Modeling Cardiac SARS-CoV-2 Infection with Human Pluripotent Stem Cells

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Abstract
Although SARS-CoV-2, the causative virus of the global COVID-19 pandemic, primarily affects the respiratory tract, it is now recognized to have broad multi-organ tropism and systemic effects. Early reports indicated that SARS-CoV-2 infection could lead to cardiac damage, suggesting the virus may directly impact the heart. Cardiac cell types derived from human pluripotent stem cells (hPSCs) enable mechanistic interrogation of SARS-CoV-2 infection in human cardiac tissue.

Purpose of Review
To review the studies published since the emergence of the COVID-19 pandemic which utilize hPSCs and their cardiovascular derivative cell types to interrogate the tropism and effects of SARS-CoV-2 infection in the heart, as well as explore potential therapies.

Recent Findings
Recent studies reveal that SARS-CoV-2 is capable of infecting and replicating within hPSC-derived cardiomyocytes and sinoatrial nodal cells, but not as extensively in their non-parenchymal counterparts. Additionally, they show striking viral effects on cardiomyocyte structure, transcriptional activity, and survival, along with potential mechanisms and therapeutic targets.

Summary
Cardiac models derived from hPSCs are a viable platform to study the impact of SARS-CoV-2 on cardiac tissue and may lead to novel mechanistic insight as well as therapeutic interventions.

Keywords
Pluripotent stem cells · iPSC · SARS-CoV-2 · COVID-19 · Disease modeling · Cardiovascular diseases

Introduction
Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, was first reported in December 2019. As of April 2022, more than 567 million cases of COVID-19 have been reported globally, resulting in over 6.3 million deaths [1]. SARS-CoV-2, a member of the beta coronavirus family, is a positive sense single-strand virus related to SARS-CoV and Middle Eastern respiratory syndrome-related coronavirus (MERS-CoV). The spike glycoprotein studding the outer surface gives the virus its name due to its crown-like appearance in electron micrographs. The spike protein also determines the viral tropism, as it binds to angiotensin-converting enzyme 2 (ACE2) to enter target cells, among other putative entry factors [2]. ACE2 is expressed widely throughout the body, including in the heart [3, 4]. Cardiac expression of ACE2 increases in patients with heart failure, which may confer greater SARS-CoV-2 infection risk to patients with pre-existing heart disease [5].
COVID-19 is primarily a respiratory disease, with major symptoms including shortness of breath, fever, cough, and loss of taste and smell. These initial symptoms can progress into acute respiratory distress syndrome (ARDS), which is a serious complication with high mortality rates, often requiring mechanical ventilation [6]. However, COVID-19 induces serious effects across multiple organs, including the brain [7, 8], the gastrointestinal tract [9], the urinary system, and the cardiovascular system [10–13]. Early reports indicated severe cardiac symptoms in up to 20% of COVID-19 patients, with manifestations including myocardial injury, arrhythmia, acute coronary syndrome, venous thrombembolism, myocarditis, abnormal echocardiograms and cardiac MRIs, and even heart failure [14–22].

While rare, postmortem COVID-19 clinical reports have detected viral particles within cardiomyocytes via transmission electron microscopy, even in patients without cardiac complications, suggesting the possibility of direct infection [23–26]. However, systemic effects from infection could also contribute to impaired cardiac function. SARS-CoV-2 triggers a hyperactive immune response, or cytokine storm, which may damage the lungs and extrapulmonary tissue. Strain from pulmonary damage-induced hypoxia, microvascular thrombosis, or the cytokine storm itself may all contribute to the myocardial injury observed in patients.

There is a critical need to elucidate the pathogenesis of SARS-CoV-2 in the cardiovascular system and to develop effective cardioprotective therapeutics. However, there is no way to tease apart direct versus indirect effects of infection in patient cardiovascular tissue. The only way to directly assess viral presence in the heart of patients is through endomyocardial biopsies, which have been rare in the setting of COVID-19. Animal models, such as mice, are not susceptible to SARS-CoV-2, so human ACE2 must be overexpressed to enable infection. Additionally, animal models fail to recapitulate many aspects of human cardiovascular physiology, limiting their translation to effective clinical therapeutics. The heart is one of the most inaccessible organs, and even if primary cardiac tissue is obtained, it is difficult to maintain in culture. Pluripotent stem cells (PSCs) are derived from human cells and can be differentiated into almost any cell type in the body, including cardiac cell types. Cardiac cells derived from human-induced pluripotent stem cells (hiPSCs) or human embryonic stem cells (hESCs) have become powerful tools for cardiac disease modeling. Both two-dimensional (2D) and three-dimensional systems (3D) comprised of multiple cell types have been used to model and interrogate myriad cardiac disease, including congenital heart defects, cardiomyopathy, and arrhythmias [27]. Stem cell-derived cardiac tissue has also been used to model viral myocarditis induced by coxsackievirus B3 [28]. Thus, ex vivo human stem cell models offer a promising human platform to directly interrogate the effects of SARS-CoV-2 infection in the heart, which is required to develop effective cardioprotective therapies. Here, we summarize the use of PSC-based models to investigate viral tropism, cellular effects, and therapeutic development.

### Modeling SARS-CoV-2 Infection in Cardiac Cell Types Using hPSCs

The severity and array of cardiac complications in COVID-19 patients have posed a significant question to the field: does SARS-CoV-2 directly infect cardiac cells, and if so, which cell types are susceptible? Multiple studies addressed this question by differentiating PSCs into many of the cell types found in the heart, including cardiomyocytes (CMs), cardiac fibroblasts (CFs), endothelial cells (ECs), macrophages (MPs), sinoatrial nodal cells (SANs), and vascular cells (Table 1) (Fig. 1A). Across the board, studies found that PSC-CMs were highly susceptible to viral infection, as measured by qPCR for nucleocapsid or spike RNA or immunostaining for double-stranded RNA (dsRNA), which is a unique intermediate formed during viral replication [29, 30•, 31–33, 34•]. Infected cultures of PSC-CMs displayed severe cytotoxic effects within 24 to 72 h, including fragmentation, apoptosis, and impairment in contraction and beating [33, 34•, 35]. Sinoatrial nodal cells and capillary organoids were also found to be susceptible to infection, leading to disrupted calcium signaling and ferroptosis in the former [36, 37]. However, none of the other cell types tested (CFs, ECs, MPs) showed significant signs of direct viral infection. There was slight luciferase expression in PSC-ECs following infection with pseudotyped virus, but no viral proteins were found with immunostaining [29]. This insusceptibility is striking given the critical role of these cell types in cardiac pathogenesis, as well as the clear signs of cytotoxicity observed in these cells after viral exposure. Despite lack of dsRNA, spike or nucleocapsid upon staining, both CFs and ECs exhibited increased cell fragmentation and death after incubation with SARS-CoV-2 [34•]. Heat-inactivated virus did not have any toxic effect on the cells, suggesting that the cytotoxicity is caused by another mechanism of live virus, such as abortive infection or paracrine signaling from surrounding infected cells. However, further investigation is required to understand how cardiac stroma responds to SARS-CoV-2 and contributes to the cardiac dysfunction observed in patients.

The high susceptibility of PSC-CMs and PSC-SANs suggests the possibility of direct infection and enables a promising platform to rigorously interrogate the pathogenesis of SARS-CoV-2 infection. Multiple groups observed consistent pathological effects in PSC-CMs within 24 h of infection, including sarcomeric disruption, cessation of coordinated beating, and a shift to glycolytic metabolism [30•, 33,
| References | Title                                                                 | Cell type | Cell model | Cell types | Permissivity to SARS-CoV-2 | Cell/tissue effects                                                                 | Effective compounds                                                                 |
|------------|----------------------------------------------------------------------|-----------|------------|------------|----------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Bailey JACC. 2021 [32] | SARS-CoV-2 Infects Human Engineered Heart Tissues and Models COVID-19 Myocarditis | hiPS      | 2D, EHTs   | Cardiomyocytes (CMs) | Yes | Cytokine production,  myeloid-rich inflammatory infiltrate, contractile deficits, sarcomere disruption, cell death | E64d (CTSL inhibitor), camostat mesylate (TMPRSS2 inhibitor) was not effective |
| Bojkova ESC. 2020 [31] | SARS-CoV-2 infects and induces cytotoxic events in human cardiomyocytes | hiPS      | 2D, 3D cardiospheres, living human explants, biopsy | Cardiomyocytes | Yes | Loss of alpha-actinin, sarcomeric disruption, cessation of beating, activation of interferon pathways | thACE2, ACE2 neutralizing antibody, remdesivir, N-acetyl-L-leucyl-L-leucyl-L-23 methionine (ALLM) |
| Yang Stem Cell Reports 2021 [40] | Cardiomyocytes recruit monocytes upon SARS-CoV-2 infection by secreting CCL2/ | hiPS and hES | 2D, 2D co-culture, adult CMs, hamster, autopsy | Cardiomyocytes, Monocytes, Macrophages | Yes | Induction of chemokines and inflammatory signaling, CCL2 secretion, monocyte recruitment, macrophage infiltration, decreased expression of CM genes, increased expression of ROS | Macrophages |
| Garcia Cell Rep. 2021 [45] | Antiviral drug screen identifies DNA damage response inhibitor as potent blocker of SARS-CoV-2 replication | hiPS      | 2D         | Cardiomyocytes | Yes | Reduced beating, non-synchronous twitching, disrupted troponin T fibers | Berzosertib (DDR inhibitor), remdesivir |
| Han Circ. Research. 2022 [36] | SARS-CoV-2 Infection Induces Ferroptosis of Sinoatrial Node Pacemaker Cells | hESCs     | 2D, hamster | Sinoatrial nodal cells | Yes | Dysfunction, ferroptosis, upregulation of chemokine and inflammatory genes, disrupted calcium cycling | Deferoxamine (ferroptosis inhibitor), imatinib |
| Huang HAL 2022 [38] | Sars-Cov-2 Spike Protein-Induced Damage of hiPSC-Derived Cardiomyocytes | hiPS      | 2D patch   | Cardiomyocytes | Yes | Sarcomere disruption, dispersed connexin 43 expression, transiently impaired beating, nuclear fragmentation | NA |
| References | Title | Cell type | Cell model | Cell types | Permissivity to SARS-CoV-2 | Cell/tissue effects | Effective compounds |
|------------|-------|-----------|------------|------------|---------------------------|---------------------|---------------------|
| Li *Proc Natl Acad Sci.* 2021 [41] | SARS-CoV-2 induces double-stranded RNA-mediated innate immune responses in respiratory epithelial-derived cells and cardiomyocytes | hiPS | 2D | Cardiomyocytes | Yes | Activation of oligoadenylate synthase ribonuclease L and protein kinase R antiviral pathways, minimal interferon stimulation | NA |
| Marchiano *Stem Cell Rep.* 2021 [33] | SARS-CoV-2 Infects Human Pluripotent Stem Cell-Derived Cardiomyocytes, Impairing Electrical and Mechanical Function | hiPS and hES | 2D, 3D EHTs | Cardiomyocytes, Smooth muscle cells | Yes, Minimal | Shift to glycolytic metabolism, upregulated chromatin modification and RNA splicing pathways, downregulated oxphos and mitochondrial pathways, disrupted sarcomeres, impaired electrophysiology and contraction, cell death | NA |
| Mills *Cell.* 2021 [35] | BET inhibition blocks inflammation-induced cardiac dysfunction and SARS-CoV-2 infection | hiPS and hES | 2D, 3D human cardiac organoids (hCOs) | Cardiomyocytes, Epicardial cells, Fibroblasts/pericytes, Endothelial cells | Yes | Systolic dysfunction (impaired contraction), diastolic dysfunction (prolonged time to peak) | INCB054329 and JQ-1 (BETis), apabetalone (BD-2 specific BETi) |
| Monteil, V. *Cell.* 2020 [37] | Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2 | hiPS | 3D capillary organoids | Capillary organoids | Yes | Not explored | hrsACE2 |
| Perez-Bermejo *Sci Transl Med.* 2021 [34•] | SARS-CoV-2 infection of human iPSC-derived cardiomyocytes reflects cytopathic features in hearts of patients with COVID-19 | hiPS | 2D, 2D co-culture | Cardiomyocytes, Cardiac fibroblasts, Endothelial cells | Yes, No | Fragmented cell bodies, myofibrillar fragmentation, proteosomal dysfunction, reduced myofibrillar and metabolic expression, nuclear loss, cell death | ACE2 neutralizing antibody, E-64d, remdesivir, IFN-β, apilimod, bafilomycin, Z-FY(tBu)-DMK |
| References          | Title                                                                 | Cell type | Cell model | Cell types | Permissivity to SARS-CoV-2 | Cell/tissue effects                                                                 | Effective compounds                                      |
|---------------------|-----------------------------------------------------------------------|-----------|------------|------------|-----------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------|
| Samuel *Cell Stem Cell*. 2020 [44] | Androgen Signaling Regulates SARS-CoV-2 Receptor Levels and is Associated with Severe COVID-19 Symptoms in Men | hiPS      | 2D         | Cardiomyocytes | Yes                         | Not explored                                                                        | Ketoconazole, finasteride, dutasteride                   |
| Sharma *Cell Rep Med*. 2020 [30•] | Human iPSC-Derived Cardiomyocytes Are Susceptible to SARS-CoV-2 Infection | hiPS      | 2D         | Cardiomyocytes | Yes                         | Cessation of beating, apoptosis, innate immune and antiviral response, impaired metabolism | Anti-ACE2 antibody                                        |
| Williams *Comm Bio*. 2021 [43] | Human embryonic stem cell-derived cardiomyocyte platform screens inhibitors of SARS-CoV-2 infection | hESCs     | 2D         | Cardiomyocytes | Yes (pseudotyped)            | Minimal death, minimal impact on beating                                              | Anti-ACE2 antibody, DX600, camostat, E64d, benzotropine |
| Yang *Cell Stem Cell*. 2020 [29] | A Human Pluripotent Stem Cell-based Platform to Study SARS-CoV-2 Tropism and Model Virus Infection in Human Cells and Organoid | hES       | 2D         | Cardiomyocytes, Endothelial cells | Yes, No                      | Not explored                                                                        | NA                                                      |
| Yang *Circ Res*. 2021 [42•] | An Immuno-Cardiac Model for Macrophage-Mediated Inflammation in COVID-19 Hearts | hiPS      | 2D, 2D co-culture | Cardiomyocytes, Macrophages | Yes, No                     | Increased ROS, increased apoptosis                                                   | Ranolazine, tofacitinib                                  |
Addition of spike protein alone was enough to induce altered localization of connexin 43, sarcomeric disruption, and impaired beating [38]. Expressions of genes forming the sarcomeric units that enable cardiac contraction were decreased after infection, suggesting loss of these proteins. Sarcomeres are striated rope-like structures composed of overlapping thick and thin filaments, formed primarily by alpha-actinin and troponin proteins, respectively. Viral infection induced physical fragmentation of these sarcomeres into strikingly periodic subunits, untethered and unaligned, in up to 20% of cells [34•]. These fragmented units were composed of one alpha-actinin 2 unit (comprising the Z band) flanked by two troponin T units, suggesting that the thick and thin bands of the sarcomere were cleaved apart. While most sarcomeric genes were downregulated after infection, multiple myosin heavy chains were conversely upregulated. Myosin heavy chain 6 (MYH6) contains an LKGGK site that overlaps with the cut site for the SARS-CoV-2 papain-like protease that cleaves proteins during viral processing. Thus, it is possible that off-target proteolytic activity from the papain-like protease leads to the cleavage of MYH6 and the subsequent dissolution of sarcomeres, potentially contributing to impaired contractility. One group did find that papain-like protease can cleave bovine MYH6 in vitro [39], but further investigation is needed to determine if something similar occurs in patients.

Alongside contractile deficits, infected PSC-CMs also displayed increased cytokine and chemokine production and inflammatory signaling [32, 40]. While PSC-CMs increased the expression of inflammatory genes after infection, they barely stimulated the interferon response, which is one of the earliest effective antiviral responses [41]. This is in line with previous studies that have found that SARS-CoV-2 suppresses the host antiviral response. However, PSC-CMs were found to activate other antiviral pathways, such as oligoadenylate synthase ribonuclease L and protein kinase R [41]. Infected PSC-CMs were also found to secrete CCL2, a chemokine that recruits monocytes, which then differentiates into macrophages to fight the infection [40]. While macrophages cannot be infected themselves, they secrete IL6 and TNFa after viral exposure, which are two of the primary cytokines associated with severe COVID-19. These cytokines in turn lead to increased ROS and apoptosis in PSC-CMs [42•].

Arrhythmias are common cardiac complications in COVID-19 and may be caused by damage to sinoatrial nodal cells, the pacemakers of the heart. PSC-SANs are productively infected by SARS-CoV-2 and generate increased chemokines, like CCL2, inflammation signaling, and ROS production [36]. Unlike PSC-CMs, PSC-SANs do not show increased apoptosis, but they do manifest increased ferroptosis, as indicated by decreased GXP4 expression. SARS-CoV-2-induced ferroptosis has not been observed in other cell types, highlighting the importance of cell type-specific disease modeling.
Investigating Therapeutic Development with hiPSC-Derived Models

Due to their physiological relevance and accessible nature, PSC-CMs are an ideal platform to screen and interrogate potential cardioprotective treatments (Fig. 1B). Multiple studies confirmed the necessity of ACE2 as an entry factor by blocking ACE2 with recombinant ACE2 protein or a neutralizing antibody, which led to significantly decreased infection [34•, 37, 43]. ACE2 serves important protective functions in cardiovascular biology, and ACE2 levels decrease after infection with SARS-CoV-2, which may further exacerbate cardiac symptoms. However, blocking the other putative receptors TMRPSS2 (via camostat mesylate) and furin did not reduce infection in PSC-CMs [32, 34•], indicating these proteins are not essential for viral entry. Inhibiting cathepsins, which are involved in endolysosomal processing, via E64d or acetyl-L-leucyl-L-leucyl-L-23 methionine (ALLM) led to a marked reduction in viral entry [31, 34•]. Inhibition of cathepsin B via CA-074 did not prevent infection, while inhibition of cathepsin L with Z-Phe-Tyr(tBu)-diazomethylketone (Z-FY-DK) did, suggesting that the virus enters PSC-CMs through a cathepsin L (CTSL)-dependent endosomal route [34•]. This differs from other cell types in the body, such as alveolar cells, which do depend on TMRPSS2, highlighting the importance of using cell type-specific platforms to assess drug efficacy.

As ACE2 is a necessary receptor for viral entry, one group screened an FDA-approved library for compounds that reduce ACE2 expression in PSC-CMs. Most hits converged on androgen receptor (AR) signaling, steroid metabolism, and peptidase activity [44]. Finasteride and dutasteride inhibit AR signaling by blocking 5-alpha reductases, leading to a concomitant decrease in ACE2 and TMRPSS2. These antiandrogenic drugs lowered SARS-CoV-2 infection, an unexpected outcome given that being male and having prostate cancer are both risk factors for severe COVID-19. These ACE2 modulating effects were not seen in Vero cell lines, indicating the importance of using more physiologically relevant screening platforms.

After viral entry, inhibition of viral replication with remdesivir was found to reduce infection [31, 34•, 45]. Through high-throughput screens, multiple compounds targeting different aspects of viral infection have been found to reduce infection or ameliorate the downstream consequences. BET inhibitors, such as INCB054329 and JQ-1, were found to prevent the cardiac dysfunction caused by viral-induced cytokine storm [35]. One study cultured PSC-CMs with epicardial cells, CFs, and ECs along with TNFα, IFNγ, and IL-1β to recapitulate the cytokine storm. They found that cytokines alone could induce diastolic and systolic dysfunction through a BRD4-dependent mechanism, which could be blocked through BET inhibition [35]. In addition to adding cytokines, multiple studies attempted to model the immune cell infiltration observed in the cardiac tissue of COVID-19 patients by co-culturing PSC-CMs with PSC-derived macrophages and monocytes [40, 42•]. Infected PSC-CMs secrete CCL2, which recruits macrophages to the site of infection. The macrophages then secrete inflammatory chemokines and cytokines, such as IL-6 and TNFα, and trigger increased ROS production, which may contribute to the increased apoptosis observed in PSC-CMs after infection. Inhibiting the JAK/STAT pathway with ranolazine and tofacitinib decreased ROS and apoptosis by inhibiting ROS production and blocking IL-6 and TNFα secretion, respectively.

Kinase inhibitor screens identified effective compounds that modulated the mTOR-P13K-AKT and DNA damage response (DDR) pathways. The ATR kinase inhibitor berzosertib blocked SARS-CoV-2 post-entry in PSC-CMs, rescued impaired function, and had a synergistic effect when combined with remdesivir [45]. Berzosertib was also able to inhibit the closely related SARS-CoV-1 and MERS viral strains, indicating it targets a conserved aspect of the viral life cycle.

As the primary toxicity seen in PSC-SANs was viral-induced ferroptosis, a screen for ferroptosis inhibitors found deferoxamine and imatinib to be most effective in reducing toxicity in PSC-SANs [36]. As these studies show, SARS-CoV-2 not only differentially infects different cardiac cell types, but it also induces differential toxic effects on each. Thus, it is critical to use cell type-specific platforms to screen for viral-induced toxicity and effective therapeutics.

Limitations of hiPSC Cardiac Systems to Study SARS-CoV-2 Infection

While PSCs are a powerful platform to model and interrogate disease mechanisms, they have limitations for disease modeling. As iPSCs are reprogrammed from adult cells, there may be epigenetic memory leftover from prior cell states. There can also be significant variability between batches of differentiations, particularly in 3D cultures, which can confound reproducibility. It is also challenging to control the various cell types that emerge during each differentiation, leading to a variable mix of ventricular, atrial, and non-parenchymal cell types. This variability may also arise from differences in differentiation protocols, reagents, and conditions used by each lab. Some groups reduce cell type variability by performing targeted differentiation into atrial or ventricular cardiomyocytes, enabling chamber-specific modeling [46].
Most cell types derived from PSCs are more immature than their adult counterparts given their pluripotent starting point. However, this challenge is particularly acute in the cardiac field, as PSC-derived cardiomyocytes fail to progress beyond a fetal phenotype, despite extended time in culture. While the field is pursuing many avenues to address this issue, including 3D culture, electrical stimulation, and metabolic conditioning, the immaturity barrier limits the use of PSCs to model adult cardiac tissue [47, 48]. These immature phenotypes are especially limiting for models of COVID-19, where cardiac complications and mortality are often seen in adults and are associated with older age.

Finally, PSC-derived models are often composed of one or more cell types but lack the complexity of an in vivo organ system. Most in vitro models lack an immune system, such as T cells and circulating monocytes. As the immune response and resulting cytokine storm is a critical feature of COVID-19, this over-simplicity limits the utility of PSC-derived systems. Some studies have attempted to address this through co-culture with macrophages [42••], but it is still unclear how closely that recapitulates the presence of the immune system.

Conclusions

SARS-CoV-2 and the resulting COVID-19 pandemic have completely altered the landscape of the world, leading to a heartbreaking number of cases and deaths. While primarily a respiratory disease, many organs are severely impacted by infection, including the heart. As every cell type is different in its physiology and response to viral infection, employing a cell type-specific and physiologically relevant model is critical when studying viral infection. Human pluripotent stem cell-based models are promising platforms that have been used to model cardiac-specific SARS-CoV-2 pathogenesis and interrogate potential cardioprotective therapies. These models have enabled the discovery of striking cardiac-specific effects of infection and the identification of many classes of therapeutic compounds that reduce infection and rescue function. Together, these findings would be immensely beneficial to patients if translated to the clinic.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent No approval of research ethics committees was required to accomplish the goals of this article because no studies with human or animal subjects were performed by any of the authors.

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