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SARS-CoV-2 infection in cardiovascular disease: Unmet need of stem cell models

LUCA ANNA BORS, BARBARA ORSOLITS, NORAH MAHNOOR AHMED, HYUNSOO CHO, BÉLA MERKELY and GÁBOR FÖLDES*

Heart and Vascular Center, Semmelweis University, Budapest, Hungary

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ABSTRACT

This review aims to summarise new approaches in SARS-CoV-2-related research in cardiology. We provide a head-to-head comparison of models, such as animal research and human pluripotent stem cells, to investigate the pathomechanisms of COVID-19 and find an efficient therapy. In vivo methods were useful for studying systemic processes of the disease; however, due to differences in animal and human biology, the clinical translation of the results remains a complex task. In vitro stem cell research makes cellular events more observable and effective for finding new drugs and therapies for COVID-19, including the use of stem cells. Furthermore, multicellular 3D organoids even make it possible to observe the effects of drugs to treat SARS-CoV-2 infection in human organ models.

KEYWORDS

SARS-CoV-2, human pluripotent stem cells, cardiovascular, disease modelling, personalised medicine, drug development

INTRODUCTION

There are still many questions about the origin of SARS-CoV-2, but several theories have come to light since its outbreak. The epicentre of the infections was recognised as the Huanan marketplace in Wuhan back at the end of 2019. Most evidence shows that this coronavirus was

* Corresponding author. Heart and Vascular Centre, Semmelweis University, 68 Városmajor Street, Budapest, H1122, Hungary. Tel.: +36 20 825 0558. E-mail: foldes.gabor@semmelweis.hu

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originally a zoonotic pathogen that mutated and transmitted to its first human hosts from wild animals sold as goods on the market [1]. Since then, the virus has gone through multiple variants, such as the more severe delta or the mild but extremely infectious omicron variant.

It is a well-known fact that the main target of SARS-CoV-2 spike protein is the angiotensin-converting enzyme 2 (ACE2) receptor. Although it is disputed if the virus had this high affinity to ACE2 receptor as it thought from the beginning [1]. In most cases, COVID-19 affects the lungs and the cardiovascular system [2, 3] as these organs have the most dense ACE2 receptor expression [4].

Almost ten thousand publications recognise that patients with increased cardiovascular risk are disproportionately more affected by COVID-19 than healthy individuals. Clinical statistics currently identify cardiac arrhythmia, cardiomyopathy, myocarditis, and cardiac arrest are often terminal events in patients with SARS-CoV-2 infection [3]. On the other hand, a dilemma arises regarding the vaccination with messenger RNA-based vaccines against SARS-CoV-2. Rare adverse effects like vaccine-associated immune thrombosis and thrombocytopenia (VITT), which resemble heparin-induced thrombocytopenia (HIT) prothrombotic disorders and myocarditis, occurred short after vaccination [5, 6] The reason behind VITT cases that young women on hormonal contraception were more susceptible to thrombosis, however, in the case of myocarditis, a direct causal relationship cannot be established due to the lack of viral genomes or autoantibodies in the cardiovascular tissue samples. Nonetheless, studies showed that these symptoms are much more frequent and severe in unvaccinated people infected by SARS-CoV-2 than as an adverse effect of vaccination. After reviewing the published papers, multiple methods and models were collected from animal models to clinical therapies to show the available tools for COVID-19 research in cardiology.

CARDIOVASCULAR RISKS IN SARS-COV-2 INFECTION

SARS-CoV-2 main targets in the cardiovascular system are Toll-like receptor 4 and ACE2. The virus binds to these proteins and often disrupts different signalling pathways in the cardiovascular and immune systems, for example, uncontrolled cytokine cascades and platelet activation, causing metabolic and coagulation abnormalities, arrhythmia, cardiomyopathy, ischemia and cytokine storm [7–12].

On the vascular side, it has been suggested that COVID-19, particularly in the chronic stages of the disease, may represent a primarily endothelial disease [2]. SARS-CoV-2-induced pneumonitis incorporates the notion of endothelial dysfunction, such as defective endothelial barrier function and disruption of vascular endothelial (VE)-cadherin responsible for the integrity of tight junctions [13]. In small vessels, like those in the alveoli of the lung, impaired barrier function leads to capillary leak and subsequent lower oxygenation of the blood. In addition to increased vascular permeability, endothelial damage is further characterised by vasodilation and leukocyte recruitment, culminating in pulmonary injury, hypoxemia, and cardiovascular stress. Indeed, endothelial dysfunction and thus a loss of the endothelial protective mechanism may contribute to multi-organ failure in the advanced stages of infection. This is particularly dominant in a cytokine storm, where cytokines affect the homeostatic function of endothelial cells, contributing to thrombosis and local tissue injury and thereby in numerous complications of COVID-19 [14]. The vascular component is not confined to the disease but also its prevention.

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A rare clinical constellation associated with vaccines against SARS-CoV-2 is cerebral venous thromboembolism and thrombocytopenia that has resulted in death [15]. Again, direct links between infection and abnormal clotting remain vague in patients with suspected vaccine-induced thrombosis and thrombocytopenia. Many clinical teams now share this interesting hypothesis that highlights the key underlying role of the vasculature; it provides hands-on guidance for effective therapeutic strategies against this still not particularly well-understood infection.

ANIMAL MODELS IN COVID-19 RESEARCH AND THEIR LIMITATIONS

Animal models are widely used in SARS-CoV-2 research to understand systemic mechanisms of infection and pathogenesis. Several species have been used in various studies, such as hamsters, African green monkeys, rhesus macaques, minks, ferrets, cats, dogs and transgenic mice [16]. For example, mouse models showed the pathomechanism whereby the SARS-CoV-2 spike protein 1 (S1) interacting with Toll-like receptor 4 (TLR4), resulting in an innate immune response to the virus [17]. This increased inflammatory state leads to cardiac hypertrophy and heart damage. The limitation of animal models is that strict regulation is required to keep the 3R rule: replace, reduce and refine. Besides the general drawbacks of in vivo models, there is another problem that SARS-CoV-2 only infects cells with human-like angiotensin-converting enzyme 2 (ACE2) receptors. Three sequences of ACE2 receptors must be present for the virus to infiltrate host cells: the first α-helix of the protein containing Lys31 and Tyr41 and the amino-acid sequences between 82–84 and 353–357 [18]. This further narrows the applicable species and brings up ethical dilemmas about whether to use transgenic rodents or wild animals that were not kept in general for research purposes like bats, masked palm civets, ferrets, raccoon dogs, and minks [16, 19]. Arguments against in vitro and in vivo experiments are shown in Table 1.

DISEASE MODELLING AND PERSONALISED MEDICINE USING HUMAN PLURIPOTENT STEM CELLS

Human cell lines can provide an additional platform to study pathomechanism. However, human cardiomyocytes and endothelial cells from the myocardium are difficult to culture, costly to obtain and limited in number. Furthermore, for drug discovery in COVID-19 therapies, alternatives for these cell types represent a great immediate need. In line with this, the concept of personalised medicine has been recently articulated, which calls for basing medical treatment on a patient's genetic makeup and specific disease characteristics to increase therapeutic benefits and decrease adverse effects [21]. The drug discovery also translates the concept even in SARS-CoV-2 into the related premise of "precision medicine". Precision medicine aims to integrate both clinical and molecular information to understand the biological basis of disease better and select better disease targets. These new approaches or treatments focus on a particular subgroup of patients with certain genotypic and/or phenotypic characteristics that make them more likely to benefit or, conversely, to experience side effects. To address this need, immortalised human cells [16], embryonic stem cells (hESC) [22], induced pluripotent cell (hiPSC)-derived gut [19, 20, 23], brain [24], lung [25], cardiac [22, 26–29] and other cells and organoids [22, 26, 27, 29]

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Table 1. Limitations of in vitro and in vivo in SARS-CoV-2 research [16, 20]

Arguments against		Possible solution
IN VITRO	It does not show whether or not the observed cells are major targets of SARS-CoV-2	Analysis of primary patient-derived samples
	It does not show systemic reactions like immune response	Include immune system components in the model
	Only shows direct toxicity and damages	Comparing results to primary patient-derived samples
IN VIVO	Most model animals do not produce clinical symptoms of SARS-CoV-2 infection when infected	Transgenic animals
	In vitro is ethically more acceptable	Follow the rule of 3R
	Using appropriate species for the SARS-CoV-2 infection model can be problematic (wild species, time and resource consuming, hard to translate to human disease)	Complementary in vitro studies

have been proposed. These can be infected with the virus or treated with drugs that induce inflammation, which can help in understanding how SARS-CoV-2 works and causes diseases like cardiac dysfunctions [30], thrombosis [31] or cytokine storm [26]. A collection of different drug research on cardiovascular cell and organ models are shown in Table 2.

Important details about viral inclusion can be understood by investigating molecular mechanisms, which is easier to observe in vitro. Studies have shown that in addition to ACE2, Transmembrane Serine Protease 2 (TMPRSS2), cathepsin-L (CTSL) and cathepsin-B (CTSB) can also be potential targets for COVID-19 treatment [27]. Regarding the cardiovascular system, the potential of stem cell-derived cardiomyocytes for disease modelling has been enhanced by realising that cardiomyocytes from human induced pluripotent stem cells (hiPSC-CM) can be obtained with disease-specific genotypes and phenotypes. These cells are suggested to have many of the properties of authentic cells, and their phenotypes provide validation that characteristics of the disease can be reproduced *in vitro*. An important development is to use these cells to model long-term disease processes. In this regard, the pluripotent stem cell-derived cells have the critical advantages of stability in culture over months and greater ease of genetic manipulation, providing immediate superiority over the classical rodent neonate preparation in addition to their human genotype. A game-changing advantage of the hiPSC-CM is their derivation from a wide range of patients and healthy subjects, allowing them to dissect genotype/phenotype relationships.

3D and multicellular models

The SARS-CoV-2 has a clear vasculature disrupting effect; thus, endothelial cells may play a key role in SARS-CoV-2 pathogenesis; however, the exact underlying mechanisms remain unknown. To support their participation, Schimmel et al. found endothelial cells actively replicating the virus in a monoculture [33]. Yet, the presence of epithelial cells in a co-culture setting inhibited

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Table 2. hiPSC and human embryonic stem cells derived cardiovascular cell cultures and organoids in COVID-19 drug research (Abbreviations: CM: Cardiomyocytes, EC: Endothelial cells, CF: Cardiac fibroblasts, FB: Fibroblasts, PC: Pericytes, SMC: Smooth muscle cells, ECC: epicardial cells, TLR: Toll-like receptor, ATR: ataxia telangiectasia and Rad3 related, BET: Bromodomain and extra-terminal motif)

Model	Origin	Cell types	Drugs and treatments	Mechanism of effect	Efficiency against SARS-CoV-2	Ref
Human cardiomyocytes	hiPSC	CM	Remdesivir	Decrease viral RNA production	Reduced spike protein expression ($P < 0.05$)	[32]
			N-acetyl-L-leucyl-L-leucyl-L methionine	Potent inhibitor of cathepsin-L and B	Reduced spike protein expression ($P < 0.05$)	
			Recombinant human ACE2 protein	Inhibition of virus binding to host cells	Reduced spike protein expression ($P < 0.0001$)	
			ACE2 neutralising antibody	Inhibition of virus binding to host cells	Reduced spike protein expression ($P < 0.0001$)	
Human cardiac cells	hiPSC	CM, CF, EC	ACE2 blocking antibody	Inhibition of virus binding to host cells	Reduced viral detection ($P < 0.05$)	[27]
			Apilimod	Inhibition of phosphotransferase activity of TLR-4 regulator	Reduced viral detection ($P < 0.05$)	
			Bafilomycin	Autophagy inhibitor	Reduced viral detection ($P < 0.05$)	
			Z-Phe-Tyr(tBu)-diazomethylketone	Cathepsin-L inhibitor	Reduced viral detection ($P < 0.01$)	
			Aprotinin	Small protein bovine pancreatic trypsin inhibitor	Not significant	
			CA-074	Cathepsin B inhibitor	Not significant	
			Camostat	Anti-inflammatory, antifibrotic, and potential antiviral	Not significant	
			Remdesivir	Decrease viral RNA production	Reduced viral detection ($P < 0.01$)	
			Interferon (IFN)-β	Modulate functions of the immune system (antiviral)	Reduced viral detection ($P < 0.01$)	

(continued)

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Table 2. Continued

Model	Origin	Cell types	Drugs and treatments	Mechanism of effect	Efficiency against SARS-CoV-2	Ref
			E-64d	Cathepsin inhibitor; interferes with autolysosomal digestion	Reduced viral detection ($P < 0.01$)	
Human cardiomyocytes	hiPSC	CM	Berzosertib	Inhibitor of ATR enzyme	Rescued beating, reduced inflammation, apoptosis and viral detection	[28]
			Remdesivir	Decrease viral RNA production	Rescued beating, reduced viral detection	
			Hydroxychloroquine	Inhibits stimulation of the TLR 9 family receptors	Rescued beating, reduced apoptosis and viral detection	
Human cardiac organoids	hESC and hiPSC	CM, ECC, FB, PC, EC	INCB054329	BET inhibitor	Decreases hace2 expression and reduces SARS-CoV-2 detection	[22]
Human blood vessel organoids	hiPSC	EC, PC	Human recombinant soluble ACE2	Inhibition of virus binding to host cells	Blocks early entry of SARS-CoV-2 infections in host cells	[29]

the appearance of detectable viral proteins in the endothelial cells. Furthermore, the endothelium remains uninfected in vivo. These conflicting results confirm that a better understanding warrants complex, multicellular models; 3D models mimicking the in vivo-like tissue structure and cellular composition can offer such experimental tools. We and others have shown that endothelial cells from stem cell origin (hPSC-derived cells or endothelial colony-forming cells, *ECFC*) along with leukocytes can be used to screen drug toxicity. These improved bioassays are applied to cytokine storm modelling, detect cytokine storm-inducing drugs, biologics, and other viral triggers [34]. Also, using these cells in a dish reflects innate immune receptor-mediated viral responsiveness, such as those with NOD1 and the associated RIP2 signalling [35]. Understanding the mechanisms of these unwanted innate immune receptor (TLR or NOD)-mediated vascular inflammation may offer a potential therapeutic or preventive advantage. Our transcriptomic analysis of stem cell-derived endothelial cells also showed highly abundant expression of ACE2 (Foldes et al., unpublished observation). ACE and ACE2 ratios can also explain the heterogeneity of cases among COVID-19 patients. A study has shown that a higher ACE/ACE2 ratio might be a factor of the severity of COVID-19 [36]. An increased ACE/ACE2 ratio, more prevalent in stem cells derived from older patients [111], causes increased oxidative stress and inflammation leading to cytokine storm and ARDS. Therefore, stem cells from older donors have insufficient immunomodulatory and regenerative functions.

To identify small molecule inhibitors of infection and subsequent endocytosis, the combination of 3D cellular models with a quantitative automated high-content imaging and analysis system appears to be the most appropriate method (shown in Fig. 1). Traditionally, high-content analyses have been performed on two-dimensional images due to the prohibitively complex 3D high-content image processing. However, virus-induced cell death, endocytosis into endothelial cells, and overall infection quantitation may require 3D measurements. This can be facilitated by spinning disk confocal high content microscopy if equipped with optical sectioning and suitable subcellular resolution capability. We can explore the timing and severity of apoptosis, cellular integrity of primary (for example, human umbilical vein endothelial cells, HUVEC) or stem cell-derived endothelial cells and transcriptional and intracellular/membrane-bound factors involved in the inflammatory processes. Collection and image analysis of infection-related features can be derived from a preliminary workflow designed especially for hPSC-CM and hPSC-CM phenotype evaluations. For validation of clinical value for these in vitro findings from pluripotent stem cell-facilitated drug screening and 3D disease modelling, we can run a head-to-head comparison with *ex vivo*, cellular, histological and RNA samples from blood vessels affected by chronic/acute inflammation. This allows us to get a primary picture of vascular events and responsiveness during the inflammation *in vitro* and *ex vivo*.

CLINICAL TRIALS AND STEM CELL THERAPIES

Numerous drugs have been in the spotlight in the search for new treatments for SARS-CoV-2 infection, but the COVID-19 Treatment Guidelines Panel recommends against them for various reasons. For example, convalescent plasma therapy appears to be effective in severe cases but not significant in milder cases; furthermore, the plasma sources are limited and expensive. Additionally, most interferon treatments are ineffective in clinical studies. Some promising drugs

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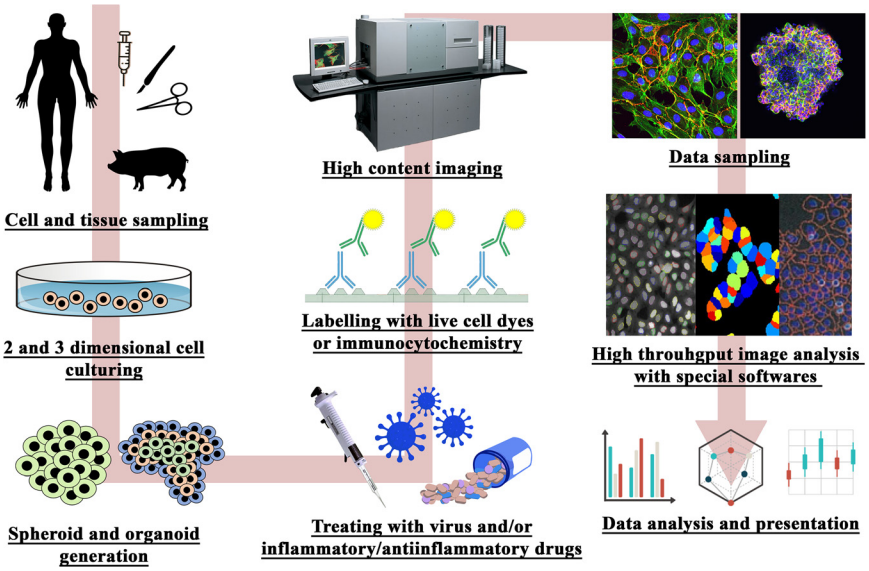

Fig. 1. Workflow of stem cell-based disease model and drug development assay using High Content Screening (HCS) analysis. This method can be personalised by collecting cells and tissues from patients or specific model animals. Primary cell culture or reprogramed induced pluripotent stem cells can then be used in assays to investigate the pathomechanism of the virus or cytokine storm or to find new treatments with the help of immunocytochemistry and High content imaging. At the end of the process, a vast amount of raw data goes through software analysis to create statistics

have already been used as antiviral compounds to treat diseases such as malaria or HIV. The detailed list of ongoing clinical trials for different types of COVID-19 medications and vaccines is available on the website of Milken Institute [37]. Stem cells have been proposed not only for disease modelling but also as a logical measure to tackle virus-induced immune responses directly. For COVID-19 therapy, more than 130 stem cell-based clinical trials have been registered at clinicaltrials.gov to date. Mesenchymal stem cells have received major attention as potential cell therapy products. MSCs have also produced growth factors and other humoral factors for tissue repair. MSCs are safe and well-tolerated in clinical use, with limited or no adverse effects in systemic lupus erythematosus or graft-versus-host disease [38, 39]. Recent studies have aimed at leveraging their immunosuppressive activity, including the inhibition of adaptive immune cell activation and blockage of mononuclear inflammatory infiltration, dominated by lymphocytes at the damaged tissues. Intravenous administration of MSCs in moderate or severe COVID-19 patients was also safe and well-tolerated [40]. In a later phase 2 double-blind, randomised, controlled trials, their efficacy to control inflammation and pulmonary fibrosis and reduce mortality was also tested. Yet, the COVID-19 Treatment Guidelines Panel [41] recommends using MSC to treat COVID-19 only in clinical trial settings. In addition to MSC [42], other stem cell types, such as hESC-derived immunity- and matrix-regulatory cells (hESC-IMRCs), have also been utilised to treat COVID-19 patients in first-in-man studies [43].

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CONCLUSION

SARS-CoV-2 showed us that we still have much to learn about viruses. We also need to push back the illegal trade of wild animals as pets and food to prevent future outbreaks like COVID-19. Poor cardiovascular health is a significant risk factor of severe COVID-19 cases as arrhythmia, cardiomyopathy, myocarditis, thrombosis, and cardiac arrest are the most frequent terminal events of COVID-19.

In vitro methods are important in COVID-19 research due to the species specificity of ACE2. Most popular animal models were unaffected by the virus or had different or less severe symptoms than humans. To create viable models for COVID-19, hiPSC derived cardiovascular cells were used, from monocultures to 3D organoids. Big data collected from assays using these in vitro models and high throughput methods can increase the potential to study cardiovascular diseases and find treatment.

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