organoid [48]
	Microfluidic device	• human airway Chip microfluidic culture device [52] • airway model within the PREDICT96-ALI platform [53]	-	-	• alveolus chip [54] • intestine-on-chip [55] • human blood–brain barrier [72]
	3D bioprinting	• 3D lung models with A549 cells [66]	-	-	-

Conclusion

To fight viral diseases such as COVID-19, it is necessary to design more efficient *in vitro* models, which produce more reliable results in a shorter time. For this purpose, it is important to study the strengths, weaknesses, and various applications of the *in vitro* models used in previous research on similar viruses (influenza A, SARS-CoV, MERS-CoV). A collection of the application of these models helps to develop new mixed models for further viral studies. It is also expected that in the future, 3D models especially microfluidics and 3D bioprinting will be more frequently used in virology. In addition, *in vitro* models will greatly reduce the need for human and animal trials. Thus, we will be prepared for future viral outbreaks.

Author Contributions

Minoo Alavi researched the literature and wrote the article. Afra Hajizadeh and Samira Tajvar discussed, reviewed and edited the manuscript.

Competing Interests

None.

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