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# Human stem cell-based models for studying host-pathogen interactions

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## Abstract

The use of human cell lines and primary cells as in vitro models represents a valuable approach to study cellular responses to infection. However, with the advent of new molecular technologies and tools available, there is a growing need to develop more physiologically relevant systems to overcome cell line model limitations and better mimic human disease. Since the discovery of human stem cells, its use has revolutionised the development of in vitro models. This is because after differentiation, these cells have the potential to reflect in vivo cell phenotypes and allow for probing questions in numerous fields of the biological sciences. Moreover, the possibility to combine the advantages of stem cell-derived cell types with genome editing technologies and engineered 3D microenvironments, provides enormous potential for producing in vitro systems to investigate cellular responses to infection that are both relevant and predictive. Here, we discuss recent advances in the use of human stem cells to model host-pathogen interactions, highlighting emerging technologies in the field of stem cell biology that can be exploited to investigate the fundamental biology of infection.

### Take Aways

- hPSC overcome current limitations to study host-pathogen interactions in vitro.
- Genome editing can be used in hPSC to study cellular responses to infection.
- hPSC, 3D models and genome editing can recreate physiological in vitro systems.

**KEYWORDS**
CRISPR/Cas9, host, infection, iPSC, macrophage, pathogen, stem cells

## 1 | CELLULAR SYSTEMS USED FOR MODELLING HOST-PATHOGEN INTERACTIONS

The study of the host-pathogen interactions has provided critical knowledge of the cellular responses to infection with bacteria, viruses, parasites and fungi. Understanding how microbes can survive and replicate within host cells provides information on both the cellular mechanisms of microbial pathogenesis and fundamental aspects of cell biology. This understanding of microbial–host-cell interactions at the cellular and molecular level can lead to the development of more effective therapies against infectious diseases (Cossart, Boquet, Normark, & Rappuoli, 1996; Finlay & Cossart, 1997; Welch, 2015).

One of the major challenges that investigators face while studying host-pathogen interactions is the limited options regarding in vitro models that reflect physiological and relevant environments. Ex vivo

tissue culture systems using precision-cut slices have been used to recreate complex tissue cytoarchitecture but regular access to biopsies is difficult, and scalability limited (Bryson et al., 2020). The most common models to study host-pathogen interactions in vitro utilise either non-immune cells or immune cells to mimic disease states. In general, epithelial/endothelial cells are used to study intestinal bacterial pathogens and pathogens that actively invade host cells (e.g. *Salmonella* or *Shigella*). On the other hand, macrophages and dendritic cells are often used for pathogens that are phagocytosed (Theriot, 1995). Many intracellular pathogens interact with more than one cell type in vivo and this interaction actually determines whether pathogens cause disease or are successfully eliminated. However, in vitro only one cell type is often used, and this is unable to mimic the full arsenal of the host.

Moreover, most of the cellular systems are in 2D and lack critical functions that are only present when the cells are in contact with other cell types (co-cultures) and in 3D physiological environments, causing dedifferentiation. These include the formation of tight junctions, microvilli and secretion of lipoproteins critical for the immune response to pathogens.

### 1.1 | Cell lines

Cell lines represent the most fundamental in vitro approach to disease modelling. There are many benefits of working with cell lines as they are cost effective, generally simple to work with, genetically tractable and can be passaged in culture for extensive periods of time. In particular, cell lines are easy to manipulate and expand to allow for larger or time-sensitive studies such as drug testing and screening. While cell lines may be suitable for a wide variety of studies, this approach also has severe limitations. Cancer cells or transformed cells show an increase in oncogene activation and genetic instability that results in uncontrolled cell growth and altered metabolism. Furthermore, immortalised cell lines cannot fully mimic the in vivo cell conditions that are specifically involved in host-pathogen interactions. One major issue is the incurrence of biological changes caused by the continuous passaging of the cell lines. This routine maintenance commonly jeopardises the physiology and genomic stability of the cellular model, often resulting in abnormal karyotypes (Frattini et al., 2015; Liu et al., 2019). For example, the commonly used cell line HEK-293 (Human Embryonic Kidney 293) has not a clear tissue-specific gene expression signature, making difficult to study phenotypes associated with the specific cell type and ultimately the tissue of origin (Stepanenko & Dmitrenko, 2015). Although working with cell lines have many advantages, this modelling approach very often do not reflect in vivo conditions in the presence of a full immune response.

### 1.2 | Primary cells

In contrast to cell lines, human primary cells are isolated directly from tissues and eventually retain the morphological and functional characteristics of their origin. Primary cells are physiologically relevant, but difficult to isolate and culture. The isolation and maintenance of primary cells from human tissues requires substantial time and it is costly. Moreover, primary cells have a limited, and in most of the cases absent, capacity for proliferation, undergo senescence-related processes and have restricted potential for differentiation. For many cell types, including fibroblasts, epithelial or blood-derived cells, there is also a finite number and limited supply. It is also important to consider the donor-to-donor variability and the lack of genetically tractable systems for gene deletion and protein expression available for primary cells. In this context, the possibility to have an unlimited source of cells from human origin that mimic the physiological and functional properties of the cells in vivo should be considered.

## 2 | STEM CELLS FOR IN VITRO STUDIES: AN ADVANCED MODELLING SYSTEM

### 2.1 | Stem cells

Stem cells are a relatively recent addition to the options for in vitro studies and are among the most advanced cellular systems for in vitro disease modelling. Pluripotent stem cells (PSCs) are capable to self-renew and differentiate, under specific stimuli, into virtually every cell type of the human body; with the exception of extraembryonic tissues such as the placenta (Cyranoski, 2018; Scudellari, 2016). The application of stem cell technologies to research is currently expanding rapidly, representing one of the most promising area across the life sciences and providing a wide variety of applications. Stem cells constitute a powerful tool in basic sciences to study the genetic bases of human disease and the physiological processes that occur during development. Stem cells are also a promising tool to develop diagnostic biomarkers. Undoubtedly, their ability to differentiate into various immune cell types makes stem cells extremely relevant for in vitro modelling of infection (Figure 1).

### 2.2 | Embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass of a blastocyst; a structure formed in the early development of mammals. Since 1981, it is possible to obtain ESCs and generate undifferentiated cell lines from the inner cell mass of mouse blastocysts (Evans & Kaufman, 1981; Martin, 1981). Further advancements made possible the isolation of ESCs from human blastocysts (Thomson, 1998). The scientific community has recognised the outstanding potential of ESCs, and the applications of these cells have been an important area of research in the last decades. Remarkably, due to the competence for germline transmission, the ESCs can be genetically manipulated and used to pass down the genome to the offspring (Capecchi, 2005; Robertson, Bradley, Kuehn, & Evans, 1986; Thompson, Clarke, Pow, Hooper, & Melton, 1989). This technique has

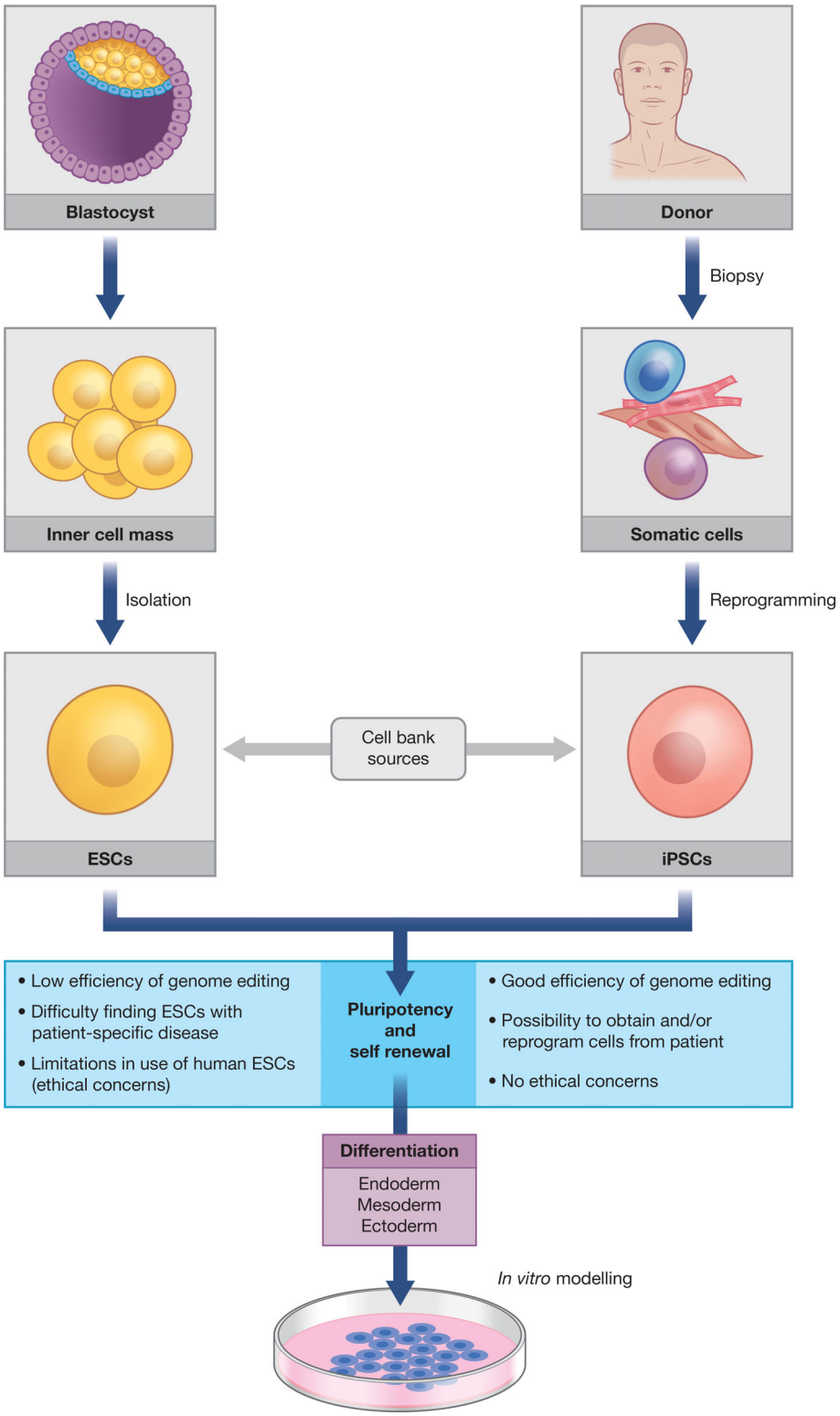


**FIGURE 1** Stem cell models for in vitro studies in host-pathogen interactions. ESCs can be generated using the inner cell mass from blastocytes and iPSCs using somatic cells from human donors. Each system has disadvantages and advantages (blue box). These cells can be differentiated into different cell types for in vitro experiments

led the way for in vivo studies of mouse genetics, development and physiology (Skarnes et al., 2011). The self-renewal potential and the ability to virtually generate any cell type of the human body, make the ESCs a useful tool for both basic research and regenerative medicine. On the other hand, limited availability of patient samples represents a practical drawback of using human ESCs (hESCs) to model disease in vitro. In addition, when compared to immortalised cell lines, hESCs are very difficult to transfect, sensitive to DNA damage and therefore less prone to gene editing. Moreover, the difficulty to generate patient- or disease-specific ESCs and ethical controversies arising from the collection and destruction of human embryos hinder their applications in medical research (Figure 1).

## 2.3 | Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are generated through reprogramming of somatic cells into a pluripotent embryonic stem cell-like state via the ectopic expression of a combination of transcription factors *Oct 3/4*, *Sox2*, *Nanog* and *Myc (OSKM) or Oct 3/4*, *Sox2*, *Nanog* and *Lyn28 (OSNL)*, avoiding the ethical issues related to ESCs (Takahashi et al., 2007; Takahashi & Yamanaka, 2006). Indeed, iPSCs are indistinguishable from ES cells with regards to morphology, proliferation, gene expression and ability to form teratomas. Mouse iPSCs can be transplanted into blastocysts to generate mouse adult chimeras, which are competent for germline transmission (Maherali et al., 2007; Meissner, Wernig, & Jaenisch, 2007; Okita, Ichisaka, & Yamanaka, 2007). The first scientists who successfully reprogrammed adult human fibroblasts to generate human iPSCs (hiPSCs) used either the OSKM (Takahashi) and OSNL (Thomas) transcription factors. Fibroblasts were the first type of cell to be used for iPSCs generation because skin biopsies are easier to obtain and handle. Nowadays, it is possible to obtain iPSCs from actively dividing somatic cells, such as peripheral blood mononuclear cells (PBMCs), T cells, B cells, neuronal progenitor cells, keratinocytes and hepatocytes (Bueno et al., 2016; W. Yang, 2014). It is also possible to reprogram into iPSCs less proliferative cells such as cardiomyocytes (Rizzi et al., 2012), indicating that most cell types can be reprogrammed (Figure 1). The potential to generate iPSCs from somatic cells is relatively high, however, the elevated cost and lengthy process of manufacturing and validating iPSCs makes this possibility not readily available for research. In this context, there is a growing number of cell banks that provide high-quality iPSCs, both from healthy and disease backgrounds, to researchers who otherwise would not be able to obtain and characterize these cells in their own laboratories (Table 1).

## 2.4 | Powerful advantages

Despite initial concerns that hiPSCs could not be functionally equivalent to hESCs, carefully controlled studies have shown a remarkable similarity between hiPSCs and hESCs derived from the same genetic background (Choi et al., 2015). Moreover, development of feeder-free human iPSC culture systems has significantly simplified the manipulation of iPSCs in vitro. Thus, unlike immortalised human cell lines, iPSCs provide an ideal model to study cellular, biochemical and developmental processes in a genetically stable cell from a single healthy donor. On the other hand, it is possible to reprogram somatic cells from patients with specific genetic diseases to perform in vitro studies. Patient-derived iPSCs have been widely used to model mechanisms of human diseases and represent a useful tool in cell-based drug discovery platforms (I. H. Park et al., 2008). In addition, genome editing technologies can be used to create isogenic cell lines from patient-derived iPSCs to conduct comparative in vitro studies (see below).

Undoubtedly, the greatest advantage of iPSCs is their pluripotency, which is the capability to develop into the three primary germ layers of the early embryo: mesoderm, endoderm and ectoderm (Figure 1). Because of their pluripotency, the combination of efficient protocols with specific cell culture media and differentiation factors allows the differentiation of iPSCs into different cell types of the human body (Passier, Orlova, & Mummery, 2016). This includes neurons, astrocytes, oligodendrocytes, retinal epithelial cells, epidermal, hair and keratinocytes (ectoderm layer), hepatocytes, pancreatic β-islet cells, intestinal epithelial cells, lung alveolar cells (endoderm layer) and haematopoietic, endothelial cells, cardiomyocytes, smooth muscle cells, skeletal muscle cells, renal cells, adipocytes, chondrocytes and osteocytes (mesoderm layer) (Oh & Jang, 2019).

**TABLE 1** Source of iPSC from worldwide cell banks

| Bank name | Location | Profit type | Ownership |
|---|---|---|---|
| California Institute for Regenerative Medicine (CIRM) | USA | Non-profit | Public |
| Coriell Institute for Medical Research (Coriell) | USA | Non-profit | Public |
| Fujifilm Cellular Dynamics International (FCDI) | USA | Profit | Private |
| WiCell Research Institute (WiCell) | USA | Non-profit | Public (University of Wisconsin-Madison) |
| European Bank for induced pluripotent Stem Cells (EBiSC) | Europe | Non-profit | Public (European Commission) |
| Stem cells for Biological Assays of Novel drugs and prediCtive toxiCology (StemBANCC) | Europe | Non-profit | Public-private partnership |
| Human Induced Pluripotent Stem Cell Initiative (HipSci) | UK | Non-profit | Public (Medical Research Council, Wellcome Trust) |
| Center for iPS Cell Research and Application (CiRA) | Japan | Non-profit | Public |
| Institute of Physical and Chemical Research (RIKEN) | Japan | Non-profit | Public |
| Human Disease iPSC Consortium Resource Center (Taiwan Human Disease iPSC Consortium) | Taiwan | Non-profit | Public |
| Korean National Stem Cell Bank (KSCB) | Korea | Non-profit | Public |

*Note:* iPSC generation, characterization and banking institutions are non-profit organisations and are largely government-funded. This list of selected cell banks that are dedicated to improving the production of stem cell research and provide unique disease cell lines for academic and industrial research.

The multitude of cell types that can be generated from pluripotent stem cells allows the scientist to recreate and model a wide variety of diseases and/or developmental stages in vitro. For example, iPSC-derived T cells, Natural Killer (NK) cells, macrophages, dendritic cells and others provide a toolset for understanding disease pathology (e.g. in cancer and infectious disease) (Bernareggi, Pouyanfard, & Kaufman, 2019) as well as developing successful immunotherapies (for adoptive therapies that include CAR-T, CAR-NK and TCR-T) (Xie et al., 2020).

The potential of PSCs to generate innate immune cells provide new approaches to study host-pathogen interactions and how infection outcomes can be influenced by human genetics. One of the first lines of protection against cancer and infection consists of NK cells and macrophages. Furthermore, due to their early response to infections, NK cells are considered innate immune cells (Morvan & Lanier, 2016). NK cells can be differentiated from human iPSCs, as well from ESCs, and are able to reproduce functional characteristics in vitro. For example, human immunodeficiency virus (HIV-1) infection is suppressed by PSC-derived NK cells by at least three distinct cellular mechanisms: direct lysis of infected cells, cell-mediated antibody cytotoxicity and release of chemokines and cytokines (Ni et al., 2011). Macrophages, on the other hand, are myeloid cells that contribute to many functions, such as tissue formation, tissue repair, homeostasis and the intrinsic immunity response to pathogens and cancer cells (Wynn, Chawla, & Pollard, 2013). Several differentiation protocols are available for the generation of macrophages from iPSC (iPSDM), including microglia (Lee, Kozaki, & Ginhoux, 2018) allowing new in vitro approaches to study host–pathogen interactions.

Overall, iPSCs provide a flexible system for disease modelling, drug screening and host-pathogen interaction study for infection research. Patient-derived iPSCs (as described above) enable the non-invasive creation of tools in an individual genetic context useful for testing new antiviral and antimicrobial therapies, aligned with personalised medicine therapies while avoiding the ethical issues associated with embryonic stem cell systems (Figure 1).

## 3 | STEM CELLS IN 3D ENGINEERED MICROENVIRONMENTS

The wide range of molecular mechanisms underlying microbial virulence strategies, such as activation of host receptors, can be investigated using two-dimensional single cell culture models in order to provide critical insights into what drives host defence strategies. In the last decades, these models have provided the basis for our present understanding of many host-pathogen interactions. The main benefit of single cell in vitro models lies in their ability to provide crucial details about the response of one specific cell type to an infectious microbe. Given the flexibility of in vitro single cell populations versus the economic and ethical limitations of in vivo models, monocultures offer an effective and inexpensive experimental system to investigate basic cellular processes. However, given the different intercellular communication networks present in tissues and the connections to the vascular systems, monocultures represent a relatively limited biological host environment. In tissues, cells are exposed to microenvironments, respond to fluctuating cues from the extracellular matrix, mechanical forces dictated by flow, supply of nutrients, interactions with other cells and gradients of oxygen and growth factors. These environments have a profound impact on the biology of cells in tissues and consequently, single cell culture systems are a poor system for modelling complex responses to infection, although their widespread usage and noted benefits.

Essential processes underlying immune responses to pathogens such as contacts between lymphocytes and antigen presenting cells or killing of cancer cells by NK cells can be studied in cellular co-cultures (Ritter et al., 2015). These approaches are increasingly used as an alternative to bridging the gap between simple in vitro models of single cells and complex biological processes in vivo.

To generate more physiologically relevant, complex model systems capable of reflecting in vivo environments, there is a growing research field focusing on developing structurally distinct 3D models of human tissues (Nickerson, Richter, & Ott, 2007). A very simple model constitutes the air-liquid interface (ALI), an in vitro lung cell model suitable to study more physiological and pathophysiological responses of the epithelial cell barrier and to investigate interactions of different cell types by co-culturing approaches. Most of the classical ALI systems mimic better a polarised barrier, transport and differentiation properties. Simple methods for recreating 3D environments consist of scaffolds combined with controlled microfluidics that supply a homogeneous source of nutrients. However, the use of flow can induce stress in the cells and high cell density can cause differences in oxygen availability. Moreover, the composition, stiffness of the extracellular matrix in these 3D cell culture systems is critical and responses vary when using collagen, matrigel or gelatin, commonly used biological hydrogels.

More complex systems include organ on a chip models that include microfluidics with either cell lines or primary cells. Organs on a chip are emerging as predictive tissue modelling tools and reliable alternatives to animal testing for pre-clinical studies. These models also allow for direct access to hypothesis-based and unbiased studies together with experimental image analysis. Some of these systems have been successfully used to investigate the interactions between *M. tuberculosis* and the alveolar space (Thacker et al., 2020). Moreover, there are some infections where no mouse models are available and the infection is restricted to humans, for example with the bacterium *Helicobacter pylori*. For this, long-term 3D cultures of human gastric stem cells have been generated to study the epithelial response to infection with *H. pylori* (Bartfeld et al., 2015). These methods mimic in vivo biology more closely, with the rearrangement of cells into proto-organic structures, beyond simple co-culturing of various cell types.

In order to produce different organoids, which are stem cell-derived 3D culture systems, mammalian iPSCs, ESCs, neonatal tissue stem cells or ex vivo adult progenitors are used as starting materials. Despite the combination of stem cells with organs-on-chip has been proposed as the "best of both worlds" system (Nawroth et al., 2019),

only recently stem cells have been used for the generation of organoids and organs-on-chip. A complete multicellular lung organoid has been established using human embryonic and patient-specific iPSC for infectious disease modelling and therapeutics validation (Leibel, McVicar, Winquist, Niles, & Snyder, 2020) and three-dimensional alveolar human stem cell culture models developed to study infection responses to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Youk et al., 2020). Furthermore, these 3D models of infection combined with stem cell technologies are very useful to study the impact of environmental toxins, microbial pathogens, alterations in the microbiome or the efficacy and safety of drugs (Nawroth et al., 2019). Human adult stem cell-derived organoid has also emerged as an option to the organoid approach. Unlike the time-consuming process to obtain, culture and differentiate iPSCs into specific cell types, the adult stem cell-derived organoid can be directly generated from the tissue obtained from a healthy or diseased donor. In this model, there is a limitation related to the availability and accessibility to the organ of interest beside the difficulties to culture already differentiated tissue. Many of these restrictions and limitations can be overcome by using PSC. Moreover, PSCs can be used to generate different cell types after induction with defined stimuli from the same genetic background, making possible the design of complex environments and a complete characterization of the response of multiple cell types through the use of *omics* studies, such as single cell RNA-seq (Figure 2).

## 4 | GENETIC ENGINEERING APPROACHES IN PSCS

Interest in patient-specific therapies (personalised medicine) based on iPSCs has increased with the rapid advances in precise DNA editing technologies. Classical genetic approaches such as retroviral, lentiviral and transposon vectors (e.g. sleeping beauty and piggyBac) are still useful tools for genome editing in mammalian cells (Vargas et al., 2016). However, the real breakthrough in genome editing came with the programmable nucleases that can specifically recognise and cleave genomic DNA. The programmable nucleases take advantage of natural cellular pathways of DNA repair and include meganucleases, zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs) and RNA-guided engineered nucleases (RGENs), which are mostly used for the introduction of changes in targeted sequences (H. Li et al., 2020).

The TALEN approach has been used in the last decades as genomic engineering tool for in vitro and clinical studies. However, the discovery of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) microbial adaptive immune system has completely revolutionised the genome engineering field, emerging as a simple and effective alternative to induce targeted genetic modifications. More than 20 years of research have given life to the most powerful genome editing technique and its use has been reported in many organisms, including yeast, bacteria, nematodes, fruit flies, zebrafish, mice, monkeys and humans. In brief, CRISPR/Cas9 is comprised of two major elements: an endonuclease Cas9 protein and a synthetic single-guide RNA (sgRNA). Unlike the conventional nuclease-mediated DNA editing techniques, ZFNs and TALENs, DNA recognition by CRISPR/Cas9 is not specified by protein but rather by the 20-nt guide RNA sequence (Doudna & Charpentier, 2014). Target recognition by Cas9 nuclease strictly requires the presence of a short protospacer adjacent motif (PAM) flanking the target site, a short-conserved sequence in the target DNA that lies adjacent to the 3′ end of the 20 bp guide RNA sequence. In particular, for type II *S. pyogenes* Cas9 system, the PAM is 5′-NGG-3′, where N stands for any nucleotide. Furthermore, the multidomain and multifunctional DNA endonuclease Cas9 consists of two distinct nuclease domains: an HNH-like nuclease domain that cleaves the DNA strand complementary to the guide RNA sequence (target strand), and a RuvC-like nuclease domain responsible for cleaving the DNA strand opposite the complementary strand (non-target strand) (Jinek, 2012). Initially demonstrated with meganucleases (Rouet, Smih, & Jasin, 1994), the introduction of a double strand break at a genomic site can be repaired through different cellular DNA repair processes (Carroll, 2014). These mechanisms can be broadly subdivided into (a) non-homologous end-joining (NHEJ) and (b) homology-directed repair (HDR). These two possible repair pathways are taken into consideration during the experimental design in order to obtain a genetic modification: for example, knockout (KO), knock-in (KI) and even more complex allele manipulation (conditional mutagenesis).

The potential of stem cell technologies combined with CRISPR/Cas9 genome editing for studying host pathogen interactions and infectious diseases is very high. Genetic knockouts are widely used to inactivate a particular gene, either by interrupting an exon's reading frame shared by all transcript variants of the interested gene or by deleting part of the gene by using multiple sgRNAs at once. These genetic manipulations are frequently used to study the function of specific genes in phenotypes (Kang et al., 2015).

On the other hand, it is possible to introduce specific genetic changes by using an exogenous DNA repair template containing homologous sequences to the region around the DSB and inducing the cells to go under a less effective homology-driven repair pathways. With gene targeting, it is now possible to produce almost any type of DNA modification by using different DNA donor templates, such as a plasmid or single strand molecule (ssODN), allowing scientists to establish the roles of individual or multiples genes in health and disease. For example, a knock-in approach could be used to tag critical cellular components in the native locus with fluorescent proteins to perform localization studies (Hoffman, Fernandez, Groves, Freed, & van Engelenburg, 2019) or with epitope tags, such as V5-tag, Myc-tag, HA-tag and affinity tags, like strep-tag and poly(His) tag, to isolate or immobilise a protein for additional proteomic studies (Anton, Karg, & Bultmann, 2018). On the other hand, it could be possible use CRISPR/Cas9 to model and study specific mutations, defined from a wealth of genome-wide association studies (GWAS), which confer increased susceptibility or resistance to specific infections (Davila & Hibberd, 2009). Gene editing approaches allow the generation of isogenic pairs of cells harbouring or not a specific disease-causing mutation (I. H. Park et al., 2008). Thus, the commonly observed variability when comparing patient-derived cells with non-isogenic controls is minimised by performing experiments on cell lines

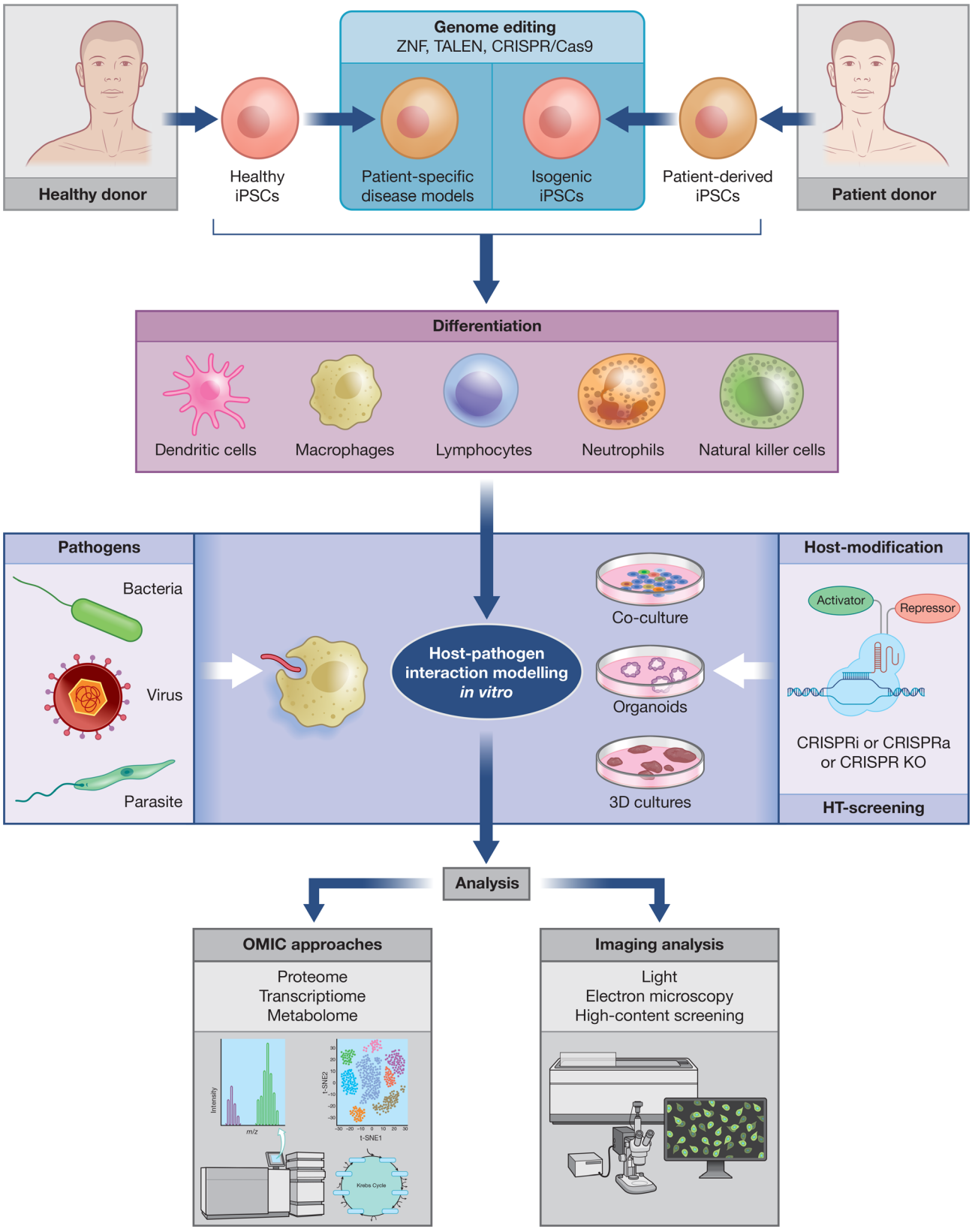


**FIGURE 2** iPSC models and technologies to study disease and infection. In the iPSC system, cells can be generated from both healthy or patient with specific diseases and these iPSC can be additionally modified using genome editing technologies (see text for more details). The different iPSC can be now differentiated into immune cells (e.g. macrophages, neutrophils etc) for *in vitro* studies. These cells could be used to investigate cellular interactions with bacteria, viruses and parasites and utilised in different systems from single cell cultures to 3D environments. These systems are also amenable for high throughput screenings for example. Because the number of cells that can be generated and differentiated from iPSC is unlimited, these systems are very useful for high content analysis and "omics" studies. Figure created in Biorender

with the same genetic background (Choi et al., 2015). On the other hand, it is also possible to edit iPSCs reference lines by introducing specific mutations associated with a particular disease (Hockemeyer & Jaenisch, 2016). In this way, using the same genetic background, it is possible to compare different disease variants and effectively determine the associated phenotype (Soldner et al., 2011). Many human diseases are caused by a single nucleotide variant (SNV), and modification of one or both alleles is often required for in vitro modelling. in order to introduce a single base pair change, different CRISPR strategies rely on introducing a "silent" mutation, primarily in the PAM region, to prevent the Cas9 protein from cutting the targeted allele. Since this non-physiological modification can cause undesirable effects, current efforts are focused on developing protocols to increase the efficiency of HDR and achieve "scar-free" SNV alleles (Skarnes, Pellegrino, & JA, 2019). In summary, these precise editing techniques for the creation of patient-derived cell lines make it possible to bypass reprograming and biopsy procedures to reduce variability of iPSC lines. In addition, isogenic iPSC lines eliminate the variability associated with donor genetic backgrounds. This allows scientists to more accurately mimic host environments when modelling disease, and in the cellular microbiology field, to investigate the interplay between pathogen infection and host genetics (Figure 2).

## 5 | STEM CELLS IN GENOME-WIDE SCREENINGS FOR HOST-PATHOGEN INTERACTIONS

The use of forward genetic screens has provided a powerful strategy for the discovery and the study of gene function in both health and disease condition. A CRISPR-based genetic screen is a pooled screening based on different CRISPR-based tools, including CRISPR knockout, CRISPRi and CRISPRa (Figure 2).

### 5.1 | CRISPR knockout screenings

One of the first methods of a CRISPR/Cas9 knockout screen is now commonly used in different biological fields (Shalem et al., 2014). A CRISPR-based screen consists of knocking out a single gene per cell that could be important in the pathway subject of study. For this propose, frequently, a library of lentiviruses is produced from a pool of oligos, containing DNA to encode mostly the entire sgRNA, designed to cover all genes of interest. Thus, lentiviruses are produced in batch containing thousands of CRISPR targeting sequences, with one targeting sequence per viral particle. Then, the lentivirus-CRISPR library is used to infect mammalian cells with no more than one virion per cell (and theoretically one sgRNA sequence per single cell). The outcome of the screening is obtained by the type of selection applied based on survival, proliferation or sensitivity to stimuli, differential gene expression or on cellular functions. After selection, next-generation sequencing (NGS) allows the determination of sequences that are present, depleted or absent on the entire mixed population of cells. Genome-wide screens have been exploited to discover host and bacterial and/or viral virulence factors important during microbial infection (Pacheco et al., 2018; Winter, Zychlinsky, & Bardoel, 2016). Novel insights into previously unrecognised mechanisms of pathogenesis were successfully revealed through the use of CRISPR screening techniques (Chang, Jin, Jiao, & Galan, 2019; Lai et al., 2020; R. J. Park et al., 2017; Tao et al., 2016; Tromp et al., 2018; Yamaji et al., 2019). CRISPR screening approaches have been applied not only to study bacterial infection but also to investigate virus–host interactions (Baddal, 2019; B. Li et al., 2020; Thamamongood et al., 2020). CRISPR-Cas9 knockout screening combined with hPSC-derived neural progenitors (NPs) identified insights into host-dependent mechanisms for Zika virus infection. This work clearly shows the immense possibilities of CRISPR/Cas9 genome editing in iPSCs and how the combination of these tools offers a multitude of different approaches for biological investigations. CRISPR screens combined with the potential to recreate a specific cell types can enormously help to our understanding of host-pathogen interactions and to the identification of new pharmacological targets for treating infections (Y. Li et al., 2019).

### 5.2 | CRISPRi and CRISPRa

The CRISPR/Cas9 system can also be used to transiently perturb gene function, without changing the sequence of the genome. This can be achieved with mutated nuclease domains of Cas9 from *S. pyogenes* to generate a nuclease deficient Cas9 (Qi et al., 2013), also referred to as a "dead Cas9" (dCas9). The dCas9 is not able to cleave DNA but can target and bind DNA with the same precision guided by specific sgRNAs. Thus, dCas9 is targeted to specific genomic sequences and it can serve as a recruitment platform for other proteins that affect gene expression. Indeed, CRISPR interference (CRISPRi) can be used to recruit transcriptional repressor domains (most commonly the KRAB domain, or epigenetic modifiers) to transcription start sites in the human genome to repress gene transcription (Lara-Astiaso et al., 2013). On the other hand, the dCas9–sgRNA complex can also serve as a recruitment platform for transcriptional activator domains to specific transcription start sites in the genome. The latter approach is referred as CRISPR activation (CRISPRa) (Lara-Astiaso et al., 2013; Perez-Pinera et al., 2013). Using CRISPRa, endogenous genes can be induced and overexpressed to varying degrees to evaluate functional consequences. For example, a CRISPR interference strategy provided a framework for systematic investigation of whole-genome interaction networks of the microorganism *Bacillus subtilis* (Peters et al., 2016), important to identified modes of action for uncharacterized antibiotics.

## 6 | STEM CELLS IN CELLULAR MICROBIOLOGY

### 6.1 | Viruses

After their discovery in 2007 (Takahashi & Yamanaka, 2006), it took some time until iPSCs were used for in vitro studies with microbes.

Cellular models based on iPSC were initially developed to study viruses-host cell interactions. Because hepatitis viruses have a host tropism for human cells, iPSC were used to establish a iPSC-derived hepatocyte-like cell (iHLC) model for hepatitis C virus (HCV) infection (Yoshida et al., 2011). Similar to primary hepatocytes, iHLCs supported the entire HCV life cycle, introducing iHLCs as a new tool for studying HCV infection (Schwartz et al., 2012). Hepatocellular systems based on iPSC have been exploited to model host cell interactions with hepatitis B virus (HBV), demonstrating the utility of iPSC-based systems for studying the interactions between and host hepatocytes (Shlomai et al., 2014). Human iPSCs have also been used to generate functional liver organoids and evaluated as model to study HBV–host interactions. These functional liver organoids were more susceptible to HBV infection than hiPSC- derived hepatic cells and produce infectious HBV for relatively long periods of time (Nie et al., 2018).

Highlighting the potential of iPSC in disease modelling, iPSCs from toll-like receptor 3 (TLR3) and UNC-93B-deficient patients differentiated into neural stem cells (NSCs), neurons, astrocytes and oligodendrocytes shed light into response to herpes simplex virus type 1 (HSV-1) infection. In these cells infected with HSV-1, the induction of interferon beta (IFN-β) and interferon lambda (IFN-λ) is selectively impaired in UNC-93B-deficient neurons and oligodendrocytes (Lafaille et al., 2012).

In an important methodological advance, a protocol for deriving monocytes and macrophages from human iPSCs, with macrophage colony-stimulating factor (M-CSF) in entirely defined, feeder- and serum-free culture conditions, was developed to study HIV infection (van Wilgenburg, Browne, Vowles, & Cowley, 2013). By using genome editing, macrophages that were derived from $^{CCR5}$-knockout iPSC (Kang et al., 2015) and haematopoietic stem/progenitor cells (HSPCs) were used to show resistance to infection by HIV in vitro and in vivo (Xu et al., 2017).

iPSC lines also represent a suitable cell culture model for early embryonic virus infection such as coxsackievirus B3 (CVB3), measles virus (MV) and rubella virus (RV). Interestingly, the metabolism of infected iPSCs was the only common phenotype observed after infection with these three viruses, suggesting that changes in metabolic activity of iPSCs represents an important phenotypic feature induced by viral infection (Hubner et al., 2017).

An iPSCs cell system model was developed to study human cytomegalovirus (HCMV) latency and reactivation in different cell types after their differentiation into the myeloid lineage. This model is suitable to study other viruses, such as HIV and Zika virus (ZIKV) that also infect cells of the myeloid lineage (Poole et al., 2019). In fact, iPSDM have been used as a model to investigate the pathogenesis of ZIKV and Dengue virus (DENV) infections (Lang et al., 2018). For example, to investigate the pathogenesis of ZIKV infection, a knockout of the entry receptor AXL in human iPSCs was generated to show this receptor has no role in ZIKV entry or induction of cell death in human iPSC-derived neural progenitor cells (NPCs) (Wells et al., 2016).

Stem cell models using hiPSC-derived cardiomyocytes (hiPSC-CMs) have been developed to study cardiac targeting by SARS-CoV-2. SARS-CoV-2-infected hiPSC-CMs in vitro opening the possibility to use these cells as a model to study the mechanisms of infection and to test novel antivirals (Sharma, Garcia, Arumugaswami, & Svendsen, 2020). Moreover, hPSC-derived cells and organoids are emerging as relevant models to study cellular responses of human tissues to SARS-CoV-2 infection and for COVID-19 disease modelling (L. Yang et al., 2020).

## 6.2 | Bacteria

Human embryonic stem cell-derived-endothelial cells (hESC-EC) have been used to investigate pathogen sensing pathways and the role of NOD1 receptors. hESC-EC are toll-like receptor 4 (TLR4) deficient and respond to *Haemophilus influenzae* via NOD1, suggesting that hESC-EC are protected from TLR4-dependent vascular inflammation (Reed et al., 2014). Cell-based immunotherapy approaches have been proposed, for example the mass production of phagocytes from iPSCs in stirred-tank bioreactors. These bioreactor-derived iPSDM were able to rescue mice from *Pseudomonas aeruginosa*-mediated acute infections of the lower respiratory tract and reduced bacterial load (Ackermann et al., 2018). Neutrophils have also been differentiated from iPSC and shown to produce reactive oxygen species (ROS) and phagocytose *E. coli* at similar levels than peripheral blood neutrophils. Importantly, this study shows that iPSC- based technologies can be used to generate human neutrophils for potential use in neutropenic patients (Trump et al., 2019).

Human gastric organoids derived from both human ESCs and iPSC lines systems were used to study the pathogenesis of *H. pylori* infection. These studies showed association of the *H. pylori* virulence factor cytotoxin-associated gene A (CagA) with the c-Met receptor and activation of downstream signalling leading to cell proliferation (McCracken et al., 2014). Human gastric organoids generated from iPSCs have been also used to study *H. pylori* interactions with the gastric epithelium. Using this system, it was shown that *H. pylori* significantly increased programmed death-ligand 1 (PD-L1) expression in organoid cultures after infection, which was dependent on the *H. pylori* effector CagA (Holokai et al., 2019).

iPSDM were also used as a cellular system to study *Salmonella*-host cell interactions. iPSDM efficiently phagocytosed live *Salmonella* Typhi and S. Typhimurium, indicating that iPSDM support productive *Salmonella* infection (Hale et al., 2015). Moreover, interactions of *S.* Typhimurium with intestinal organoids derived from hiPSC showed that hiPSC-derived organoids represent a promising model to study interactions of enteric bacterial pathogens with the intestinal epithelium (Forbester et al., 2015). Intestinal organoids derived from hiPSCs restrict *S.* Typhimurium infection and revealed a role for IL-22 in phagosome biology. Altogether, this work clearly shows that stem cell-derived intestinal organoids are a very powerful tool to investigate the effect of mutations on immune responses to pathogens (Forbester et al., 2018). iPSCs have been generated from an immunodeficient patient with severe infantile-onset inflammatory bowel disease (IBD) lacking a functional interleukin-10 receptor B (IL10RB)

gene. Macrophages differentiated from these IL-10RB$^{-/-}$ iPSCs showed defects in their ability to restrict *S*. Typhimurium replication, a phenotype that was rescued by introducing functional copies of the IL10RB gene (Mukhopadhyay et al., 2020).

Transcriptomic and proteomic profiling of iPSDM also helped to identify novel host factors required for the infection with *Chlamydia trachomatis*. These studies highlighted the role of type I IFN and interleukin 10 (IL-10)-mediated responses. Using CRISPR/Cas9 genome editing, knockouts of IRF5 (Interferon Regulatory Factor 5) and IL-10 receptor subunit alpha (IL-10RA) were generated in iPSCs showed that these genes are important in limiting chlamydial replication in iPSDM (Yeung et al., 2017).

Impaired interferon gamma (IFN-γ) signalling and mycobacterial clearance was observed in interferon gamma receptor 1 (IFNγR1)-deficient human iPSDM, showing that iPSCs represent a disease-modelling platform, in this case Mendelian susceptibility to mycobacterial disease, and to gain insights into cytokine signalling in macrophages (Neehus et al., 2018). iPSDM were probed for immune responses to Bacillus Calmette-Guérin (BCG) infection showing and increase in apoptosis and the production of nitric oxide (NO) as well as the expression of tumour necrosis factor alpha (TNF-α) after infection (Hong et al., 2018). iPSDM showed a similar gene expression profiles and characteristic immunological features of classical macrophages and supported *Mycobacterium tuberculosis* infection. After scaling up, this system was used to perform phenotypic screening of compounds against intracellular *M. tuberculosis*, highlighting the potential of iPSC-derived cells for high-throughput (HT) screens for new anti-tuberculosis drugs (Han et al., 2019). Although iPSDM with IFN-γR1 and IFN-γR2 deficiency showed residual induction of downstream signalling pathways, these cells did not significantly restrict BCG replication (Haake et al., 2020). iPSDM-restricted *M. tuberculosis* replication in vitro by >75%. However, IFN-γ increased iPSDM reactivity to LPS, but did not increase iPSDM mycobactericidal capacity. Finally, iPSDM have been used to investigate the cell biology of the response to *M. tuberculosis*, particularly autophagy. This system, combined with live cell imaging and three-dimensional electron microscopy (3D-EM), was used to show autophagic responses to *M. tuberculosis* in human macrophages (Bernard et al., 2020).

Induced pluripotent stem cell-derived brain endothelial cells (BMECs) were used to model bacterial interactions between the Group B *Streptococcus* (GBS) and the blood–brain barrier. Because immortalised or primary cell models lack substantial tight junctions, human iPSC-derived were used in these studies to model the blood–brain barrier (BBB). Notably, iPSC-derived BMECs consistently display BBB properties, such as the expression of tight junctions, which are key components for the investigation of bacterial effects on the BBB. Virulent GBS efficiently interacts with iPSC-derived BMECs and specifically disrupts tight junctions (Kim et al., 2017). Moreover, BMECs have been also developed as a cellular model to investigate the host-pathogen interactions during *Neisseria meningitidis* infection. This model recapitulated many previously observed interactions including the recruitment of the pilus adhesin receptor CD147 under meningococcal microcolonies (Martins Gomes et al., 2019).

### 6.3 | Parasites

iHLCs were also used to investigate in vitro the liver-stage of the parasite *Plasmodium spp*. This model allowed the assessment of donor-specific drug responses and host genetics on host-pathogen interactions. Establishment of in vitro liver-stage malaria infections in iHLCs were used for *Plasmodium berghei*, *P. yoelii*, *P. falciparum* and *P. vivax* and showed that differentiating cells acquire permissiveness to malaria infection at the hepatoblast stage (Ng et al., 2015).

Human iPSC-derived neurons were also used as a model for cerebral toxoplasmosis and to study both tachyzoite and bradyzoite stages of *Toxoplasma gondii* in human neurons (Tanaka, Ashour, Dratz, & Halonen, 2016). Human iPSC-derived cardiomyocytes (hiPSC-CM) represent a robust model to study genetic links to infection and to test novel drugs. For example, it has been shown that hiPSC-CM are a suitable model to study *Trypanosoma cruzi* infection in the heart and to test for efficacy of anti-*T. cruzi* compounds (da Silva et al., 2018).

### 6.4 | Fungi

In the case of Chronic mucocutaneous candidiasis (CMC), there is a susceptibility to chronic or recurrent infections with Candida *spp*. due to mutations in genes of the interleukin 17 (IL-17) signalling pathway. A hiPSC line from a patient suffering from CMC due to a heterozygous gain-of-function (GOF) mutation was successfully reprogrammed from CD34+ cells and probed as a model for the disease (Haake et al., 2020).

### 6.5 | Prions

Human stem cell-derived astrocytes were also established as a model that replicate human prions from brain samples of Creutzfeldt–Jakob disease (CJD) patients in a human prion protein (*PRNP)* gene genotype-dependent manner. This study filled a long-standing gap in the repertoire of human prion disease field and showed that iPSC-based models can be extremely useful for mechanistic studies and drug discovery in the prion field (Krejciova et al., 2017).

## 7 | PERSPECTIVES

Stem cell technologies are developing at a very fast pace and represent a revolution in cell biology for disease modelling. To address these shortcomings of the current approaches for in vitro disease modelling, advanced systems have been developed using stem cell-derived lines combined with either genome editing technologies or engineered 3D microenvironments. These stem cell-based models offer immense potential of physiologically relevant and predictive systems for investigating cellular responses to infection. In this context, differentiation protocols development and sharing are critical as well as developing systems that are more affordable. In the case of

immune cells, and particularly macrophages, we need a better understanding of the functional type of cells obtained by growth and differentiation factors-dependent protocols. A multi-step model has been proposed for many cell types. In the case of iPSDM, a first differentiation step based on their ontogeny and then a second step conditioned by their tissue-specific environment. In fact, phenotypic and functional characterization of the differentiated cells is critical to validate the system and often this information is lacking in human cells. Finally, stem cells combined with mouse models (e.g. engraftment of stem cells in mice) represent a very powerful system for understanding the contribution of immune cells to infectious disease (Takata et al., 2017).

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## DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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