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Review

Organoids from pluripotent stem cells and human tissues: When two cultures meet each other

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SUMMARY

Human organoids are a widely used tool in cell biology to study homeostatic processes, disease, and development. The term organoids covers a plethora of model systems from different cellular origins that each have unique features and applications but bring their own challenges. This review discusses the basic principles underlying organoids generated from pluripotent stem cells (PSCs) as well as those derived from tissue stem cells (TSCs). We consider how well PSC- and TSC-organoids mimic the different intended organs in terms of cellular complexity, maturity, functionality, and the ongoing efforts to constitute predictive complex models of *in vivo* situations. We discuss the advantages and limitations associated with each system to answer different biological questions including in the field of cancer and developmental biology, and with respect to implementing emerging advanced technologies, such as (spatial) -omics analyses, CRISPR screens, and high-content imaging screens. We postulate how the two fields may move forward together, integrating advantages of one to the other.

INTRODUCTION

Organoids are 3D culture systems that are widely used as physiologically relevant *in vitro* models to address an array of biological questions, ranging from developmental principles to translational prediction of patient responses.[1–3] Organoids are appealing for multiple reasons. They can capture different physiological tissue states (homeostatic/healthy and diseased) and present an increased level of cellular and architectural complexity as compared with classical cell culture models, while still being amenable to longitudinal experimental manipulation, more complex readouts, and higher-throughput applications. The value of these innovative models and their continued optimization is also highlighted by the recent implementation of the FDA to bolster adopting non-animal methods, including organoids, as validation in pre-clinical testing.[4]

A univocal definition for an ''organoid'' is difficult to provide,[5] as a wide array of different models with distinct features that are constantly evolving can be grouped under this same term. A general classification can be based on the organoid origin: those derived directly from tissue, generally referred to as tissue stem cell (TSC)-derived[5] and those derived from the differentiation of (either embryonic or induced) pluripotent stem cells (PSCs).[6] Historically, both cultures can find their origin in classical developmental biology experiments of cellular dissociation and reaggregation, which initially illustrated the principles of tissue self-organization.[7] In the following decades, essential contributions, such as the establishment of PSC cultures, the growth of 3D cellular structures in extracellular matrices, and the understanding of stem cell niches paved the way toward organoid cultures around the end of the first decade of the 2000s (as reviewed in Lancaster and Knoblich[7] and Kretzschmar and Clevers[8]). These initial cultures from TSCs or PSCs were developed simultaneously[7] and inspired by each other. Since then, both PSC- and TSC-organoid fields provide exponential advances for basic research and translational applications, also facilitated by the advent of novel technologies. In this regard, both fields seem for a large part self-sustainable, but sharing common goals. Yet, PSC- and TSC-organoids provide us with complementary platforms that may synergize to provide novel insights.

In this review, we will compare TSC- and PSC-organoids and evaluate their main features, strengths, and limitations, with a focus on organoid models of human origin. We will discuss their main applications, recent advances, and current directions of both fields, also in the context of adopting state-of-the-art technologies. Finally, we provide an outlook on how the two fields may jointly move forward to provide even better sophisticated experimental models or ''next generation organoids.''

A CELLULAR COMPARISON BETWEEN TSC- AND PSC-DERIVED ORGANOIDS

Multiple criteria could be defined when comparing TSC- and PSC-organoids (Figure 1; Table 1). Given that one of the main purposes is to recapitulate aspects of human tissue biology to allow its study *in vitro*, tissue similarity can be considered the prime criterium to benchmark to. This is demarcated at multiple levels including cellular complexity (Figure 1A), the functionality of the different cell types that are present and their maturation level (Figure 1B), similarity to different organ regions (Figure 1C), tissue architecture, and cellular organization. Moreover, how well an organoid system allows reproducibility of

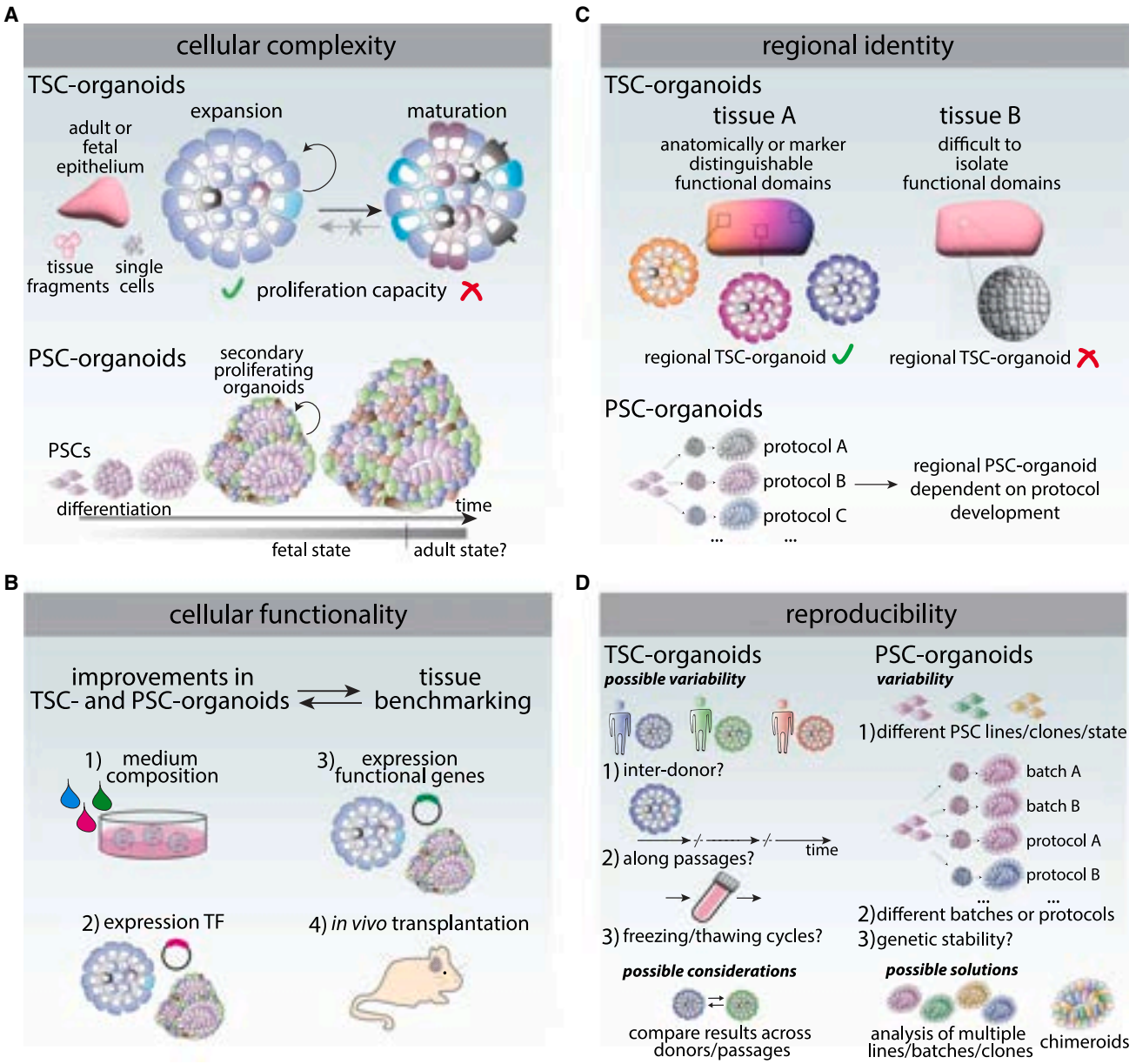

Figure 1. Main features of PSC- and TSC-organoids and associated considerations

Different organoid systems derived from pluripotent stem cells (PSCs) or tissue stem cells (TSCs) possess inherently different features that have implications for their use to model human organ functionality.

(A) TSC-organoids capture the epithelial components of an organ, in an expanding state. Cellular complexity can be modulated by shifting to maturation medium. In PSC-organoids, instead, cellular complexity might not be limited to epithelial cells and changes over time during organoid development.

(B) Approaches that have been used to improve cellular functionality in TSC- and/or PSC-organoids.

(C) The ability to generate regional organoids from TSC- and PSC-organoids is methodologically different and depends on the specific organ considered.

(D) Possible sources of variation in reproducibility using TSC- and PSC-organoids. PSC-organoids may associate with variation across lines, batches, and clones, as well as PSC line genetic stability. Reproducibility concerns in TSC-organoids may arise by using organoids from different donors, along repeated passaging, as well as during freeze-thaw cycles.

results and the genetic stability of the organoid in culture (Figure 1D) are important features.

Cellular complexity

TSC-organoids are mostly "epithelial-selective" cultures relying on specific culture conditions to recapitulate the epithelial niche specific to each organ to sustain the maintenance and growth of the stem cell/progenitor populations from which the organoids are initially derived. In PSC-derived organoids, the PSC potency needs to be progressively reduced to reach germ layer and organ cellular specification. In this way, PSC-organoids can also co-develop non-epithelial components in addition to the epithelial cells. The different rationale behind the two culture types has a great impact on the cellular representation and complexity

Table 1. Main characteristics of PSC- and TSC-organoids

	PSC-organoids	TSC-organoids
Cellular and architectural complexity	possibility of off-target cellular populations	exclusive presence of epithelial lineage(s) and tissue-specific cell types
	tissue identity externally specified using varied chemical cocktails	broadly maintain natively established tissue identity
	higher representation of tissue cellular complexity from different lineages but cellular proportions can be distinct from *in vivo*	more restricted cellular complexity linked to epithelial selectivity
	usually recapitulates more complex tissue architecture	usually characterized by simpler, but uniform, architecture
	virtually model any tissue type	model establishment relies on self-renewal or (activatable) regenerative capacity of the organ
	capacity to generate proliferating secondary organoids is dependent on the PSC-organoid type	can be cultured at any time in conditions promoting expansion or maturation
	maturation is usually limited to fetal states and can be lengthy	can capture in principle adult and fetal tissues
	ongoing efforts to identify optimal tissue specification conditions	ongoing efforts to identify optimal expansion and maturation conditions
Regional identity	require ad hoc protocols to specify different functionally or anatomically distinct organ regions	require capacity to isolate different cell types/organ functional domains based on anatomical distinction or specific markers
Reproducibility	variability across different PSC lines, clones, states	possible inter-donor variability
	batch-to-batch variability	possible variability of the organoid culture along passages
	possible differences linked to specific protocol used	not fully characterized how freezing/thawing cycles could affect line variability
	important to compare/validate findings across multiple batches, clones, PSC lines	important to compare/validate findings across multiple donors and engineered clonal lines (when applicable)
	necessity to regularly check maintenance of genetic stability and use of low-passage PSC cultures	possible occurrence of clonal dominance over culturing time

A side-by-side summary of the main general features of PSC- and TSC-organoids in relation to representation of cellular and architectural complexity, capacity to capture organ regional identities, and their reproducibility.

(Table 1), and consequently in their use and efforts for further improvements. For most of the TSC-organoid models, maturation conditions are needed to stimulate the stem cell/progenitor pools to augment production of the differentiated progeny or to induce a switch from a proliferative to a more mature cellular state. In the mature state, TSC-organoids are often not capable of further expansion (Figure 1A). In TSC-intestinal organoids, it has been shown that cells can be differentiated using a short pulse, and after that can return to a stem/proliferative state, postulating that it is possible to reach an intermediate stage in which the organoids can either proceed to terminal maturation or go back to a more undifferentiated state.[9,10] Conditions to improve maturation therefore can increase cellular complexity in TSC-organoids[11–13] and may promote the generation of a specific differentiated progeny.[14–16] This has been shown e.g., in TSC-airway organoids, where ciliated cell fate can be induced with a specific maturation protocol to study primary ciliary dyskinesia.[14]

The cellular populations that can be obtained lie within the potency of the tissue-resident epithelial stem/progenitor cells, leading to a limitation that TSC-organoids may only be derived from tissues that possess an active or activatable stem/progenitor pool. While this lineage-specificity on the one hand ensures the lack of "off-target" cell types, as instead sometimes observed in PSC cultures (derived from unintended co-developing of different germ layers or tissues), the cellular complexity that can be reached with TSC-organoids is limited to epithelial and specific lineage components. Taking the liver as an example, TSC-organoids cannot capture non-parenchymal cells such as stellate cells, which are crucial disease components, such as in liver fibrosis.

In PSC-organoids, cellular complexity is dictated by the developmental trajectory (Figure 1A). Cellular composition over time is determined by sequential media switches aimed at reproducing organ development and maturation. At any given time, therefore, cellular complexity and the degree of maturation in PSC-organoids changes, directed toward a more differentiated state in which multiple mature cell types are present, though often not reaching an adult-like state. Depending on the organoid system, this process can take some weeks to a few months (e.g., Giandomenico et al.,[17] Spence et al.,[18] Thompson and Takebe,[19] and Nakano et al.[20]). While usually a "completion" of maturation is assumed upon reaching the protocol's end, maturation levels may continue to increase

upon prolonged culturing. For example, very prolonged cultures of PSC-brain organoids displayed increased electric activity[21] and possible acquisition of a post-natal transition, as judged by NMDA receptor isoform shifts.[22] Given that the starting material is a PSC, this methodology allows to achieve a higher cellular diversity, that can simultaneously include cell types of different lineages. The case of PSC-kidney organoids is striking: from tissue, the tubular epithelium can be propagated in culture as organoids (termed tubuloids),[23] but kidney organoids generated from PSCs can recapitulate multiple segments in complex structures.[24,25] Correct representation of cellular proportions as well as cellular distribution can represent a challenge for PSC-organoids. For example, PSC-liver organoids may underrepresent hepatocytes, while they generally overestimate cholangiocyte and stellate cells.[26,27] In the PSC-organoid field, protocols are constantly evolving to increase cellular complexity and tissue resemblance, as well as to reduce off-target cells.[28] Yet, the high *in vivo* complexity and precise spatially and temporally regulated human organ specification remains inevitably difficult to fully recapitulate *in vitro*.

PSC-organoids are a more versatile proxy of human organs and represent organs/cell types for which TSC-organoids do not exist, such as the heart (e.g., Hofbauer et al.,[29] Drakhlis et al.,[30] and Lewis-Israeli et al.[31]). As mentioned, this likely links to the existence of adult TSCs/regeneration capacity, which is unlikely in the case of the mammalian heart,[32] and culture conditions are primarily optimized to sustain epithelial cell expansion. Generally, for both PSC- and TSC-organoids, cellular complexity can be limited and frequently misses tissue components, including stromal compartments, vasculature, immune components, and systemic organ-to-organ crosstalk, which can be improved using multiple approaches as we will discuss later. As an important consideration, while increasing organoid complexity and similarity to tissue is advantageous to obtain more *in-vivo*-like models, it often does so at the expense of reproducibility and simplicity of readouts and data analyses. Therefore, in a more reductionistic view, a less complex 3D system can be more suitable for certain applications, for example, to study a specific cell type, in which confounding factors may want to be excluded.

Cellular functionality

How can the functionality of PSC- and TSC-organoids be assessed? Various -omics techniques are often used to assess gene expression and regulation as a proxy for cellular state and maturation. This can be linked to cell function, through expression analysis of specific functional genes, such as receptors, transporters, and enzymes. These indirect approaches can be biased in capturing cellular heterogeneity, as some cells can be easier to retrieve than others. Specific to the organ, functional assays are also used, such as secretion assays, electrophysiological tests, transporter assays, and these can inform on disease phenotypes (e.g., Dekkers et al.,[33] Uchida et al.,[34] Trujillo et al.,[35] and Bannier-Hélaouët et al.[36]). For the application of human organoids as platforms for functional biological studies and as preclinical models, their suitability should be carefully evaluated depending on the specific use. This can include proper expression of receptors needed for e.g., microorganism entry[37–39] or drug metabolism enzymes.[40,41]

For an in-depth analysis of cell function, maturation conditions in both TSC- and PSC-organoids can be adjusted to favor shifting between cell types (e.g., Low et al.[42]) or to enrich for rare cell types (e.g., Beumer et al.[43]) (Figure 1B). If tweaking culture conditions is not sufficient, additional approaches have been used, such as overexpression of master transcription factors, as shown with NGN3 to promote enteroendocrine cell differentiation in TSC-intestinal organoids[10,44] (Figure 1B). Forced overexpression of functional genes, as shown for the NTCP receptor to allow hepatitis B virus entry has also been utilized.[45] *In vivo* transplantation can also be pursued to foster further maturation, as well as to assess the impact of cellular integration on host tissue[46–50] (Figure 1B). For real-time assessment of cellular abundancy, functionality, maturation, as well as to isolate specific cell types, endogenous gene tagging applied to organoids has been instrumental,[9,51] as exemplified both in TSC-organoids (e.g., Beumer et al.,[10] Huang et al.,[16] and Lin et al.[52]) and PSC-organoids (e.g., Howden et al.,[53] Vanslambrouck et al.,[54] and Mithal et al.[55]).

Since differentiated cells in a certain tissue are the "effector" or functional blocks, assessment of their functionality is often the primary interest. However, also evaluating how well the stem cell *in vivo* properties are maintained in culture is of interest, but this has not been done systematically both in TSC- and PSC-organoid models. This might concern both stemness as well as their differentiation capacity. TSC-organoids attempt to capture the expansion/regeneration capacity of the TSC pool (or cell populations with regenerative capacity, as for example, in the liver). The human intestine has a very-high turnover rate, and perhaps not coincidentally TSC-intestinal organoids can be expanded virtually without culture time limit.[56] Likewise stem cells isolated from PSC-derived intestinal organoids have a durable expansion capacity *in vitro*.[57] In comparison, some TSC-organoids derived from organs, which are not usually under constant proliferation have robust expansion capacity supporting multiple passaging steps but within a more defined culture period. This is the case for cholangiocyte organoids,[58] which might reflect the ductular reaction occurring only upon injury *in vivo*.

Given that PSC-organoids capture a developmental process, these models may possibly reflect the tissue stem cell behavior temporally taking place during organogenesis when analyzed along the differentiation process. A recent analysis of the mode of stem cell division in PSC-brain organoids revealed similarity with fetal tissue, although slightly favoring direct differentiation (neurogenesis), suggesting also the potential existence of inherent organoid phenotypes.[59] Stem cell functionality in organoids may be improved by optimization of culture conditions, as shown by addition of IGF1 and FGF2[12] or NRG1[60] in TSC-intestinal organoids, resulting in better clonogenic capacity and improved differentiation potential.[12]

Regional identity

How well different organoid models can capture organ regional heterogeneity deserves further discussion. In this respect, TSC- and PSC-organoids present a fundamental difference. While for PSC-organoids defined differentiation protocols are needed to develop regional organoids, TSC-organoids could in principle be generated from functionally and anatomically

different organ regions, though still requiring tailored culture condition establishment. This can imply some different technical challenges for both organoid systems (Figure 1C). Sometimes, functionally distinct organ regions cannot be easily isolated from tissue biopsies, and the absence of defined markers can pose problems for the establishment of pure, regionalized TSC-organoids. That said, defining protocols to reproduce regional identity in PSC-organoids has not yet been achieved for all tissues. To give an example, functional zones are established in the liver between the portal and central veins (liver zonation). Hepatocytes in the different zones each present with specialized functions. TSC-murine hepatocyte organoids have not been able to be derived in a zone-specific manner, but expression of some zonation genes can be modulated by using specific differentiation media.[61] PSC-liver organoid protocols are currently also unable to capture liver zonation, and the hepatocyte populations rather represent more mixed states.[62,63]

For other organs, such as the brain, some distinct regions can be isolated and cultured as TSC-organoids while broadly preserving regional identities,[64] but it might be difficult to isolate more nuanced subregions. In this respect, the PSC-brain organoid field has developed iterative protocols to reflect many different brain regions.[65]

For organs, such as the gut, instead, it may be easier to isolate anatomically-distinct portions of the tissue. Regional identity is well described across different regions of the human small intestine.[66,67] Regionalization in TSC-intestinal organoids derived from different tissue segments is maintained at transcriptomic level,[68,69] and possibly at functional level,[70] and methylation profiles are well preserved.[69] For PSC-organoids, instead, protocols currently exist to model broadly the small intestine[18] and colon.[71,72] Derivation of regional organoids from both PSCs and TSCs is therefore highly dependent on the considered organ (Figure 1C).

Architecture and cellular organization

Architecture and cellular organization are essential for proper tissue homeostasis and their disruption can cause disease, such as cancer.[73] PSC-organoids in general are considered to allow for a higher level of tissue architecture, given that the different cell types from the same organ can more easily co-develop within one structure. This however does not automatically translate to proper cellular organization. Here, the liver serves as an example. TSC-organoids can be grown from either hepatocytes[74,75] or cholangiocytes[58] as two separate culture systems, while PSC-liver organoids can allow simultaneous representation of both cell types.[26,41,76] Yet, in current protocols, their overall spatial organization remains rather distinct from the *in vivo* topology.

Finding the right conditions to properly establish and maintain 3D architecture in different organoid models is important for organoid growth and can translate into functionality. Proper cell-cell contacts as well as interactions with the extracellular matrix (ECM) are essential for tissue integrity and thus architecture. We recently showed that maintenance of tissue integrity was vital to establish TSC-human fetal brain organoids.[64] Importantly, this favored the endogenous generation of a tissue-like ECM niche, possibly underlying organoid formation and their expansion. Most TSC-organoid cultures that can be grown from single cells are cultured in the presence of exogenously provided ECM mixtures, mainly Matrigel, which are an essential component to expand stem cells and to form complex 3D structures.[56] Likewise, most PSC-organoid protocols include exogenous ECM steps, either as an important component of organoid establishment (e.g., intestine[18]) or can be optionally included to promote differentiation (e.g., brain[77,78]). Beyond providing physical support, ECM components also instruct important processes in development and organ function, and ECM composition differs per organ. Indeed, using tissue-specific ECM was shown to be beneficial to organoid phenotypes. Embedding PSC-brain organoids in decellularized human fetal brain ECM promoted their functional maturation.[79] Similarly, functional maturation was observed when including tissue-relevant ECM in PSC-kidney organoids.[80] In parallel, defined ECM hydrogels are being developed, which, unlike Matrigel, can be applicable for regenerative medicine that necessitate GMP compliance and may be cost effective.[81–85]

Changes in the architecture of organoid cultures have been shown to influence cellular identity and phenotypes. Dissociating PSC-brain organoids and allowing subsequent reaggregation perturbed cellular identity in the reformed structures.[86] Also morphogens can define tissue organization, as subtle temporal changes in morphogen exposure improved tissue architecture and ultimately enhanced regional identity in PSC-brain organoids.[87] Bioengineering with scaffold-guided approaches can direct the spatial organization of TSC-intestinal and colon organoids and can potentially lead to more mature cell types.[88] Tissue structure can thus be considered an important *in vitro* consideration for organoid cultures.

Reproducibility

Increased cellular heterogeneity and complexity in organoids might come at the cost of reproducibility. Assessing and enhancing reliability has been an ongoing effort, especially in the PSC-organoid field.[89] Reproducibility can concern organoid-to-organoid, batch-to-batch, and additionally can vary depending on the PSC line, and even the state of the PSCs can influence organoid quality outcomes[90] (Figure 1D; Table 1). Early studies showed a variable efficiency across multiple iPSC lines in neuronal differentiation,[91] which was later extended also to distinct tendencies to reach different regional identities.[92] Similar observations were reported regarding iPSC-hepatocyte differentiation capacity.[93] Different levels of endogenous pathway activities (including Wnt[92]) and epigenetic variation[94,95] could partially explain iPSC differentiation biases. A degree of variation in the generation of PSC-organoids is also observed across lines, as shown for PSC-kidney organoids,[96,97] which interestingly is mitigated upon organoid xenotransplantation due to diminished off-target cells.[97] While a certain number of ESC lines (e.g., H1, H9, and H14) are widely used, the wide employment of different iPSC lines makes comparisons across studies more difficult. Due to this variability, the PSC-organoid field is increasingly demanding to reproduce/evaluate results across multiple lines. Using several lines can also be advantageous as it can be considered to reproduce inter-individual variability on a small population scale.[63] Alternatively, pre-mixing of progenitor cells from multiple PSC lines to form ''chimeroids'' represents another way in which donor-to-donor variation can be simultaneously accounted for.[98]

Where does the field of TSC-organoids stand regarding testing of reproducibility and variability (Figure 1; Table 1)? This is dependent on the considered application. TSC-organoid lines can usually be consistently established across donors. In functional studies, multiple donors and/or multiple clonal lines are often used to reproduce results, e.g., in cholangiocyte,[99] hepatocyte,[100] lacrimal gland,[101] breast,[102] and stomach[103] organoids. When more complex genetically engineered TSC-organoid lines are generated, e.g., based on (multiple) gene tagging steps, these experiments often rely on a single or few organoid lines.[9,10,13,52] Line reproducibility has not been considered a major concern in the TSC-organoid field, yet multiple aspects that could be linked to variability within different donors have not been systematically characterized. These include capacity of maturation but also experimental variables, such as the capacity of being genetically engineered. There are indications that TSC-organoids may suffer from some in-between line variation. For instance, a specific differentiation protocol can induce branching morphogenesis in human cholangiocyte organoids, but not across all lines tested.[11] The reasons for these discrepancies are unknown. Could subpopulations in the cell-of-origin generating the different organoid lines lie at the apex? In general, TSC-organoid lines from different organs are established through tissue processing and culturing the whole-cell suspension, rather than growing specific cell populations based on FACS approaches, which is less often used.[104,105] While the cell-of-origin for TSC-organoids is usually assessed through (stem) cell marker positivity, this does not exclude the existence of subpopulations. For example, the EpCAM+ liver cell population originating the cholangiocyte organoids[58] was shown to consist of TROP2 low-to-high cells, with differences in organoid formation efficiency.[106] Moreover, since TSC-organoids can be expanded long term and biobanked, assessment of reproducible characteristics over repeated passaging for TSC organoids could be important (Figure 1D; Table 1). Evidently, inter-donor variability due to patient-specific intrinsic factors may also add a level of biological information, and therefore discrimination between reproducibility and inherent inter-donor heterogeneity for both organoid types requires consideration.

Genetic stability

Preservation of genetic stability in prolonged culture of healthy human cells is desirable, especially when considering possible therapeutic applications. Yet, all dividing cultures can be prone to genetic insults. Possible emergence of genetic alterations has been well documented in PSC lines. Researchers thus resort to using low-passage PSC lines, performing routine karyotype analysis and adhering to good stem cell practices (e.g., Pamies et al.[107]) to ensure "healthy" cultures. Nonetheless, a large-scale study documented the prevalence of cancer mutations (especially *TP53*) in a relatively high percentage of PSC lines.[108] Thus, surveillance of cancer-related mutations is important, given the potential impact on cellular differentiation.

Genetic stability has been assessed in some human TSC-organoids, such as those from the liver,[58] stomach,[109] or pancreas.[85] Generally, wild-type TSC-organoids are considered genetically stable long-term in culture. A recent study, however, showed a clonal dominance occurring in TSC-intestinal organoids cultured for more than 6 months, with a few occurrences of clonal enrichment for cells carrying mutations in *ARID1A* or *RNF43*.[110] These analyses postulate the value of extending whole genome sequencing to other types of human organoid cultures. The observation of a non-neutral clonal dominance during prolonged culture might have implications for the use of wild-type TSC-organoids for functional studies, especially when relying on genome editing approaches that subject organoid cultures to (repeated) selection for clonal populations.

FIT FOR PURPOSE APPLICATION OF ORGANOIDS

Recently, in many studies, biological insights from experiments in animal models are further investigated in human-based organoids or they are even used directly as the starting point. A question that arises is which organoid system is most fit for purpose for the specific biological question in consideration, or perhaps if both systems are equally suitable (Figure 2). Below, we discuss opportunities and challenges related to PSC- and TSC-organoids for selected applications.

Gene function and disease

Specific gene functions can be relatively easily studied in initially wild-type TSC-organoids through inducing gene mutations, employing CRISPR-Cas techniques, usually starting from a clonal population[111] (Figure 2A). Yet, this is based on the premise that the TSC system can capture the cell type(s) in question and that the organoid cells can be readily genome engineered. This is not for all systems necessarily the case. TSC-organoids from airway[38] and cholangiocytes[112] remain rather difficult to engineer, while for example TSC-intestinal organoids can be efficiently edited.[113] A standardization for genome engineering in all TSC-organoid systems does not exist yet. For some genetic diseases, TSC-organoids can also be directly established from the diseased organs, such as cystic fibrosis,[33] when biopsy material can be obtained. Some TSC-organoids can be conveniently derived from non-invasive specimen samples, e.g., broncho-alveolar lavage fluid,[114] urine,[23] and very recently amniotic fluid.[115]

PSC-organoids can be generated from iPSC lines, which can be non-invasively obtained from patients with specific (rare) genetic diseases. These are often used to create matched diseased and gene-corrected iPSC lines, allowing to study the effect of a specific mutation within the same donor. PSCs can also be engineered at their pluripotent state to introduce a desired mutation, and this can be necessary to study gene mutations that induce lethality before birth (Figure 2A). At the same time, such approaches can be potentially confounded by the role(s) the gene may have during the PSC differentiation trajectory, thereby posing a challenge in extracting pure gene-phenotype observations at the organ level. To circumvent this, more sophisticated approaches aimed to genome engineer "in-organoid" could provide solutions, for example, through using inducible constructs (e.g., Ungricht et al.[116]), or editing of a subset of cells *in situ* at the PSC-organoid level (e.g., Ballabio et al.[117]) (Figure 2A).

When considering organoids for disease modeling, different strengths and limitations for PSC- and TSC-organoids can be identified. The derivation of isogenic organoids reflecting

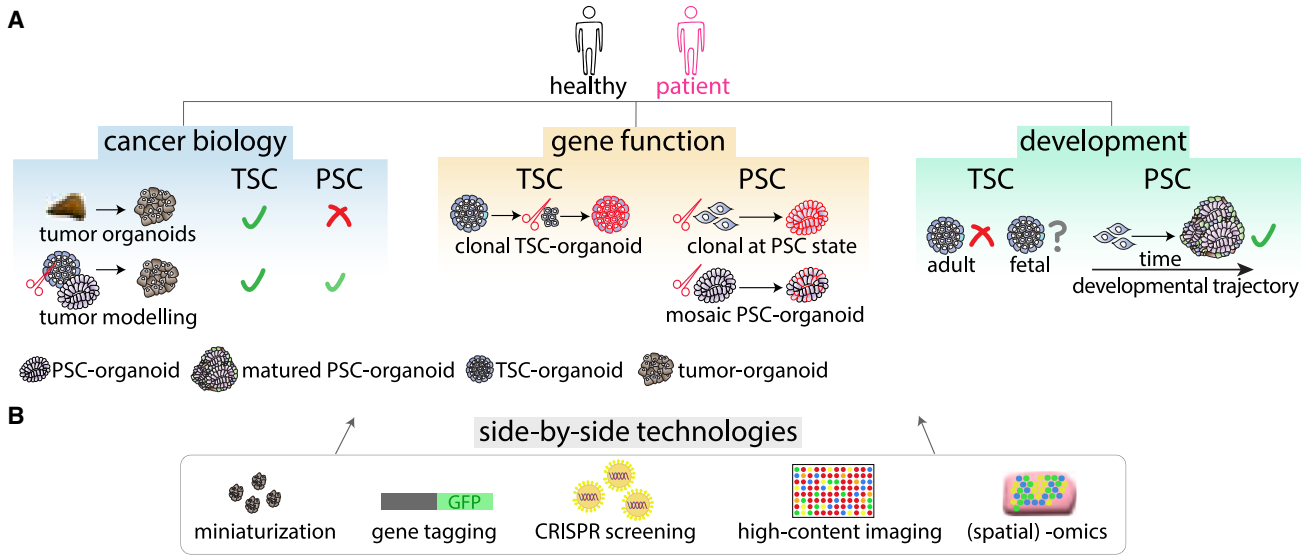

Figure 2. Main applications for which PSC- and TSC-organoids are used and state-of-the-art technologies applied to organoids

(A) Three main applications for which organoids are used. TSC-organoids are widely chosen for cancer research given the ability to derive organoids directly from tumor tissue as well as the relative ease in engineering bottom-up tumor models from wild-type organoids. PSC-organoids are broadly used to study developmental trajectories. Human fetal tissue-derived TSC-organoids may offer novel complementary developmental models but require further investigation. Both PSC- and TSC-organoid systems are often chosen as a model to study gene and cellular function in the context of both homeostasis and disease, using model-specific genetic engineering strategies or by deriving iPSCs or TSC-organoids (when available) from patient material.

(B) Different advanced techniques that have recently been applied to organoid systems. Adaptation may require optimization to circumvent organoid-specific limitations, such as the (speed of) organoid differentiation capacity, clonal outgrowth capacity, ease of genome engineering, and their reproducibility.

different organs from a single PSC line may be a strong approach to identify and evaluate genes with multi-organ importance, such as shown for CIART in SARS-CoV-2 infection.[118] Given the generally higher cell type complexity in PSC-organoids, disentangling cell-specific gene function or contribution to disease might be more difficult than in TSC-organoids. However, it also offers addressing gene function at the level of more complex cellular crosstalk, for example, between epithelial cells and non-epithelial components. In this regard, PSC-organoids capture multi-cellular aspects of genetic liver fibrosis.[119] PSC-organoids often reflect a more fetal state and therefore could be more appropriate to model developmental diseases rather than adult ones, that could potentially be more faithfully modeled in TSC-organoids. For instance, direct comparison between modeling of polycystic kidney disease in TSC-tubuloids versus PSC-kidney organoids discovered disease-resembling cyst formation and drug response only in those of TSC-origin.[104] The lack of response of PSC-organoids was attributed to absence of expression of the drug target receptor, putatively linked to a more immature state. The suitability of different organoid platforms to model certain diseases can thus not be determined a priori and should be evaluated on a case-by-case manner.

Cancer biology

The unique ability to grow TSC-organoids paves the way to establish organoids directly from tissues with acquired genetic alterations, such as cancer. TSC-organoids from patients' tumor samples often capture molecular and cellular properties and the genomic landscape of the original tumors (with intra-tumor resolution)[120] and allow to assess and predict drug responses.[121] Several side-by-side studies have emphasized the potential of tumor-derived organoids to predict *in vivo* patient drug responses,[122–125] suggesting their promise in personalized medicine. In parallel, efforts are invested in devising organoid miniaturization strategies to reduce the amount of tumor starting material and to accelerate drug screening.[126] Analysis of some tumor-derived organoids of adult and pediatric origin showed that they can capture tumor cell heterogeneity,[127–129] holding great promise to assess the behavior and sensitivity of different cell populations. In this context, combination of tumor-derived organoids with CRISPR-based gene tagging could be a powerful approach to define distinct cellular behavior, as shown for cancer stem cell plasticity in colorectal cancer organoids.[130,131] Nonetheless, not all populations that could have functional effects in tumor behavior and drug response are captured in simple tumor organoids, including immune components and cancer-associated fibroblasts, and co-culture approaches could provide solutions, as discussed later.

How well tumor heterogeneity is preserved over time in culture may be influenced by the culture medium and the specific mutations, as well as the tumor's genomic stability state. Fujii et al. noted that tumor organoid cultures derived from microsatellite-instable colorectal tumor display a drift toward clonality early on in culture,[132] implying that this could affect fidelity in downstream readouts (e.g., drug screening). This feature however may help to define aspects of tumor chronobiology. Prolonged organoid culturing could indeed capture trajectories of tumor evolution,[133–135] including karyotype alterations,[136] which might match the clonal evolution observed *in vivo*.[133,134] In organoids from rare neuroendocrine tumors, patterns of evolution differed between low-grade versus high-grade organoid lines.[135] Future

direct longitudinal comparison of organoid-to-patient tumor evolution upon treatment and *in vivo* relapse could further unravel the potential of organoids in this regard.

Wild-type organoids can be genome engineered to model cancer using bottom-up approaches, usually expanding clonal lines from single engineered mutant cells. Base and prime editing has facilitated introducing, and therefore mimicking, the precise mutations found in patients, rather than relying on simpler gene knockout or overexpression.[111] Cancer modeling in TSC-organoids enables to build mutation-defined models, in one-step[137] or sequentially[138] to identify aspects of the initial tumorigenesis and its progression. These include studying the effect of cancer gene mutations or mutational interactions driving aggression *in vivo*.[99] It also offers to study rare cancer types for which patient samples are scarce,[139,140] or to investigate rare mutations in common cancers.[141] The ability to grow engineered mutant organoids long term also opens doors to address genomic evolution from a preneoplastic state,[142] recapitulate benign-to-malignant tumor transformation from a predisposed background,[143] and to discover mutational signatures arising in cancer-susceptible genetic backgrounds or upon exogenous challenges.[144,145] Nonetheless, interaction with the wild-type environment may be a crucial determinant in tumorigenesis, which is not reflected when generating mutant cell-exclusive TSC-organoid lines,[111] with some exceptions such as TSC-brain organoids.[64]

How well the engineered models resemble primary tumor tissues of a similar mutational make-up at the molecular level has not been extensively assessed. We recently showed that human fetal hepatocyte organoids engineered to reflect two different genetic backgrounds of pediatric fibrolamellar carcinoma clustered with their respective genetic tumor subtypes at the whole transcriptome level, suggesting recapitulation of specific molecular properties.[140] These molecular comparisons should be envisioned in the future, not necessarily to merely prove similarities but also to identify aspects needed to determine the aggressiveness of tumors. As mentioned, the lack of some non-epithelial cellular components may influence organoid-to-tumor tissue similarity.

Bottom-up cancer modeling has also been explored in PSC-organoids.[146,147] Modeling of the same mutations in pancreatic acinar and ductal PSC-organoids showcased lineage-dependent mutation permissiveness.[148] Approach-wise, generating (clonal) mutant lines carrying specific mutations has been achieved by editing at the PSC or early progenitor stage, sometimes conjugated with inducible strategies (e.g., Wang et al.[149] and Breunig et al.[150]). Alternatively, by sporadic introduction of mutations at the organoid level, the outgrowth and capacity of (often mosaic) mutant cells within a healthy environment can be modeled (e.g., Bian et al.[151] and Ogawa et al.[152]). Interestingly, some rare germline mutations can cause developmental defects and can predispose to tumor formation later in life, such as mutations found in tuberous sclerosis complex. In fact, employing iPSC lines from patients with a *TSC2*$^{+/-}$ background (or engineering the mutation in wild-type PSCs) can be valuable to understand the developmental mechanisms underlying the vulnerability to develop tumors using organoids from brain[153,154] and kidney.[155,156]

In general, TSC-organoids are more widely employed in the context of cancer biology (Figure 2A). This is, as mentioned, in part due to the ability to grow organoids directly from tumor tissue. Moreover, the robust TSC-organoids' expansion capacity further facilitates the generation of clonal engineered lines directly at the organ level.

Developmental trajectories and cell type response

PSC-organoid models offer a unique opportunity to study the mechanisms underlying organ development (Figure 2A). Along the developmental trajectory, intermediate states of organoid differentiation can be analyzed to assess cell state transitions and to identify associated regulatory mechanisms. To this end, "temporal" single-cell RNA sequencing and other -omics analyses (e.g., epigenomics) have been conjugated to many PSC-organoid systems.[157,158] Such strategies bypass the difficulties in obtaining human fetal tissues from different developmental stages, especially of relevance for very early and later gestational ages. Computational methods also allow prediction of cellular developmental trajectories and population dynamics based on pseudo-time reconstruction.[159] The level of accuracy of information inherently depends on human tissue similarity. Therefore, PSC-organoids are increasingly benchmarked to human (developing) tissues (e.g., Uzquiano et al.,[160] Wu et al.,[161] Yu et al.,[162] and Ziffra et al.[163]), in terms of cellular similarity but also in terms of cell fate trajectories. The continuously larger availability of human tissue atlases has facilitated this process.

Besides offering a descriptive measure of human organ development, PSC-organoids offer powerful experimental value. Genetic manipulation and subsequent PSC-development can shed light on developmental mechanisms and associated gene defects (e.g., Fleck et al.[164]). Coupling PSC-organoid development with culture medium perturbations and subsequent single-cell RNA sequencing can also be used for protocol optimization, as done recently to increase cellular heterogeneity in PSC-retinal organoids.[165] Nonetheless, readouts relying on -omics remain a bottleneck to larger applications. Miniaturization of the organoid cultures conjugated with multiplexed single-cell RNA sequencing may improve the screening capacity and reduce costs. Miniaturization approaches for high-throughput screening by imaging of differentiation conditions have been optimized in some PSC-organoid models, such as the kidney.[166]

As compared with PSC-organoids, the use of TSC-organoids has been limited for studying development. By default, adult TSC-organoids cannot inform on fetal developmental processes. Comparison of expansion and maturation states in adult TSC-organoids conjugated with single-cell RNA sequencing can instead be used to infer mechanisms underlying adult stem cell differentiation into mature progenies. This approach was used to study the role of signaling pathways on cellular differentiation, such as how BMP signaling influences enterocyte and goblet cell differentiation in the human intestine.[167] TSC-organoids offer a rapid readout to test the effect of maturation/morphogen conditions. How well the response and plasticity of cells in TSC-organoid cultures reflect the state in human organs cannot be validated directly, though transplantation experiments can provide answers, as done with tumor organoids.[130,131]

Recently, various TSC-organoids have been derived from fetal tissues.[64,74,168–172] Beside representing a broad alternative model to adult TSC-organoids (if similarity to adult features is assessed in the context of the desired application), they are

potentially emerging as novel systems to study organ development (Figure 2A). As an example, cellular plasticity and progenitor potency across gestational weeks was reported in an elegant study by establishing fetal lung TSC-organoids from different developmental times, and identifying NKX2-1 as a key transcription factor involved in the bifurcation between alveolar and airway fate.[105] TSC-organoids from fetal intestine could capture different developmental stages depending on the tissue age and could be used to model necrotizing enterocolitis, a disease affecting preterm infants.[173] Altogether, this suggests that fetal TSC-organoids of different developmental stages could capture temporal snapshots of human organogenesis. For many fetal TSC-organoid systems, it would be interesting to explore whether and how much of the organ developmental trajectory can be captured in culture by further differentiation, either spontaneously or induced by switching to maturation medium. Human fetal TSC-organoids derived from different intestinal regions preserve the regional methylation profiles, but after long-term culturing can acquire higher similarity to pediatric and adult tissues, highlighting a degree of maturation in culture while preserving some regional information.[69]

Recently, direct comparison of human fetal brain-derived TSC-organoids with PSC-brain organoids on the effects of morphogen signaling on neural stem cell fate demonstrated distinct responses across these models, and also between different PSC-organoid protocols.[64] Thus, understanding potential differences or similarities, including potency of the stem cell/progenitor population, by direct comparison between adult and fetal TSC-organoids and PSC-organoids would be fundamental to further understand developmental and regenerative stem cell mechanisms and cell type responses as well as organoid model-to-model capacity.

EMERGING TECHNOLOGIES APPLIED TO ORGANOIDS

Technological advances allow the gathering of information at increased speed, deriving large amounts of data (and at single-cell level), often in an unbiased and systematic manner. Applying such technologies to organoids holds promise for novel biological insights. High-content imaging techniques together with miniaturized chemical screening approaches, CRISPR screens, and (spatial) single-cell multi-omics approaches have been conjugated to both PSC- and TSC-organoids (Figure 2B), but with slightly different technical challenges and biological considerations, given the inherent differences between the two systems, which will be discussed below.

High-content imaging-based screening

Both PSC- and TSC-organoids have been explored in higher-throughput chemical screens, for example, to identify disease-drug sensitivities (e.g., Driehuis et al.[174]) and to discover viral therapeutics (e.g., Han et al.[175]), more classically relying on single readouts (e.g., cell survival or metabolic assays). The desire to obtain a comprehensive understanding of compound effects has led to explore high-content imaging-based approaches tailored to 3D cellular structures.[176] The power of imaging-based chemical screening conjugated with high-throughput methodologies relies on the capacity to simultaneously assess thousands of conditions with complex multi-parameter phenotypic readouts based on advanced computational analysis in a cost-affordable manner. An exemplar study analyzed nearly half a million organoids to interrogate factors governing TSC-intestinal organoid development.[177] Within the many considerations for the design and analysis of such experiments,[176] a key aspect for feasibility is the availability of starting organoid material. Given their continuous expansion, TSC-organoids may offer an easier and more immediate use, and are readily scalable. However, miniaturizing approaches[166] would also make high-content imaging-based approaches more feasible in PSC-organoids. Recently, conditions were established to accommodate imaging-based screening using PSC-liver organoids.[63] In this study, genetic susceptibility to steatohepatitis in 24 different PSC-donors was screened. To reach sufficient starting material, after foregut differentiation, the initial organoids were dissociated into single cells to regrow multiple organoids, as an intermediate expansion step. These PSC-organoids derived from different donors were pooled and imaged in the same well, which however required an additional step of integration of imaging data with SNP analysis to match phenotype-donor information.[63]

Using patient-derived tumor organoids, imaging-based screening of drug sensitivity has also shown potential,[129,178] as exemplified using morphological multi-parameters to determine sensitivity to EGF signaling,[179] or to discriminate cell death from cytostatic states, which cannot be distinguished by metabolic ATP assays.[180] These applications may be further advanced by tagging functional genes, as recently shown in a proof-of-principle phenotypic screening based on fluorescence tagging of PLIN2, a coating protein of lipid droplets, to live trace the effect of steatosis-reducing drugs in TSC-hepatocyte organoids.[100]

Developing novel live-imaging strategies and computational imaging analyses that are optimized to specific PSC- and TSC-organoid systems is crucial.[181–183] Recently, live imaging followed by retrospective antibody-based cellular identification and machine learning, showed lineage relationships and cellular interactions during TSC-intestinal organoid differentiation.[184] Such approaches could be useful especially when applied to human-derived organoids, where real-time cellular identification would otherwise require multi-gene tagging approaches. Since most TSC-organoids can be rapidly regrown from single cells, long-term live imaging as relatively small organoids to understand principles of stem cell differentiation *in vitro* is relatively easy.[184–187] PSC-organoids can capture organ development processes *in vitro*, but the development from PSCs to mature organoids can be lengthy, requiring subsequent media switches, and can generate bigger tissue structures. These aspects might pose a technical challenge for the window of live imaging. In a recent study, this was addressed by generating relatively smaller PSC-brain organoids with mosaic labeling of multiple cellular structures, followed by several days of imaging based on an optimized experimental setup, which was conjugated to cellular demultiplexing, to achieve a real-time description of brain organoid morphogenesis.[188]

Large-scale CRISPR screens

CRISPR screens have already been adapted to both PSC- and TSC-organoid systems, based vastly on CRISPR-Cas9 gene

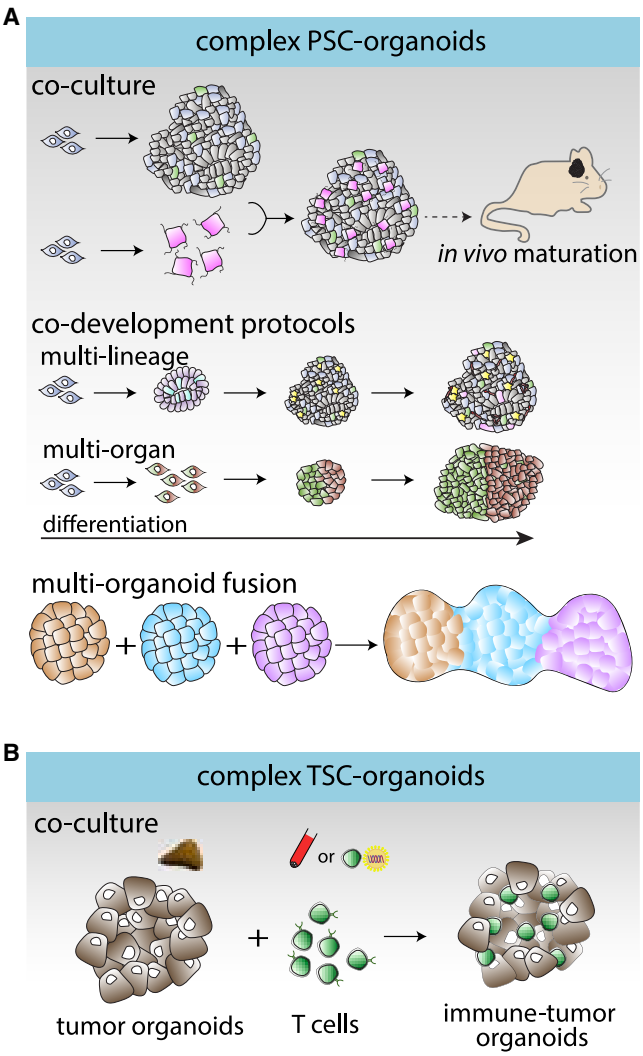

Figure 3. Main approaches taken to improve the complexity of PSC- and TSC-organoids

(A) In the PSC-organoid field, diverse approaches are used to increase cellular as well as organ-like complexity. These include co-culturing of organoids with in parallel-generated other cell types, generation of novel protocols to induce co-development of cell types resulting in multi-lineage/multi-organ organoids, as well as organoid fusion strategies ("assembloids"). Hybrid *in vitro-in vivo* strategies can promote additional cellular incorporation as well as further organoid maturation.

(B) In the TSC-organoid field, co-culture approaches have so far mainly focused on integrating immune cells within tumor organoids, but its adaptation to wild-type organoids is ongoing.

knockout. PSC-organoids have been used to screen for gene involvement in neurodevelopment using focused libraries (e.g., Esk et al.,[189] Meng et al.,[190] and Li et al.[191]) or to identify genes controlling nephrogenesis.[116] In TSC-organoids, CRISPR screens have found their value by means of discovering novel genes involved in disease development/progression[192,193] and disease targets,[100] or factors controlling maturation of cell types.[52] A commonality between these different genetic screens is the focus on a certain phenotype (e.g., presence of lipid droplets or organoid growth) or cell type (e.g., enteroendocrine cells or interneurons). Technically, as a possible difference between the two organoid systems, PSC-based CRISPR screens mostly employ inducible Cas9 strategies so to screen at later steps during the developmental trajectory and often rely on mosaic cell editing in single organoids (especially in PSC models undergoing terminal differentiation), Statistical power is then obtained by employing several independent organoids from different batches (e.g., Li et al.[191]). TSC-organoid cultures usually consist of hundreds of organoids (each deriving from a single targeted cell), conferring the possibility to screen directly at the organoid population level, which might be less challenging from a technical point of view.

Omics approaches with spatial information

We have already discussed the power of single-cell sequencing approaches applied to organoids to disentangle cell composition and cellular differentiation. These, however, lack spatial information, which is informative to understand cellular and molecular aspects related to tissue architecture. Spatial transcriptomics has been applied to study the topology of human PSC-brain organoid development[160] or to assess the effect of optogenetic-based perturbation of signaling pathways.[194] Recently, Wahle et al. optimized a pipeline that could integrate single-cell epigenetic and transcriptomic information with multiplexed antibody-based high-content imaging analysis, allowing to reconstruct the differentiation trajectory of PSC-retinal organoids at higher definition.[195] Integrating this approach with mosaic genetic perturbation identified transcription factor involvement during retinal development.[195] Future development of these technologies is particularly relevant to organoid systems showing spatiotemporal evolution and/or complex cellular organization. Moreover, characterization of the 3D cellular organization in human tumor-derived organoids can be envisioned to assess preservation of tumor heterogeneity and functional domains in a spatial context.

NEXT GENERATION ORGANOIDS: CO-CULTURES AND MULTI-ORGAN APPROACHES

There have been active efforts to enhance features of human organoid models, such as multicellularity and cross-organ interactions (Figure 3). Certain PSC-organoid differentiation protocols can "naturally" enable the co-development of multiple cell types, including those from different lineages.[18,196] As a route to further increase complexity, two conceptually alternative approaches are being explored (Figure 3A). On the one hand, continued improvements of protocols are aimed at directly promoting co-development of cellular identities within the same PSC-organoids (e.g., Low et al.,[42] Harrison et al.,[62] Holloway et al.,[197] and Múnera et al.[198]). For instance, this could lead to the appearance of vasculature in PSC-liver organoids[62] or development of resident macrophages in PSC-colon organoids.[198] Secondly, complexity has been achieved through "forced" co-cultivation techniques, relying on the pre-mixing of different individually specified PSC-derived cells or cell lines prior to organoid formation. Such strategies have been successful in generating vascular-like networks within PSC-organoids of heart,[199] or to include an enteric nervous-like system in PSC-gastrointestinal organoids.[200,201] Similarly, liver bud formation was induced through the assembly of mesenchymal, endothelial, and

PSC-hepatic epithelium components.[202] Further inclusion of Kupffer cells was recently achieved by additional co-culturing with PSC-derived erythro-myeloid progenitors.[203] PSC-gut organoids were among the first system to be explored to broadly include immune cells, perhaps given its extensive crosstalk in host-pathogen interactions and chronic inflammatory diseases. Transplantation of organoids within an *in vivo* environment can also result in increased cellular complexity. Using such a hybrid *in vitro-in vivo* approach, PSC-intestinal organoids were transplanted in mice with a humanized immune system, resulting in the infiltration and population of various human immune cells, including M cells, T cells, and B cells within the organoids.[204] Exclusively *in vitro* approaches, offering a more controlled and higher-throughput platform, have achieved co-cultures of PSC-intestinal organoids, e.g., to study maturation of innate lymphoid cells within PSC-gut organoids.[205]

The case of microglia-enriched PSC-brain organoids is an interesting example showcasing how the different methodological routes can be directed to the same aim. Since PSC-brain organoids generated using "unguided" protocols leads to the presence of cells from different germ layer origins, it was reported that cells present from the mesodermal lineage can co-develop and later on mature into microglia.[206] Alternatively, microglia-enriched organoids can be achieved by co-culturing separate PSC lines in different proportions: one overexpressing the transcription factor PU.1, which will preferentially give rise to microglia, and unmodified PSCs, which instead will develop the brain organoids.[207] Finally, to study microglia-neuron interactions in an *in vivo* vascularized environment, a recent study colonized PSC-brain organoids with PSC-derived yolk sac myeloid progenitors, followed by xenotransplantation in the mouse brain, which ultimately resulted in the maturation of functional, homeostatic microglia.[47]

Co-culture strategies have also begun to be explored in TSC-organoids, but to a lesser extent (Figure 3B). As they are mostly epithelial-selective systems, co-culture strategies are required to capture additional cellular entities. Incorporation of components of the tumor microenvironment in patient-derived tumor organoids has been a primary interest, perhaps given the promise of T cell therapy in cancer treatment, and the unique possibility to derive tumor organoids directly from tissue and immune cells from (the same) patients. Using air-liquid interface culture, organoids from diverse tumors could preserve stromal components and tumor-specific immune cells, although only stable short term after tissue isolation (ca. 1 month).[208] In parallel, tumor-immune organoid co-cultures have been established, creating *in vitro* platforms to induce and evaluate (autologous) T cell responses in tumors from different tissues (e.g., Dijkstra et al.[209] and Ou et al.[210]), to evaluate T cell-based therapies (e.g., Jacob et al.,[127] Schnalzger et al.,[211] Yu et al.,[212] and Harter et al.[213]), and showcased behavioral CAR-T cell heterogeneity.[214] Co-cultured tumor organoids with matched cancer-associated fibroblasts have also been reported, identifying different drug sensitivities as compared with organoid monocultures, suggesting that these more complex models could offer promise for precision medicine.[215] In the scope of investigating other diseases in which immune response is crucial, two recent studies described TSC-organoid co-cultures with tissue-resident immune cells. This setup was used to study aspects of celiac disease[216] and to investigate cancer-treatment-triggered gut inflammation.[217]

Organoid co-cultures of both PSC and TSC origins evidently require a more tedious setup. Such constructions may also come with challenges in reproducibility, especially of relevance for self-assembling co-cultures.

Beyond increasing cellular complexity, organoids can also be assembled to reflect multi-region organoids, which may improve their *in-vivo*-like functionality. Here, the PSC-organoid brain field is at the forefront (Figure 3A). Fusing different pre-specified organoids enables to model interactions between two or more brain regions, so-called assembloids.[218] Multiple regions within the same organoid can also naturally co-develop based on minimally-guided medium.[219] Fusion strategies can also generate multi-organ PSC-organoids. Fusing pre-specified anterior and posterior gut spheroids allows to model organogenesis of hepatic, biliary, and pancreatic organ components and associated organ segregation errors upon loss of HES1.[220]

Many organoid cultures rely on tissue- or even cell-type-specific medium and creating conditions to support growth/maturation of multi-cellular or multi-tissue organoids may require optimization. Whether fusion approaches could be translated to widely generate multi-regional PSC- and TSC-organoids depends on whether it is easily experimentally attainable. Several organoid types are grown with support of ECM, which may limit the level of interaction and thus would largely rely on self-organization events or require engineering approaches. Indeed, organ-on-chip or other bioengineering approaches have been harnessed for this purpose, which are reviewed elsewhere.[221,222] Interestingly, spontaneous co-development of two tissues has also been shown in the form of PSC-neuromuscular organoids.[223] In this case, the intermediate generation of a bipotent neuro-mesodermal progenitor, capable of generating both spinal cord neurons and skeletal muscle tissue, was an essential step to capture the self-organization into organoids with functional neuromuscular junctions.[223]

A JOINT FUTURE OF THE ORGANOID FIELD

Both PSC- and TSC-organoid fields have witnessed important advances in recent years, with both proceeding in parallel. Can we envision a future in which more crosstalk, integration, and knowledge exchange will be greatly beneficial for both systems, ultimately allowing major progress in understanding human biology and biomedical applications (Figure 4)? The TSC-organoid field has had a major emphasis and widespread understanding of cellular requirements to promote expansion. We could foresee that adapting such knowledge to multiple PSC-organoids may allow their improved expansion at the level of tissue-specified stem cells, when desired. A few pioneering studies already point in this direction. The use of TSC-inspired expansion medium applied to PSC-kidney organoids could propagate kidney epithelium for several months in culture and, importantly, this led to the disappearance of off-target cells.[224] Promoting a step of expansion in PSC-liver organoids could improve reproducibility and allowed to obtain sensible organoid amounts for further screening.[63] Similarly, in PSC-brain organoids, reproducibility as well as better representation of cellular diversity was favored by introducing

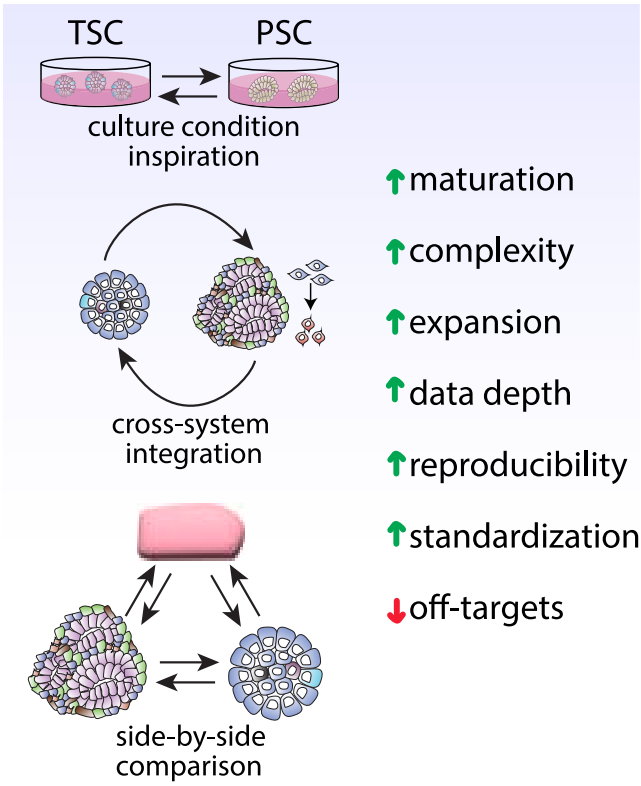

Figure 4. Future crosstalk between PSC- and TSC-organoids to address current limitations and to advance both technologies

Cross-integrating knowledge from both types of organoid systems can be envisioned to lead to further improvements of PSC- and TSC-organoids. These may include designing novel culture media, establishing hybrid (co-culture) systems, and making continued comparisons of both organoid model types with tissue benchmarking. Side-by-side experiments in both organoid models may further increase biological depth.

an organoid expansion cycle.[225] Conversely, the PSC-field has had a major emphasis on defining sequential media conditions to promote gradual maturation of unspecified cells toward more mature populations specific per organ. Could the TSC-field benefit from this knowledge? Traditionally, most TSC-maturation protocols are designed to be completed in a short time frame and with a single medium switch. Adapting strategies to resemble PSC differentiation methodology could be beneficial to achieve better maturation and ultimately functionality and perhaps architecture in TSC-organoids. In this regard, differentiation of human TSC-intestinal organoids in a two-step manner, encompassing a patterning and maturation step, could induce budding structures and improved cellular diversity.[13]

Going forward, mixing components of the two systems in the same culture could on the one hand increase cellular complexity in TSC-organoids or possibly promote maturation in PSC-organoids. Hybrid co-cultures of TSC-organoids with cells from PSC-cell lineages could promote incorporation of cellular entities not retained in TSC-organoids. Transition of PSC-organoids to a more adult state may perhaps be achieved upon integration of PSC-organoid cells to TSC-organoids by means of co-culture/organoid fusion, or use of conditioned media.

Human organoid studies are most often done employing one specific organoid system (i.e., either TSC-organoids, or using a single, specific PSC protocol). Could results potentially be confounded by the organoid model used? When possible, side-by-side use of PSC- and TSC-organoid systems could ensure robustness and increase the depth of biological insights by leveraging the strengths of each system, for instance, to compare gene function across development and differentiation in adulthood, and at the same time would address variation across protocols and donors. To ensure a wider application and to promote cross-integration of the two systems, devising cost-reducing culture conditions[226] for both organoid types will be important. Continued benchmarking with tissue samples and direct PSC-to-TSC organoid comparisons, as done in the context of intestine and associated organoid models,[227] in conjunction with bioinformatic pipelines,[228] will help standardization, assess the physiological level of current organoid models, could identify areas for future improvement, and will facilitate deciding which organoid system is best fit for the question in place.

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DECLARATION OF INTERESTS

B.A. and D.H. are inventors on a filed patent related to organoid technology.

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