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Progress, obstacles, and limitations in the use of stem cells in organ-on-a-chip models

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ABSTRACT

In recent years, drug development costs have soared, primarily due to the failure of preclinical animal and cell culture models, which do not directly translate to human physiology. Organ-on-a-chip (OOC) is a burgeoning technology with the potential to revolutionize disease modeling, drug discovery, and toxicology research by strengthening the relevance of culture-based models while reducing costly animal studies. Although OOC models can incorporate a variety of tissue sources, the most robust and relevant OOC models going forward will include stem cells. In this review, we will highlight the benefits of stem cells as a tissue source while considering current limitations to their complete and effective implementation into OOC models.

Contents

1. Introduction

In recent years, pharmaceutical research has become increasingly inefficient, with costs associated with preclinical work and clinical trials skyrocketing as the number of successful drug candidates plummets. In 2014, the cost of producing one new drug was estimated to be as high as $1.2 billion, with the combined research and approval processes taking an average of 10 years [1]. A sizeable portion of these expenses is associated with preclinical drug testing in cell culture and animal models. As evidence mounts that both types of preclinical models are limited in their ability to faithfully replicate human pathophysiology, it is becoming clearer that translational science is in dire need of a

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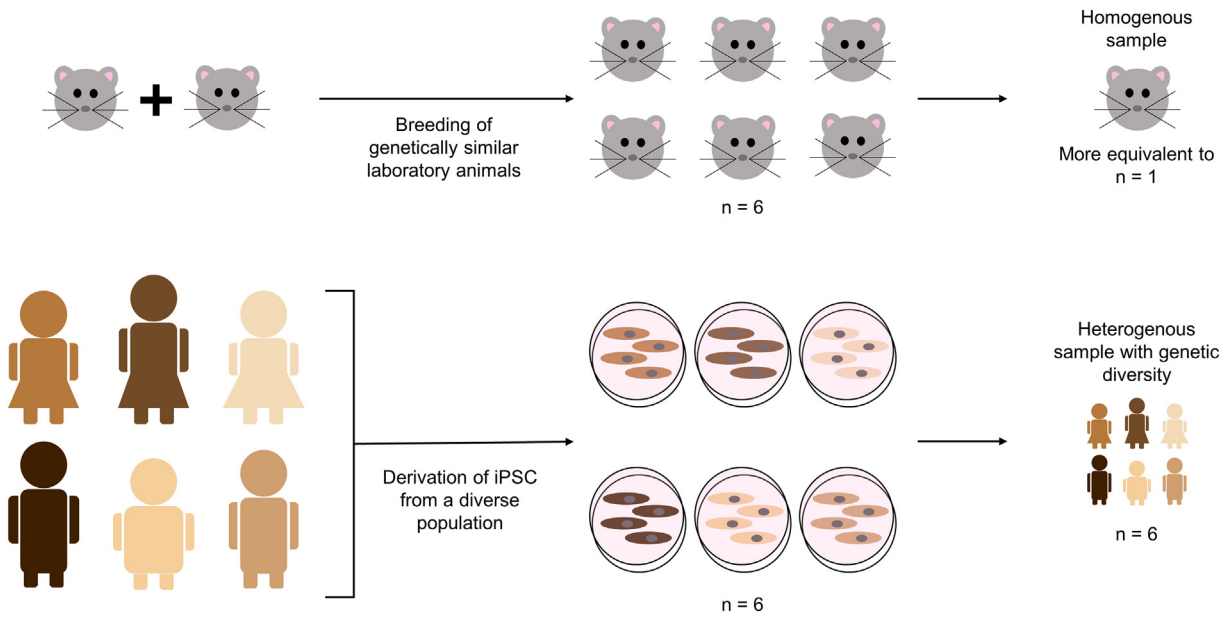

Fig. 1. Genetic heterogeneity of induced pluripotent stem cells (iPSCs) compared to laboratory animals. Laboratory animals, especially rodents, are often inbred and are thus very genetically similar. As a result, it is often difficult to reach population-level conclusions on drug safety and efficacy or disease mechanisms from animal studies. In contrast, iPSCs can be derived from a diverse patient population, producing data more representative of the relevant human population.

new model system to complement existing models that is more physiologically representative of human disease.

A variety of animal models, ranging from rats to pigs to non-human primates, have been utilized in studies of human diseases in nearly all organ systems, including the cardiovascular system and liver [2,3]. However, a number of investigational compounds that showed initial promise in preclinical trials in animals failed in human clinical trials [4–6]. The major assumption justifying the use of animals in drug discovery and toxicology research is that animal models are predictive of human response [7]. While some of the failures of animal models can be attributed to a lack of standardized experimental conditions, the majority are likely due to the failure of this assumption [8]. Animal models do currently have the advantage of allowing study of multi-organ interactions and system-wide drug effects. However, the inbreeding of many laboratory animals limits the genetic variability of these models, which is not representative of the diversity of the human population to which these results will be applied (Fig. 1).

Similarly, most commonly used *in vitro* drug testing methods do not accurately recapitulate the pathophysiology of human disease, even in the cases where human cells are used. Dissociated cell culture of primary cells and immortalized cell lines in 2D dishes is the main cell culture model currently used to evaluate drug efficacy and toxicology due to its availability, ease of use, and relatively low cost [9]. However, typical 2D culture methods are limited in that they frequently only incorporate one cell type without providing the cell matrix and mechanical cues found in *in vivo* tissue [10]. These factors have significant implications for cell morphology and phenotype, which in turn can affect drug response [11]. Even current 3D models do not always include physiologically relevant scaffold architecture or extracellular matrix components [10,12,13]. Additionally, static 2D and 3D models also do not account for the interconnectivity of organs, which becomes important when drugs of interest are metabolized by one organ into compounds that produce differing effects in downstream organs [9].

Organ-on-a-chip (OOC) technology is a promising complement to current preclinical models that can potentially combine patient-specific cell models, 3D tissue culture, microfluidics, and high throughput analysis methods to create a powerful tool for disease modeling, drug screening, and toxicity testing. Generally, the term OOC encompasses any device that incorporates cells into a microfluidic system within an engineered architecture that attempts to replicate some or all aspects of the native tissue structure. By this definition, all OOCs include a microfluidic chip with cultured cells or tissue and either a pump-driven or pumpless supply of culture media or other nutrient sources, and some incorporate 3D cell culture techniques, although this is not yet common practice [13–17]. Many OOCs also incorporate co-culture to look at interactions among different cell types within an organ [18]. This principle can be extended to the integration of multiple different OOCs into one body-on-a-chip (BOC) device, which provides a physical mimic of physiologically-based pharmacokinetic models that can be combined with computational pharmacokinetic and systems biology models to produce a better understanding of drug metabolism, bioavailability, and distribution in human systems [19–22]. Additionally, comparing human and animal cell-based BOC systems could facilitate extrapolation of preclinical animal work to human clinical trials [23]. BOC and OOC technologies are currently limited in their adoption and application due to the technical knowledge required for device design and cell culture, as well as the lack of integration with available high-throughput screening methods. However, OOC research has significantly expanded in the past several years and ongoing research both in industry and academia has the potential to increase the adoption of these technologies in drug discovery (Fig. 2) [10].

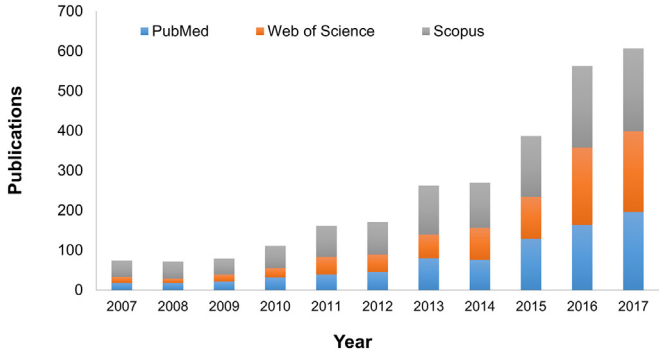

Fig. 2. Organ-on-a-chip publications. Publications related to organ-on-a-chip devices each year since 2007, defined by a search for "organ on chip" on PubMed, Web of Science, and Scopus. Publications may be duplicated across databases.

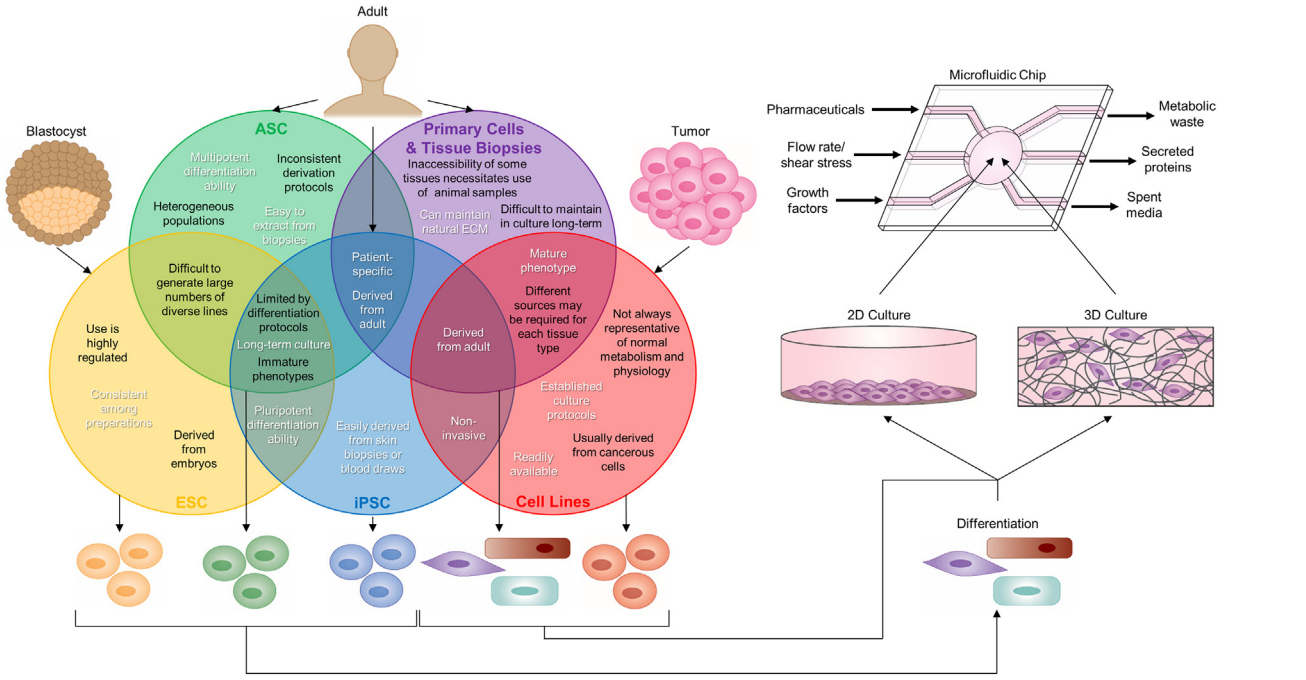

Fig. 3. Tissue sources for organ-on-a-chip (OOC) devices. Embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs) can be differentiated and incorporated into microfluidic chips in the same way as cell lines and primary cells. The Venn diagram illustrates advantages (in white) and limitations (in black) for the use of ESCs, ASCs, iPSCs, primary cells and tissue biopsies, and cell lines in OOC devices. Cell lines and primary cells have been more commonly used in OOCs because they generally have well-characterized biological responses. However, cell lines are not representative of normal physiology, and primary cells have limited culture time and inconsistent quality. In contrast, stem cells are easy to obtain and represent an essentially unlimited cell source. Even with current limitations in differentiation and maturation protocols, stem cells are a promising technology for incorporation into OOC devices.

One of the most important parameters in OOC design is the biological tissue source. Stem cells will allow us to source cells from humans without requiring a tissue biopsy. By definition, a stem cell is any cell that can self-renew and has the potential to differentiate into one or more specialized cell types. The most common types of stem cells used in biological research are embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs). These cells can be transformed into a variety of terminally-differentiated adult cell types, which can then be used for disease modeling or toxicity testing [24]. A variety of studies have already established the applicability of stem cells to drug testing and disease modeling as an alternative or additional preclinical model, demonstrating patient-specific drug responses and replicating clinical manifestations that were not observed in other preclinical models [25–28]. Stem cell technologies also allow investigation of diseases and drug effects in a more diverse population than for cell lines or animals (Fig. 1). Stem cell technology and differentiation techniques have seen substantial improvements in recent years, and although there are still some obstacles to complete implementation, stem cells will likely be widely applicable to OOC models in the near future (Fig. 3).

2. Current stem cell technologies

2.1. Adult stem cells (ASCs)

Human ASCs, the most common of which are mesenchymal stem cells (MSCs), are multipotent stem cells extracted from adult tissues. MSCs are typically derived from either the bone marrow or adipose tissue, making them an attractive option because they are relatively easy to extract from tissue biopsies [29]. They are multipotent stem cells, meaning that they can only differentiate into a limited number of cell types. MSCs are most commonly differentiated into mesodermal cells of bone, muscle, cartilage, and fat lineages [29]. In addition to being isolated from multiple different starting tissues, MSCs are isolated and cultured with a diverse range of protocols, leading to heterogeneous phenotypes in populations of MSCs differing from lab to lab and even from preparation to preparation [30]. Due to both their limited differentiation ability and the lack of consistent derivation protocols and defined biological responses, MSCs are less applicable to OOC models than their pluripotent counterparts.

2.2. Embryonic stem cells (ESCs)

Human ESCs are derived either from the blastocyst or from the inner cell mass of embryos. Depending on their source, they are either totipotent or pluripotent cells, both of which can differentiate into any type of adult human cell from any of the three germ layers [31]. An estimated 200 to 300 different ESC lines have been derived from different laboratories around the world, but unlike MSCs, comparative studies of these lines have demonstrated similar levels of pluripotency and surfaces marker gene expression among the different lines [32]. Although ESCs do have an unlimited differentiation potential and a more consistent phenotype than MSCs, the fact that human ESCs must be derived from human embryos makes their use ethically controversial, which has in turn led to increased regulation of their use in research. Due to the ethical debate surrounding ESCs and the technical difficulties generating large numbers of genetically diverse cell lines, it is harder to apply human ESCs than alternatives to precision medicine for disease modeling and therapeutic drug evaluations.

2.3. Induced pluripotent stem cells (iPSCs)

Like ESCs, iPSCs are pluripotent and can differentiate into cell types from all three germ layers [33]. Human iPSCs were first derived in 2007 by introducing four defined factors (Oct4, Nanog, Sox2, and *c*-Myc) into human adult fibroblast cells [34]. Protocols now exist to derive iPSCs using a wide selection of transfection methods [35] and adult somatic cell types including skin fibroblasts, adipose stromal cells, and blood samples [36–38]. Because iPSCs are derived from adult tissue instead of embryonic tissue, they avoid the ethical concerns

associated with ESCs. ESCs and iPSCs derived from the same genetic background have no significant differences in gene expression levels, surface marker expression, or morphology [39,40]. In addition to sidestepping ethical controversies, another advantage of iPSCs over ESCs is that they can be derived from donors with known disease phenotypes, whose cells can then be used in patient-specific disease models and drug screens.

2.4. Stem cell differentiation

The principal cells of interest for toxicity testing are cardiomyocytes, neurons, and hepatocytes, as their respective organs are the most likely to exhibit a drug-induced toxicity [24,33]. However, primary cells and tissue samples from these organs are difficult to obtain. As a result, the development of iPSC and ESC differentiation protocols have primarily focused on these three cell types [41]. Cardiomyocytes can be derived from iPSCs and ESCs with a chemically defined medium for the evaluation of drug response and cardiotoxicity using transcriptome profiling and functional analyses [25,42]. Studies have shown a predictive response of patient-specific iPSCs, including those derived from patients with known cardiac conditions, to cardiotoxic therapeutics such as tyrosine kinase inhibitors, doxorubicin, troglitazone, nicorandil, and cisapride [25,26,28,43]. Patient-specific neurons, neural stem cells, and motor neurons have been derived from iPSCs and used in drug screens for Alzheimer's disease, Niemann-Pick disease type C, and amyotrophic lateral sclerosis [44–46]. Protocols to produce hepatocyte-like cells from iPSCs have been implemented in studies of patient-specific hepatotoxicity and drug-induced liver injury [47,48]. These cell types are also relevant in disease modeling and mechanism studies, including for neurological diseases such as schizophrenia [49,50] and Alzheimer's [51] and cardiovascular diseases such as familial dilated cardiomyopathy [52].

In addition to these cell types, iPSC and ESC differentiation protocols have been expanded to include a range of cell types from retinal cells to skeletal muscle [53,54]. Although these studies all illustrate the promise of iPSC- and ESC-derived cells in disease modeling, drug screening, and toxicity testing, many of them were limited to traditional 2D culture techniques, restricting their clinical applicability due to their lower throughput, unrealistic cell microenvironment, and limited number of co-cultured cell types [10]. By incorporating iPSC- and ESC-derived cells into OOCs, researchers can take advantage of the unique ability to create a wide variety of cell types combined with the potential to create high-throughput systems that have a complete 3D cell microenvironment as well as the ability to provide multiple stimuli and asses multiple functional readouts [12,13,55].

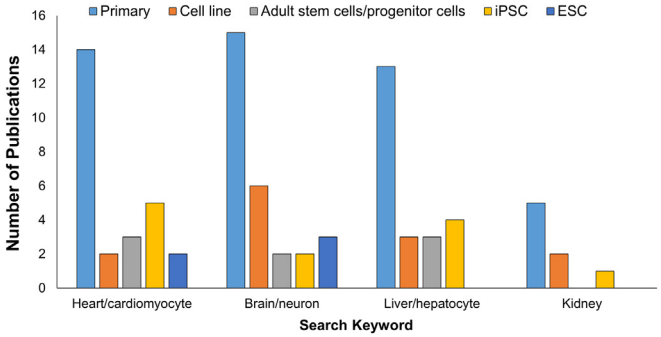

Fig. 4. Prevalence of varied tissue sources in organ-on-a-chip research. Online search was completed for the listed keywords plus "lab chip". Original research articles produced by the search were then manually screened to determine the cell types used. Review articles, editorials, and commentary papers were excluded from analysis. Primary cells and tissues were the most common tissue source used in organ-on-a-chip research publications.

3. Biological tissue sources for OOCs

Although there is a clear potential for incorporating stem cells into OOC devices, this is far from common practice. The most common types of cell used in OOC devices to date are primary cells and tissue biopsies and cell lines, with stem cell sources only occasionally used (Fig. 4) [56,57]. Cell lines have been incorporated into OOC models for organs ranging from the liver to the placenta [58,59]. They have also been used to study organ interactions in multi-organ chip systems, including combinations of the lung, liver, gastrointestinal tract, kidneys, skin, bone marrow, and adipose tissue [15,60]. Primary cells have been used in OOC simulations of a variety of tissues, including heart valves, lung, kidney proximal tubule, liver, and neurovascular units [14,61–64]. However, some of these studies were limited to using animal primary cells due to the difficulty of extracting human primary tissue and, in other cases, co-cultures with established cell lines were required to more accurately model cell- or organ-level interactions [14,61,62]. *Ex vivo* tissue culture has been used in several OOC models, including co-cultures of intestinal and liver slices and culture of endocrine tissues [65,66]. A combination of all three tissue sources was used in at least one study that modeled multi-organ interactions among a skin biopsy, an intestinal barrier model containing primary cells, and a liver cell line, demonstrating that the appropriate tissue source may vary among organs [67].

Each of these sources has a unique set of advantages, but each is also limited in comparison to stem cells (Fig. 3). Tissue biopsies and primary cells are similar in that they are derived directly from adult tissue, and thus provide potentially more accurate information on the biological properties of mature tissue [57]. Tissue biopsies have the added benefit of maintaining some of the natural extracellular matrices and three-dimensional tissue structures that are not obtainable with 2D cell cultures. As discussed previously, dissociated cells can be incorporated into a 3D architecture, but current methods often lack the organ-specific structures and matrix components found in tissue biopsies. One specific limitation of tissue-based devices is that tissue samples often do not survive more than 48 h in *ex vivo* culture, which restricts their ability to be studied long-term [57].

Cell lines are similar to primary cells in that they are a source of more mature cells, with the same limitation of lack of natural extracellular matrix. Additionally, cell lines are more likely to be a homogenous population and to produce reproducible results compared to primary cells, but, as a tradeoff, they lack the patient-specificity found in primary cells, tissue biopsies, and stem cells. The lack of patient-specificity limits the use of cell lines for disease modeling studies. For toxicity testing, the cell lines used often have induced overexpression of proteins known to be involved in specific toxicity-related pathways, and as such are limited to assessing known mechanisms of toxicity [9]. One study evaluating cell lines for organ-specific drug responses observed that the majority of compounds had similar effects in all three of the cell lines studied, with little correlation to *in vivo* organ-specific toxicities [68]. Additionally, there is growing evidence that cell lines may not accurately recapitulate tissue function. One comparison of protein expression in a hepatoma cell line and primary hepatocytes indicated that the cell line had downregulated drug-metabolizing enzymes and some normal metabolic pathways to favor cell-cycle-associated proteins [69]. Thus, while cell lines may currently be one of the more commonly used biological tissue source for OOCs, they are far from being the most applicable.

Despite the clear advantages to using primary cells, biopsies, and cell lines, stem cell-based cultures provide advantages in that they are often easier to obtain, last longer in culture, and have the potential to differentiate into many cell types. Stem cells provide the potential to recreate multiple organ-like structures with the same genetic background, which is useful in disease modeling and drug testing for genetic diseases. However, there are limitations to current stem cell technologies that will need to be resolved before they become widely applicable to OOC models for all organ types.

4. Current limitations of stem cells in OOCs

One of the biggest obstacles to the use of stem cells is the variability in efficiency and robustness of differentiation protocols. The differentiation of stem cells into distinct, mature cell phenotypes is also limited. For example, the heart is composed of a mixture of cardiomyocytes, cardiac fibroblasts, and endothelial cells. Well-defined differentiation protocols exist for cardiomyocytes and endothelial cells, but not for cardiac fibroblasts. Some studies have succeeded in producing fibroblast-like cells that recapitulate the morphology of cardiac fibroblasts [26]. However, the lack of specific cell type markers for cardiac fibroblasts, beyond myofibroblast and mesenchymal genes common to all fibroblast cell lineages, makes it difficult to determine the efficiency of differentiation protocols.

Even in cells for which clear markers exist, such as cardiomyocytes and endothelial cells, there is variability in the differentiation methods used across different research groups, including in the small molecules used to induce differentiation and the environment in which the cells are cultured [42,70–74]. Early protocols [74,75] have continually been modified to make differentiation more efficient [76,77]. This is not limited to cells of cardiovascular lineage; differentiation protocols for other cell types, such as skeletal muscle, also vary among research groups [78,79]. In part, this is because the field is constantly improving protocols and adoption of new protocols across different laboratories can be slow. Nevertheless, the constant improvement in existing protocols and the development of protocols for differentiating into new cell types, such as a recently published method which illustrated differentiation into podocytes [80], will increase the applicability of stem cells to OOC models.

Once the stem cells differentiate into the desired cell type, the next obstacle is ensuring that the cells achieve a mature adult phenotype that is representative of the adult organ [33]. For example, ESC- and iPSC-cardiomyocytes derived using current protocols are more similar to fetal than adult heart tissue on the transcriptome level, and have immature electrophysiological behaviors, metabolism, calcium-handling, and sarcomere structural features [81–83]. While this is a concern for incorporation of these cells into an OOC model, numerous research groups are developing methods for pushing the cardiomyocytes towards a more mature phenotype, such as electrical or mechanical stimulation, geometrical confinement, overexpressing maturation-related microRNAs, introducing growth hormone, and increasing culture time [84–86]. Recent publications have demonstrated that advanced maturation can be achieved in 3D cardiac tissues by electrically stimulating tissue formed from early stage iPSC-cardiomyocytes [87] and that progressively changing the afterload experienced by cardiac tissues can improve maturation or induce a disease-like state [88].

In addition to the relative immaturity of most iPSC-cardiomyocytes, another issue is separating out individual cell subtypes. Cardiomyocytes can be atrial, ventricular, or nodal, and differentiation protocols typically result in a mixture of all three subtypes. While protocols exist to derive each of these cell subtypes independently [89–91], protocols which result in a heterogeneous population are much more common. Even with differentiation into individual subtypes, it is unclear which of these subtypes would be relevant to include in OOC models and the relative proportions they would need for incorporation.

Like iPSC- and ESC-derived cardiomyocytes, stem cell-derived endothelial cells, neurons, and hepatocytes display an immature phenotype and a lack of distinctly separable cell subtypes. Endothelial cells can be consistently derived with high purity, but protocols to separately differentiate arterial, venous, valvular, and lymphatic endothelial cells are still lacking [71–73]. ESC- and iPSC-derived neuronal cells also usually contain a heterogeneous population of neural subtypes. Although several protocols for specific neuronal subtypes exist, these have low efficiency [33,49,92]. Similar to the cardiomyocytes, stem cell-derived hepatocytes are often called hepatocyte-like cells, as they express more fetal markers than adult markers and have lower metabolic activity than primary cultures [93–95].

Another limitation of iPSC- and ESC-derived cells in OOCs beyond the cell phenotype is the lack of native 3D tissue structure. Unlike tissue biopsies, which contain the native extracellular matrix, stem cell-based cultures have derivation protocols that usually utilize 2D culture conditions. A number of laboratories have approached this issue by creating microtissues, usually containing iPSC-derived cells and an extracellular matrix. Some of these microtissues have been developed for heart OOC models, thus heightening the relevance of iPSC- and ESC-derived cardiomyocytes by incorporating a 3D scaffold and improving their maturation [96–98]. Although this is a promising improvement, it is not yet common practice, and some parameters of these types of models will need to be optimized before they are fully representative of human physiology. These may involve the use of matrix components found in native heart tissue and the incorporation of the multiple other cell types, including fibroblasts and endothelial cells, at the same ratios found *in vivo*.

Another consideration for using stem cells in OOCs is whether differentiation will be conducted on or off the chip. On-chip differentiation would permit precise control of the cell microenvironment and culture conditions, including relevant chemical, electrical, and mechanical cues for differentiation, while simultaneously measuring a variety of functional outputs [99–102]. Yoshimitsu et al. described the expansion of human iPSCs in a microfluidic chip after optimizing both surface coating and media flow rate. They demonstrated differentiation of the iPSCs into extra-embryonic trophoblast lineage cells and observed that iPSCs in the microfluidic chip exhibited the expected response to anti-tumor drugs [101]. Giobbe et al. investigated the effect of perfusion frequency on iPSCs and ESCs, and found that controlling the frequency affected pluripotency gene expression of the stem cells and the extent of homogenous differentiation of the cells into ectodermal, mesodermal, and endodermal lineages once the cells were cultured in differentiation media. They also demonstrated successful differentiation into functional cardiomyocytes with approximately 65% efficiency, as well as on-chip differentiation of eight human iPSC lines into hepatocyte-like cells [103]. Similarly, Hesari et al. successfully differentiated human iPSCs into neurons on a hybrid microfluidic chip containing aligned poly(lactic-*co*-glycolic) acid nanofibers. The differentiation of neural cells on the hybrid device increased the expression of neural marker genes compared to 2D cultures and scaffolds alone, demonstrating the potential for these devices to help overcome some of the issues associated with stem cell-based cultures [104].

Taken together, these studies show the promise of microfluidics in stem cell culture and differentiation. However, they also point to some of the potential design problems for microfluidic OOC systems, especially in the context of multi-organ devices or individual OOCs incorporating more than one cell type. With different flow rates, scaffolding, culture time, and biophysical and chemical stimuli required for differentiation of different cell types, device design will become much more complex, making widespread adoption of the technology challenging. One potential solution would be to culture individual OOCs and combine them into one multi-organ system once all of the components completed their differentiation, as suggested by Loskill et al. in their μOrgano chip design [105]. However, this type of design would still be limited by the difficulty of timing the differentiation of the multiple components and by the types of connections that are possible among the organs. It also does not address the issues of how to derive multiple cell types for use within a single organ model and how to optimize the cell culture media to promote survival of all cells once the organs are connected.

Additionally, a question that is applicable to all OOC models, with special relevance for stem cell-based models, is if we care about modeling the general population, whose cells do we test? Regardless of whether primary cells or stem cells are used, there is an inherent variability in genotype from patient to patient, which can potentially translate to major differences in phenotype, including in drug metabolism and response [106]. Drug screening for efficacy can use patient-derived cells as a model, but identifying representative cell sources for

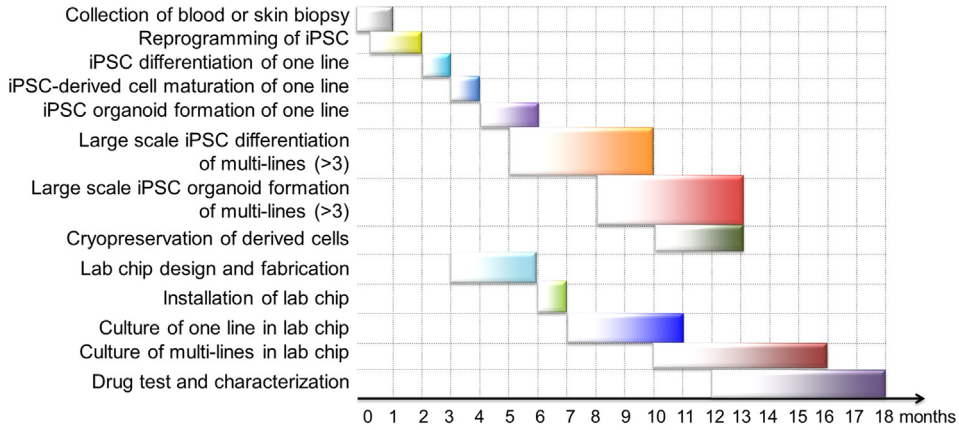

Fig. 5. Timeline of incorporation of iPSC into organ-on-a-chip devices. A representative timeline for incorporation of an iPSC line into an organ-on-a-chip device, from reprogramming of the line through drug testing or characterization studies.

toxicity testing will prove more challenging and may require the use of population-based genomics and computational modeling [107]. Disease modeling and mechanism studies using OOC models will also need to incorporate cells from multiple patients to demonstrate consistency in mechanism across patients of different genetic backgrounds.

Finally, it is important to take into consideration the greater time and cost currently associated with designing stem cell-based OOC devices. In contrast to cell lines, which are widely available, and primary cells, which can be isolated relatively quickly when tissue samples are available, stem cell-based models take a long time and a great deal of resources to develop (Fig. 5). The process of deriving iPSC from blood or skin samples can take weeks to months, and differentiation protocols often take multiple additional weeks. Traditional differentiation protocols also generally yield a limited number of cells, especially for quiescent cell types such as cardiomyocytes. These factors limit the feasibility of commercial production of stem cells and their derivatives and make it difficult for research groups new to stem cell technology to begin using it. Large scale production of stem cell-derived cells, such as recent protocols developed for iPSC-cardiomyocytes, will improve accessibility [108,109]. However, cryopreservation of differentiated stem cells is often trickier and less successful than cryopreservation of cell lines or primary cells, which increases the difficulty of widespread distribution. For these reasons, research groups interested in using stem cells in OOC development should be prepared for the slower progress rate associated with their use. Despite these challenges, incorporation of stem cells into OOC devices is not impossible, and will ultimately lead to increased applicability of these devices as clinical models.

5. Conclusions

OOC systems are a promising technology for drug screening, disease modeling, and toxicity testing applications. By incorporating multiple cell types and multiple functional analyses, they have the potential to model human pathophysiology and measure drug response more accurately than current preclinical studies, which typically employ 2D cell culture and animal models. Stem cell differentiation and maturation protocols require improvement before stem cells can be considered accurate models of human physiology. However, because stem cells are easier to obtain than many primary cell types and tissue biopsies, and because they are more physiologically representative than cell lines, it is likely that stem cells will be the primary tissue source for OOCs going forward. Continued research on methods for on-chip differentiation of stem cells into functional organ models will contribute to both improvement in stem cell methods and advancement of OOC technologies.

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