




# Elucidating effects of environmental exposure using human-induced pluripotent stem cell disease modeling

Mark Chandy<sup>1,2,3,\*</sup> , Detlef Obal<sup>1,4</sup>  & Joseph C Wu<sup>1,5,\*\*</sup> 

## Abstract

Induced pluripotent stem cells (iPSCs) are a powerful modeling system for medical discovery and translational research. To date, most studies have focused on the potential for iPSCs for regenerative medicine, drug discovery, and disease modeling. However, iPSCs are also a powerful modeling system to investigate the effects of environmental exposure on the cardiovascular system. With the emergence of e-cigarettes, air pollution, marijuana use, opioids, and microplastics as novel cardiovascular risk factors, iPSCs have the potential for elucidating the effects of these toxins on the body using conventional two-dimensional (2D) arrays and more advanced tissue engineering approaches with organoid and other three-dimensional (3D) models. The effects of these environmental factors may be enhanced by genetic polymorphisms that make some individuals more susceptible to the effects of toxins. iPSC disease modeling may reveal important gene–environment interactions that exacerbate cardiovascular disease and predispose some individuals to adverse outcomes. Thus, iPSCs and gene-editing techniques could play a pivotal role in elucidating the mechanisms of gene–environment interactions and understanding individual variability in susceptibility to environmental effects.

**Keywords** cannabis; e-cigarettes; environment; iPSC; opioids

**Subject Categories** Evolution & Ecology; Methods & Resources; Stem Cells & Regenerative Medicine

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See the Glossary for abbreviations used in this article.

## Introduction

The discovery of induced pluripotent stem cells (iPSCs) has transformed the field of stem cell biology and regenerative medicine (Yu *et al*, 2007). Induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) are both pluripotent stem cells (PSCs) capable of self-renewal and differentiation into any tissue lineage. Tissues derived from ESCs and iPSCs are largely similar from molecular and functional perspectives (Zhao *et al*, 2017). However, the use of iPSCs avoids the ethical problems associated with using ESCs, and iPSCs can be obtained readily from a blood sample to isolate peripheral blood mononuclear cells (PBMCs) or tissue samples with fibroblasts. Adult human somatic cells are reprogrammed into stem cells via transferring somatic nuclear material into oocytes, followed by cell fusion, and genetic integration of somatic cell chromatin. The development of viral transduction and overexpression of the Yamanaka factors (octamer-binding protein 3/4 (OCT 3/4; also known as POU5F1), SRY (sex-determining region Y)-box 2 (SOX2), Krüppel-like factor 4 (KLF4), and Myc proto-oncogene protein (c-MYC)) was a revolutionary innovation (Takahashi & Yamanaka, 2006; Takahashi *et al*, 2007; Yu *et al*, 2007). iPSCs can be differentiated into any tissue cell type using a cocktail of recombinant protein factors and small-molecule inhibitors, allowing the use of cardiovascular tissue for regenerative medicine, disease modeling, and drug discovery (Obal & Wu, 2020; Paik *et al*, 2020). While the financial cost and time required for reprogramming, generating, biobanking, and differentiation are high, iPSCs are a more accurate model of human disease than traditional mammalian cell culture and animal models. Leveraging individual genetic information and recent advances in gene editing (Nishiga *et al*, 2022), iPSCs are proving critical to understanding the molecular mechanisms of human disease and advancing the goals of precision medicine. In addition, with the diverse genetic backgrounds of individuals, a large cohort of iPSC lines has the potential to capture the heterogeneity of disease and drug treatments, which can help us discover the toxic effects of drugs and environmental exposures (Fig 1).

1 Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, USA

2 Department of Medicine, Western University, London, ON, Canada

3 Department of Physiology and Pharmacology, Western University, London, ON, Canada

4 Department of Anesthesiology, Perioperative, and Pain Medicine, Stanford University, Stanford, CA, USA

5 Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA, USA

\*Corresponding author. Tel: +519 661 2111; E-mail: mchandy2@uwo.ca

\*\*Corresponding author. Tel: +650 736 2246; E-mail: joewu@stanford.edu

**Glossary**

<b>AHA</b>	American Heart Association	<b>hERG</b>	Human Ether-à-go-go-Related Gene
<b>BDNF</b>	Brain-derived neurotrophic factor	<b>iKR</b>	Inward-rectifier potassium channels
<b>CB1</b>	Cannabinoid receptor 1	<b>iPSC</b>	Induced pluripotent stem cell
<b>CB2</b>	Cannabinoid receptor 2	<b>iPSC-CM</b>	iPSC-derived cardiomyocyte
<b>CRISPR/Cas9</b>	Clustered Regulatory Interspaced Short Palindromic Repeats/Cas9 system	<b>iPSC-EC</b>	iPSC-derived endothelial cell
<b>CRISPRi</b>	Clustered Regulatory Interspaced Short Palindromic Repeats Interference	<b>iPSC-NC</b>	iPSC-derived neuronal cell
<b>CRISPRa</b>	Clustered Regulatory Interspaced Short Palindromic Repeats Activation	<b>iPSC-SN</b>	iPSC-derived sensory neuron
<b>CV</b>	Cardiovascular	<b>HTS</b>	High-throughput screening
<b>DCM</b>	Dilated cardiomyopathy	<b>KLF4</b>	Krüppel-like factor 4
<b>EGFR</b>	Epithelial growth factor receptor	<b>MEA</b>	Multi-electrode array
<b>EHT</b>	Engineered heart tissue	<b>c-MYC</b>	Myc proto-oncogene protein
<b>ENDS</b>	Electronic nicotine delivery systems	<b>OSKM</b>	Oct2/4, Sox2, Klf4, c-Myc
<b>EVALI</b>	e-cigarette or vaping use associated lung injury	<b>PBMC</b>	Peripheral blood mononuclear cells
<b>FDA</b>	Food and Drug Administration	<b>PDCD4</b>	Programmed cell death protein 4
<b>GABA</b>	$\gamma$ -aminobutyric-acid	<b>PGC-1</b>	Peroxisome proliferator-activated receptor $\gamma$ -coactivator-1
<b>GDNF</b>	Glial cell-derived neurotrophic factor	<b>SCD</b>	Sudden cardiac death
<b>GPCR</b>	G-protein coupled receptor	<b>scRNA-seq</b>	Single-cell RNA sequencing
<b>HCM</b>	Hypertrophic cardiomyopathy	<b>SRY</b>	sex determining region Y
		<b>SOX2 TALEN</b>	sex determining region Y box 2
		<b>TRP</b>	Transcription Activator-Like Effector Nucleases

**Regenerative medicine**

Despite great advances in percutaneous coronary intervention and medical management, cardiovascular disease remains the world's leading cause of death, with associated long-term complications that include malignant arrhythmias and pump failure. iPSCs are a limitless source of tissue that are autologous, and their use helps avoid the need for immunosuppression (Lu *et al*, 2013). Despite their initial promise and great enthusiasm, preclinical studies have shown varying and sometimes disparate results for improving cardiac function, vasculogenesis, and reducing apoptosis (Nelson *et al*, 2009; Kawamura *et al*, 2012; Templin *et al*, 2012). Although clinical results from adult stem cells have been mixed, there are now several trials focusing on using human ESC- or iPSC-based cardiovascular therapies (Neofytou *et al*, 2015).

**Drug and toxicity screening**

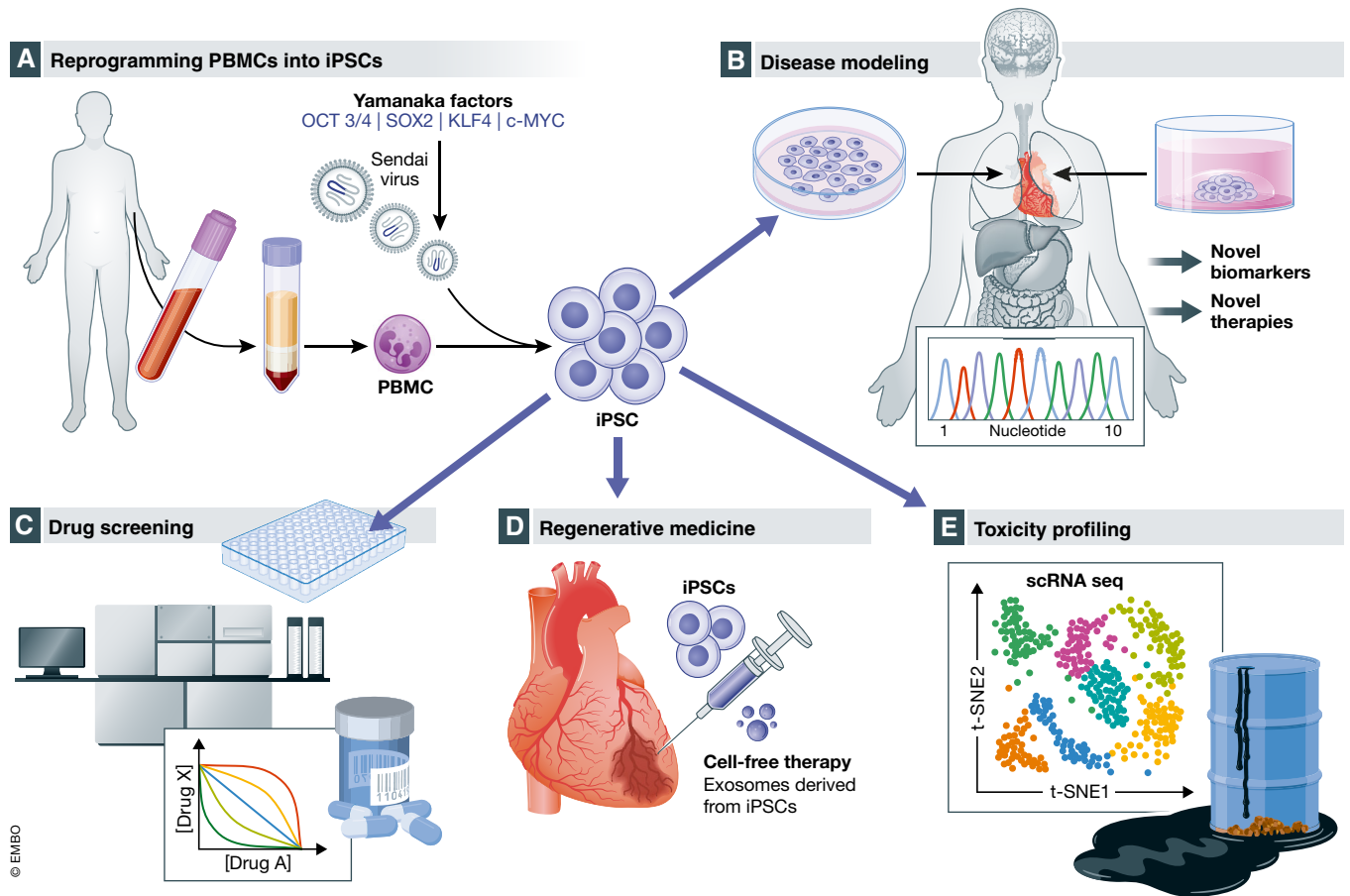
Because they contain an individual's unique genetic makeup, iPSCs are proving to be a powerful platform to discern the beneficial and adverse effects of drugs. Historically, animal models and cell culture systems have provided preclinical safety data for new drugs, and treatments and clinical trials are used to determine safety and efficacy in large heterogeneous populations. While sufficient for most individuals, this approach does not capture the precise cost-to-benefit ratio for each person. Currently, iPSCs are expensive and require months to generate. However, once created, iPSCs can be differentiated into any tissue type and provide a limitless supply of tissue for drug and toxicity testing. For example, iPSC-cardiomyocytes (iPSC-CMs) can be used to investigate the effects of new antiarrhythmic drugs or caffeine (Luo *et al*, 2021). iPSC-derived tissue can advance personalized medicine because they are derived from a single individual, making it feasible to predict which treatments are safe and effective for the individual with an enormous

potential for achieving the goals of personalized medicine (Lau *et al*, 2019).

**Disease modeling**

Traditional methods of studying human diseases using animal models or patient-derived tissue samples have been valuable, but many findings nevertheless do not translate to the clinic. Animal models do not always faithfully recapitulate the physiology of human disease, and because of interspecies variation, discoveries based on animal findings may not reflect the human pathophysiology (Matsa & Denning, 2012). Patient-derived tissue is difficult to acquire and limited in quantity, and lacks longevity in cell culture (Beqqali *et al*, 2009). Immortalized cell culture models are complicated by genes that facilitate long-term culture, and may also disrupt the transcriptome and cell function without reflecting patient-specific genetic information. Derived from terminally differentiated cells, iPSCs provide an exciting new model that has the potential to transform basic science and precision medicine.

iPSCs can be differentiated into any tissue type, including cardiomyocytes, smooth muscle cells, endothelial cells, and fibroblasts. They can be used to identify genes responsible for a disease or be modified by environmental factors in a dish. Gene-editing tools, such as TALENS, CRISPR-Cas9, CRISPR-I, and CRISPR-A, can facilitate the discovery of molecular mechanisms of a disease (Hsu *et al*, 2014; Karakikes *et al*, 2017; Ma *et al*, 2018; Nishiga *et al*, 2022). By uncovering novel disease mechanisms, iPSC-derived tissue is expected to identify novel disease-specific biomarkers and druggable targets for therapies which may eventually translate into new clinical tools for diagnosis and treatment. Novel disease-specific biomarkers have the potential to expedite the diagnosis of diseases and facilitate disease management by monitoring response to therapy. iPSCs are also a powerful tool for mechanistic studies that can identify novel drug targets, which are needed to change the scope and dimension of cardiovascular care (Fig 1).



**Figure 1. Applications for induced pluripotent stem cells (iPSCs) include disease modeling, drug screening, regenerative medicine, and toxicity profiling.**

(A) From a single blood draw, peripheral blood mononuclear cells (PBMCs) are isolated and reprogrammed into iPSCs via viral transduction and overexpression of the Yamanaka factors (octamer-binding protein 3/4 (OCT 3/4; also known as POU5F1), SRY (sex determining region Y)-box 2 (SOX2), Krüppel-like factor 4 (KLF4), and Myc proto-oncogene protein (c-MYC); Takahashi & Yamanaka, 2006; Takahashi et al, 2007; Yu et al, 2007). (B) iPSCs contain an individual's genetic information, and disease modeling offers the unique opportunity to understand patient-specific disease mechanisms that might lead to novel biomarkers and therapies. (C) Drug screening traditionally has difficulty translating from small animal and cell culture models to the clinic. iPSCs contain the genetic code of individuals and can be differentiated into any cell type allowing the determination of safety, efficacy, and possibly patient-specific responses in a dish. (D) Regenerative medicine involves iPSCs and cell-free therapy with exosomes derived from iPSCs. (E) iPSCs are also ideally suited to testing for the effects of toxins on the different tissue beds and identifying patient-specific factors that predispose to toxicity.

Patient-specific iPSCs are a limitless source of cardiovascular tissue that have been instrumental in breakthrough studies on the mechanisms of cardiovascular disease such as dilated cardiomyopathy (DCM; Sun et al, 2012), hypertrophic cardiomyopathy (HCM; Lan et al, 2013), arrhythmogenic right ventricular cardiomyopathy (ARVC; Kim et al, 2013; Asimaki et al, 2014), left ventricular non-compaction (LVNC; Kodo et al, 2016), and LEOPARD syndrome (Carvajal-Vergara et al, 2010). Imbued with an individual's genetic information, iPSCs are ideally suited for precision medicine (Grskovic et al, 2011) and will usher in a new era of biomarkers and therapies for cardiovascular disease. Simultaneously, iPSC-derived tissues are an excellent platform to evaluate the effects of drugs, toxins, and environmental exposures (Sayed et al, 2016; Lee et al, 2019). Advances in tissue engineering have allowed for the use of more complex iPSC models such as organoids or engineered heart tissue, which are expected to elucidate interactions between

different tissues and cells that contribute to disease pathophysiology (Kim et al, 2022).

Besides these applications, a central question is whether iPSCs can be used to study the impact of the environment on cardiovascular disease in this model. Murine embryonic stem cells have been used to study the effects of environmental toxins (Czyz et al, 2004a; Czyz et al, 2004b; Nikolova et al, 2005). The present review examines the advantages and challenges associated with the use of iPSCs in environmental cardiology.

## Environmental cardiology

The environment is emerging as a significant risk factor for cardiovascular disease (Bhatnagar, 2017). However, the effects of environmental toxins from natural or man-made sources remain unclear

especially in the long term. Despite attempts to curb global warming, industrial pollution, and encroachment into natural habitats, humans are continuously exposed to environmental toxins such as particulate matter less than 2.5 micrometers in size (PM<sub>2.5</sub>; Rajagopalan *et al*, 2020). After years of declining tobacco use, the rise of e-cigarettes threatens to renew cardiopulmonary disease. The legalization of marijuana has made cannabinoids more accessible around the world, but the long-term effects of marijuana on the cardiovascular system remain unclear. A recent study revealed that the psychoactive component of marijuana delta9-tetrahydrocannabinol ( $\Delta^9$ -THC) causes vascular inflammation, oxidative stress in iPSC-derived endothelial cells, and atherosclerosis in mouse models (Wei *et al*, 2022). Once mislabeled as being non-addictive, the consequences of chronic marijuana and opioid consumption are now manifesting in adverse cardiovascular outcomes (Jalali *et al*, 2021; Rohani *et al*, 2021). The haphazard use of opioids revealed that a class of medications thought to be powerful tools in preventing pain rapidly became a new environmental hazard affecting some 4% of the U.S. population (Skolnick, 2018). Human iPSCs are a powerful model system to study the toxic effects of these different compounds on the human body (Fig 2).

## Air pollution

The link between air pollution and cardiovascular disease has been described in epidemiological research (Brook *et al*, 2004; Kim *et al*, 2020a; Kim *et al*, 2020b; Kim *et al*, 2020c). About 91% of the world's population inhabits areas with poor air quality that exceeds the World Health Organization (WHO) safety limits, and air pollution is estimated to contribute to over 7 million deaths per year, or 1 in 9 deaths worldwide (Seaton *et al*, 1995; Stieb *et al*, 2002; Venkatesan, 2016). Fossil fuels are an abundant source of not only particulate matter but also carbon dioxide. Climate change mediated by carbon dioxide can lead to drought and wildfires that also exacerbate human exposure to particulate matter. The mega-fire event experienced in California culminated in a giga-fire in the fall of 2020 and led to vast amounts of particulate matter transforming the sky into a Mars-like landscape, a spectacle that is now repeated worldwide. The long-term effects of this exposure might not be evident in epidemiological studies for decades.

PM<sub>2.5</sub> are capable of traversing through the lungs and entering the circulatory system. Thus, PM<sub>2.5</sub> is thought to have adverse effects on the cardiovascular system. Studies in animal models and cell culture suggest that PM<sub>2.5</sub> can enter the circulation and has the potential to cause adverse effects on the cardiovascular system (Hoek *et al*, 2013; Feng *et al*, 2016). iPSC disease modeling can be used to uncover the toxicities of particulate matter on the body and elucidate the mechanisms of their effects. After exposure to PM<sub>2.5</sub>, iPSC-CMs were found to have increased arrhythmias due to upregulation of TRM3 (Cai *et al*, 2020). This may account for the increased incidence of sudden cardiac death associated with wildfires (Jones *et al*, 2020). Using iPSC-derived tissue, investigators may be able to assess the effects of particulate matter on different tissues. The discoveries may inform policymakers and regulatory agencies to craft decisions and laws limiting exposure to PM<sub>2.5</sub>. The continuing advances in iPSC-based drug discovery promise to an era of novel therapies for cardiovascular disease.

## E-cigarettes

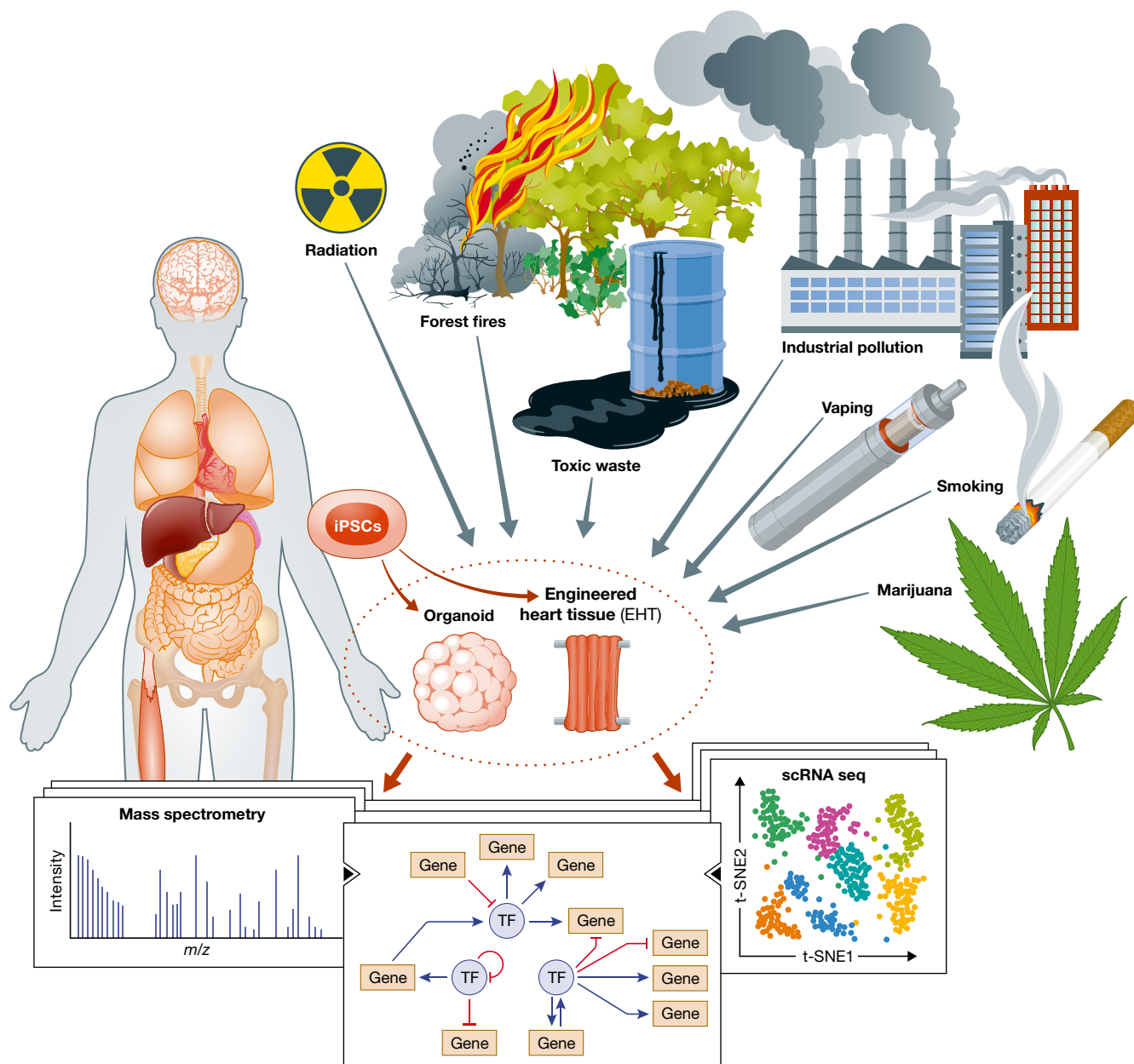
Smoking has long been associated with lung disease and cancer, and is also a major cause of the cardiovascular disease (CVD), the leading cause of death worldwide (Ambrose & Barua, 2004; Munzel *et al*, 2020). Despite its deleterious effects, tobacco is consumed by 933 million people worldwide (Collaborators, 2017). While tobacco use in developed nations has dramatically improved over the past 50 years, new tobacco products, such as e-cigarettes, have created a new generation of tobacco users. Since its introduction in 2007, e-cigarette use among high school students in the U.S. has proliferated, with a more than 900% increase from 2011 to 2018. The latest National Health Interview Survey (NHIS) estimates that 5.5 million (3.8%) US adults were using e-cigarettes in 2014. Strikingly, over 26.5% of high school students and 15.2% of middle school students were e-cigarette users (Wang *et al*, 2021).

The toxicity and mechanisms of traditional cigarette smoke on the vascular system are well documented, but the effects of e-cigarettes on the cardiovascular system have not been systematically studied thus far (Ambrose & Barua, 2004; Johnson *et al*, 2010; Polosa & Caponnetto, 2016; Benowitz & Fraiman, 2017). E-cigarettes are liquid solutions containing propylene glycol, vegetable glycerin, nicotine, tetrahydrocannabinol (THC), vitamin E acetate, and other components that are vaporized by heating and inhaled as an aerosol. As e-cigarettes do not involve combustion, they do not produce carbon monoxide or some of the other toxins associated with traditional cigarettes. Initially marketed as a smoking cessation aid, first-generation e-cigarettes had a low dose of nicotine and fewer adverse effects, but now e-cigarette and electronic nicotine delivery systems (ENDS) have evolved to have much higher doses of nicotine. For example, a single pod of Juul™ has the equivalent of 20 cigarettes (one pack) worth of nicotine (Wu *et al*, 2019). The debate about whether e-cigarettes will provide long-term benefit or harm is ongoing, with little data on the cardiac and pulmonary effects (Drummond & Upson, 2014). The emergence of e-cigarette or vaping use-associated lung injury (EVALI) has further uncovered the adverse pulmonary effects of e-cigarettes.

Epidemiology studies will likely take years to fully expose the potential adverse effects of e-cigarettes on the cardiopulmonary system, and in the meantime, e-cigarette use has grown dramatically without rigorous toxicology assessment. While studies in small animals and *in vitro* cell culture suggested adverse effects of e-cigarettes on the cardiopulmonary system, iPSC disease modeling was the first to identify that e-cigarette flavorants had deleterious effects on iPSC-derived endothelial cells *in vitro* (Lee *et al*, 2019), helping to steer policymakers in the Food and Drug Administration (FDA) to recommend a ban on flavorings.

The effects of e-cigarettes on embryonic development remain unclear. iPSC-derived embryoid bodies contain immature cells and tissues that are an ideal platform to screen for environmental toxicities. When combined with single-cell sequencing, embryoid bodies revealed potentially toxic effects of nicotine on embryonic development (Guo *et al*, 2019). A more rigorous and systematic analysis of the effects of e-cigarettes on the development is needed.

E-cigarettes include many hazardous and potentially hazardous compounds affecting the cardiopulmonary system. Nicotine has known adverse cardiovascular effects. Even without combustion, these compounds are toxic but need to be evaluated with a



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**Figure 2. iPSCs offer a unique patient-specific model to study the effect of the environment on the human body.**

New or unrecognized environmental toxins such as particulate matter 2.5 (PM<sub>2.5</sub>) from forest fires and industrial pollution, marijuana, e-cigarette, radiation, or toxic waste exposure might cause adverse effects on the human body. iPSC disease modeling is an opportunity to study the effects of environmental toxins before they result in end-organ damage or organ failure. iPSCs can be differentiated into organ-specific cells and assembled into fabricated tissues such as engineered heart tissue or differentiated into organoids comprised of organ-specific tissue. Using single-cell RNA sequencing (scRNA-seq), mass spectrometry, and other -omic analysis, iPSC disease modeling could be leveraged to discover harmful effects of environmental factors before epidemiology studies are available and prevent significant morbidity and mortality. The discovery of druggable targets and pathways might lead to the development of new biomarkers and therapies for disease.

high-throughput model that provides information on the toxicology of these compounds on human tissue. Genetic polymorphisms contribute to the development of vascular disease with traditional cigarette smoking. Investigators from the CARDIoGRAMplusC4D meta-analysis found that genetic polymorphisms make certain individuals more susceptible to cardiovascular events (van der Harst & Verweij, 2018; Larsson *et al*, 2020). iPSC disease modeling and

transcriptomics can be used to uncover single-nucleotide polymorphisms (SNPs) that exacerbate cardiovascular disease in the setting of e-cigarette exposure (Hindy *et al*, 2018; Levin *et al*, 2021). In addition to vascular disease, iPSC disease modeling may provide insights into gene and environment interactions with e-cigarette components that cause arrhythmias. Toxicity studies in iPSC-CMs have revealed that cinnamaldehyde is associated with cardiotoxicity

and altered cardiomyocyte excitability (Nystoriak *et al*, 2019). More recently, smoking and e-cigarette exposures were linked to ventricular repolarization and sudden cardiac death (SCD; Ip *et al*, 2020). The cardiotoxic effects of e-cigarettes on channels could increase the likelihood of sudden cardiac death in individuals with mutations in ion channels. iPSC disease modeling in conjunction with novel gene-editing technologies (Nishiga *et al*, 2022) are being deployed to uncover the possible causal link and identify individuals who may be at a greater risk of SCD after e-cigarette exposure, as well as possibly identify novel antiarrhythmic agents that might prevent these adverse outcomes.

EVALI underscores the importance of testing e-cigarette devices before marketing the products to the public. Clinical investigations using bronchoalveolar lavages indicated that nicotine,  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the psychedelic component of marijuana, and vitamin E acetate, a component used to emulsify  $\Delta^9$ -THC, were linked to EVALI. Studies in mouse models have found conflicting results, with one study suggesting vitamin E acetate is the causative agent. A more recent study indicates that the vehicle vegetable glycerol and propylene glycol might be sufficient to cause injury (Blount *et al*, 2019). iPSC-derived lung tissue may be used to provide a more exhaustive study of the components of e-cigarettes that cause EVALI.

## Marijuana

Associated with EVALI, marijuana is emerging as a new potential and growing threat to cardiopulmonary health. The most popular illicit drug in the world is becoming legalized for medicinal and recreational uses, with rapidly growing rates of marijuana use being reported with legalization (Cerda *et al*, 2020). The long-term effects of marijuana remain unclear because of years of restrictions on research, but it is implicated in cardiomyopathy, arrhythmias, and vascular disease from case reports and case series (Pacher *et al*, 2018). A recent large epidemiological study suggests that the odds ratio of marijuana is greater than traditional cigarettes and can predispose younger patients to premature cardiovascular disease (DeFilippis *et al*, 2018; Wei *et al*, 2022).

With legalization, the use of marijuana will increase and expose larger segments of the population to its potentially adverse effects (Pacher & Gao, 2008). Marijuana affects the body via the cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2; Pacher *et al*, 2018). CB1 activation is associated with endothelial dysfunction and atherosclerosis (El-Remessy *et al*, 2011; Rajesh *et al*, 2012), whereas CB2 activation is related to vascular quiescence (Pacher & Mackie, 2012; Pacher *et al*, 2018). Marijuana is composed of over a hundred different cannabinoids. The psychedelic component of marijuana,  $\Delta^9$ -THC, is an agonist of the CB1 receptor and causes inflammation and oxidative stress (El-Remessy *et al*, 2011; Pacher *et al*, 2018). iPSC disease modeling and CRISPR-Cas9 gene editing were used to elucidate the mechanisms  $\Delta^9$ -THC-mediated vascular dysfunction (Wei *et al*, 2022).

$\Delta^9$ -THC promotes inflammation and oxidative stress via the CB1 receptor, and the mechanisms have been previously described for MAP kinase activation and NF- $\kappa$ B pathways. Briefly, CB1 mediates increased oxidative stress and inflammation implicated in diabetic retinopathy, cardiomyopathy, and endothelial dysfunction

(Mukhopadhyay *et al*, 2010; Rajesh *et al*, 2010a; El-Remessy *et al*, 2011; Rajesh *et al*, 2012). CB1 activation occurs via the MAP kinase pathway, which causes oxidative stress, inflammation, and cell death in human coronary artery endothelial cells (Liu *et al*, 2000; Pertwee *et al*, 2010; Rajesh *et al*, 2010b). iPSC disease modeling could elucidate the mechanisms accounting for why some individuals are more likely to develop cardiovascular disease or arrhythmias after using cannabinoids. While these studies in primary cells are important for understanding mechanisms of cardiovascular disease, iPSC disease modeling with vascular cells can also be used to reveal novel mechanisms that might serve as biomarkers and lead to novel therapies for the disease. Moreover, patient-specific polymorphisms may help predict which individuals are more susceptible to adverse effects of marijuana or smoking or vaping. iPSC disease modeling could uncover polymorphisms that make an individual more likely to develop cardiovascular disease with cannabis exposure.

Some recent studies in iPSC-derived neurons suggest that THC exposure perturbs gene expression profiles and is linked to neuropsychiatric disorders such as schizophrenia (Noh *et al*, 2017; Guenewig *et al*, 2018). iPSC-embryoid body toxicity profiling is likely to reveal more global adverse effects of cannabinoids with the use of transcriptional profiling. Because it is considered an herbal remedy for nausea and vomiting, cannabis is often used by pregnant women, and epidemiological studies on prenatal exposure to cannabis indicated that it is associated with psychiatric disorders of offspring (Roncero *et al*, 2020). Recent investigations in iPSC-derived neurons have described neurotoxicity caused by physiologic doses of THC exposure (10  $\mu$ M) and perturbations in the expression of voltage-gated calcium channels (Miranda *et al*, 2020). Indeed, marijuana is associated with cardiac arrhythmias, and iPSC disease modeling can be used to elucidate the molecular mechanisms of how cannabinoids might cause arrhythmias. The long-term effects of marijuana on the cardiovascular system are not completely understood. However, a recent study showed using UK Biobank data that marijuana was a risk factor for cardiovascular disease (Wei *et al*, 2022). Here Wei *et al* (2022) used iPSC disease modeling and small animal models to investigate the mechanisms of  $\Delta^9$ -THC-mediated vascular dysfunction via the CB1 receptor. The prevalence of marijuana use is expected to increase with its growing legalization, and is likely to cause additional unanticipated, adverse health effects such as EVALI. Therefore, there is an urgent need to study the impact of marijuana in a pre-clinical model that is more translatable and relevant to the human body than existing cell culture and animal studies.

## Opioids

The current opioid epidemic in the United States has developed in three phases. In the 1990s, most of the opioid-related deaths were caused by increased prescriptions of natural and semi-synthetic opioids. A second wave began in 2010 with high usage of heroin (Rudd *et al*, 2016). The latest wave of opioid-related deaths is associated with the significant increase in use of illicitly manufactured fentanyl. Overdose-related deaths are mainly caused by bradycardia and respiratory depression. While recently oxycodone and hydrocodone have been the most commonly used opioids involved in overdose deaths (Mattson *et al*, 2021), more recently fentanyl and its

derivatives are involved in more cardiovascular complications and malignant arrhythmias. Methadone has been well described to inhibit human cardiac ether-a-go-go-related gene (hERG)-associated K<sup>+</sup> current, the rapid component of the delayed rectifier current (IKr), which determines the duration of the resting QT interval (Katchman *et al*, 2002). Inhibition of this current results in a prolonged cardiac repolarization phase of the action potential, extending the QT interval and increasing the vulnerability to arrhythmias (Roden, 2004). Nevertheless, the ambulatory data on methadone-induced cardiac arrests are limited, and perioperative data on QTc prolongation in patients undergoing non-cardiac surgery suggest that even after administration of more than nine QTc-prolonging drugs, opioids do not significantly contribute to clinically relevant QTc prolongation (Roden, 2004; Obal *et al*, 2014).

A more subtle effect may be occurring on the cardiovascular system. Epidemiological data suggest that chronic opioid administration is associated with a higher incidence of myocardial infarction and stroke (Chen & Ashburn, 2015). The American Heart Association (AHA) recently advised on the effect of chronic exposure to synthetic opioid analogs on cardiovascular (CV) function underscoring their potential detrimental impact on CV function and health (Chow *et al*, 2021; Dezfulian *et al*, 2021). Surprisingly, little is known about how chronic opioids affect endothelial and cardiomyocyte function. Distracted by the apparent short-term effects of opioids (i.e., respiratory depression and bowel obstruction), long-term effects have been lurking in the shadows and unrecognized. Recent insights into opioid receptor function and binding of different ligands have provided a broader understanding on the complex regulation of opioid signaling within the cardiovascular system (Gladen *et al*, 2016; O'Donnell & Jackson, 2017).

## Microplastics

The widespread use of plastics and microplastics is a potentially novel hazard (Matthews *et al*, 2021). A recent study revealed that the abundance of microplastics may be vastly underestimated, with over 5 trillion pieces of plastic in the world's oceans that have a combined mass of 250,000 tons (Eriksen *et al*, 2014). The United Nations Environment Assembly (UNEP) has estimated that 4.8–12.7 million tons of plastic are introduced into oceans annually (Haward, 2018). The long-term consequences of such ubiquitous pollution with plastics on the body are unknown but increasingly found in all elements of the aquatic food chain (Lehel & Murphy, 2021).

By studying the effects of microplastics *in vitro*, iPSCs might provide a window into the effects of plastics. Microplastics are consumed by plankton and are found in all aquatic species (Lehel & Murphy, 2021). The largest source of protein for humans is fish, and the microplastics do not simply transit through the gastrointestinal system but instead accumulate in the circulatory and adipose tissues (Lehel & Murphy, 2021). iPSC disease modeling can be used to understand how microplastics affect the gastrointestinal system, central nervous system, and the cardiovascular system. For example, epigenetic changes and the introduction on mutations in the genetic code may herald the initiation and progression of carcinogenesis. Initial studies with iPSCs have only revealed growth inhibition (Jeong *et al*, 2018). More recent studies have employed organoid models to understand how microplastics affect the body (Miloradovic *et al*, 2021; Winkler

## Pending issues

iPSCs are a powerful model system to investigate the adverse effects of these compounds on the body and avoid animal testing. However, 2D iPSC-derived tissues are immature and may not fully recapitulate the cellular physiology of somatic cells. With the development of 3D models such as organoids and EHT, and advances in single-cell RNA sequencing, iPSC disease modeling has improved significantly. 3D models provide a better environment that promotes cellular maturation and development. More importantly, single-cell RNA sequencing can provide spatial transcriptomics of complex cell-cell interactions. With ongoing advancements in biomaterials, biomedical engineering, and next-generation sequencing, iPSC disease modeling is continuing to evolve into a high-throughput screening platform for environmental exposures.

*et al*, 2022). Indeed, plastics are likely to modify gene expression and cause disease. More worrisome, plastics may alter epigenetic expression and be transmitted to future generations.

## Summary and conclusion

iPSC disease modeling is emerging as a powerful paradigm for understanding the patient-specific disease mechanisms. The interface with gene-editing tools and iPSCs allows investigators to elucidate the underlying molecular mechanisms that cause disease. iPSCs, gene-editing tools, and transcriptomics have the potential to revolutionize toxicology and rapidly advance our understanding of the adverse effects of environmental toxins. Because they contain the genetic code unique to each person, the use of iPSCs promises the discovery of new gene-environment interactions that can decipher why some individuals are more susceptible to environmental factors that exacerbate diseases such as cardiovascular disease.

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## Author contributions

**Joseph C Wu:** Conceptualization; resources; supervision; writing – original draft; writing – review and editing. **Mark Chandy:** Conceptualization; visualization; writing – original draft; writing – review and editing.

**Detlef Obal:** Conceptualization; writing – original draft; writing – review and editing.

In addition to the [CRediT](#) author contributions listed above, the contributions in detail are:

MC: manuscript writing and preparing figures; DO: manuscript writing; JCW: manuscript writing and final approval of the manuscript.

## Disclosure and competing interests statement

J.C.W. is a co-founder and SAB of Greenstone Biosciences, and M.C. is a consultant for Greenstone Biosciences, but this manuscript was written independently.

## For more information

For additional information on iPSC Biobanking, cell culture protocols, publications, and contact information to request iPSC lines, please visit the Stanford Cardiovascular Institute (SCVI) Biobank website: <https://med.stanford.edu/scvibiobank.html>.

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