Stem Cell Reports
Perspective
ISSCR
OPEN ACCESS

Biomedical and societal impacts of *in vitro* embryo models of mammalian development

Naomi Moris,[1,4,*] Cantas Alev,[2,*] Martin Pera,[3] and Alfonso Martinez Arias[1,4]
[1]Department of Genetics, University of Cambridge, Cambridge CB2 3EH, UK
[2]Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University, Kyoto 606-8510, Japan
[3]The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA
[4]Present address: Francis Crick Institute, 1 Midland Road, Somers Town, London NW1 1AT, UK
*Correspondence: naomi.moris@crick.ac.uk (N.M.), alev.cantas.8m@kyoto-u.ac.jp (C.A.)
https://doi.org/10.1016/j.stemcr.2021.03.023

In recent years, a diverse array of *in vitro* cell-derived models of mammalian development have been described that hold immense potential for exploring fundamental questions in developmental biology, particularly in the case of the human embryo where ethical and technical limitations restrict research. These models open up new avenues toward biomedical advances in *in vitro* fertilization, clinical research, and drug screening with potential to impact wider society across many diverse fields. These technologies raise challenging questions with profound ethical, regulatory, and social implications that deserve due consideration. Here, we discuss the potential impacts of embryo-like models, and their biomedical potential and current limitations.

The emergence of *in vitro* embryo-like models of mammalian development represents a remarkable advance in the field of developmental biology (see this issue) and ushers in a set of powerful new tools to complement the repertoire of model organisms such as mouse and non-human primates that have served as a reference for human biology over the last 50 years. Research with non-human mammalian embryos is challenging because of their intrauterine development which offers limited access to experimental material, as well as concerns driving the principles of reduction, refinement, and replacement for use of animals in research (the "3Rs" principles). Embryo-like models, often based on pluripotent stem cells (PSCs), create an opportunity for affordable examination of various fundamental principles of development in a high-throughput, accessible, and experimentally tractable manner within the 3Rs regulatory framework. In addition, such systems often utilize "bottom-up" bioengineering approaches that seek to deconstruct the complexity of the embryo by focusing on specific stages of development, tissues, or cell types, with the overall aim to provide additional and complementary insights into the guiding principles and core molecular and functional components of embryonic development (Fu et al., 2021; Heemskerk, 2020; Tewary et al., 2018)

While mouse cell-based *in vitro* systems have proved crucial to establishing the background and feasibility of embryo-like models, the greatest benefits of such systems are likely to be found in the study of human development. Historically, this field has relied on the availability of material from collections of human embryos, including the one gathered under the umbrella of the Carnegie Institution (Noe, 2004; O'Rahilly and Müller, 1987), which provide a major resource for the study of human embryology. However, the transformation of descriptive embryology into causal developmental biology in the second half of the 20th century, alongside associated advances in cell and molecular biology, raise the need for an experimental analysis of human development. While it remains possible to obtain early human embryos with consent and within ethical frameworks, these embryos are limited in availability and do not provide a wide scope for mechanistic studies of development. Research leading to *in vitro* fertilization (IVF) in the 1970s and 1980s (Steptoe et al., 1971) opened up the possibility of growing human embryos in culture and this, in turn, led to discussions of the ethical limits of such experiments. The result was the day-14 rule that acts as a widely accepted temporal limit for research with IVF-derived human embryos, and uses gastrulation and the formation of the primitive streak as a discrete, albeit somewhat arbitrary, cutoff aimed to prevent the acquisition of "individualization" in culture (Warnock, 1984, 1985).

Despite early expectations, the growth of fertilized human eggs *in vitro* beyond pre-implantation stages has proved challenging. However, inspired by work with mouse embryos, new methods have been reported to enable the culture of human and non-human primate embryos in the laboratory up or even beyond the 14th day (Deglincerti et al., 2016a; Shahbazi et al., 2016; Xiang et al., 2020). The success rates of these experiments are still low, and routine practice of such approaches is still limited by the "special status" of human embryos (Jones, 2011), for which some have argued that a 3Rs principle should also apply, similar to that used in animal research (Bioethics, 2017). Notwithstanding these issues, recent experiments and emerging observations highlighting differences between mouse and human embryos (Ghimire et al., 2021) have raised the need to modify the day-14 rule to be able to explore human development at peri-gastrulation stages (Hurlbut et al., 2017; Hyun et al., 2016). In the meantime, the search for appropriate models to study this period of development has led to an increased use of closely related species, in particular non-human primates. However, the

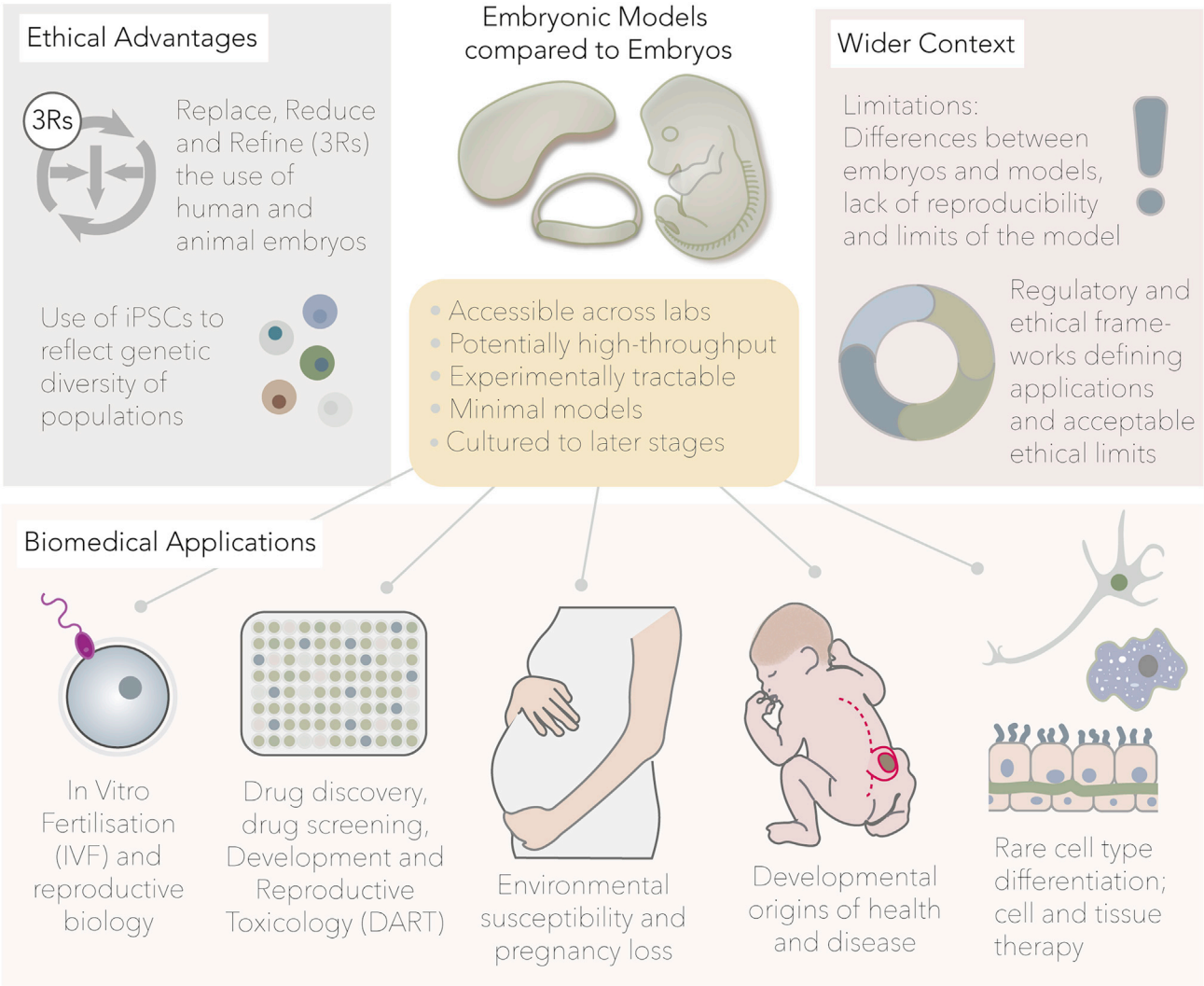

Figure 1. Schematic summary of the potential applications, benefits, and limitations of *in vitro* embryo models compared with embryos for research and therapy

cost of these experiments as well as ethical issues associated with individual jurisdictions make this research difficult. It is in the face of these challenges that recent progress with human PSC-based embryo-like models has led to their emergence as powerful alternatives to the use of embryos.

Recent technological advances that utilize human PSCs to create embryo-like models of trilineage germ layer emergence (Deglincerti et al., 2016b; Warmflash et al., 2014), peri-implantation stage epiblast (Simunovic et al., 2019), post-implantation amniotic sac embryoids (PASEs) (Shao et al., 2017b) (Shao et al., 2017a; Zheng et al., 2019), and axially organized post-implantation-like structures (Marikawa et al., 2020; Moris et al., 2020) provide a new suite of powerful tools that researchers can use to study human development without the need to use actual human embryos. Despite their obvious potential, such technologies also raise a number of serious concerns, which need to be dealt with at the outset. It is therefore imperative that we critically assess and appreciate the implications of these techniques early on so as to initiate wide discussion cognizant of the many opportunities presented as well as the ongoing and expected future challenges and questions raised. To foster this discussion, we provide here a view of the overall potential, the scientific and technological limitations, and possible ethical and societal issues raised by embryo-like model systems (Figure 1).

Biomedical applications

Model systems focusing on the recapitulation and analysis of early human embryonic development have an obvious

potential when applied to the field of reproductive biology, in particular fertility research and pregnancy loss, whereby experiments with these models might lead to improvements in assisted reproduction technologies (ART). In addition, since exposure to certain substances and medications during the early stages of embryonic development can have detrimental effects on the organization of the embryo, affecting the development of specific organs and tissues, there is potential to examine the effect of perturbations on normal developmental processes. Studying these effects is the realm of toxicology and teratology, and embryo-like models are likely to provide a valuable tool to assess the effects of such environmental and epigenetic stressors on the development of the human embryo. Also, there has been a great expansion in our knowledge of human developmental disorders during the past 5 years, including identification of the genetic basis of disease. Further exploration of the mechanisms by which genotype can be linked to phenotype could make use of model systems in which genetic manipulation is experimentally tractable and could also make use of the advent of induced pluripotent stem cells (iPSCs) to develop patient-relevant models. Additional applications might include the derivation of cell types or tissue samples which could be applied to further research (particularly for rare cell types that require multiple tissue inputs during development) or used toward cell therapy in the clinic.

Each of these fields represents an exciting new opportunity for embryo-like models to contribute beyond basic research and toward biomedical applications. However, there are also limitations and further considerations that are required of these models, which we discuss here in more detail.

Reproductive research

Only an estimated 30% of human conceptions will lead to a live birth (Zinaman et al., 1996), with the underlying mechanistic cause for such a low figure still being unclear. Most are associated with failures during the first 6 weeks of development, with an estimated 50% of these being traced to the pre-implantation stage of development. Reproductive failures are thought to be due to a variety of reasons, including gamete abnormalities that limit fertilization, karyotypic disorders, immunological dysregulation, and defects in the process of implantation (Fleming et al., 2018; Jarvis, 2016; Larsen et al., 2013). As such, demand for ART procedures has been steadily increasing over the past few decades, and an estimated 7 million babies have been born using these technologies since 1978 (Adamson et al., 2018a). However, despite these numbers, the frequency of successful pregnancies derived from ART still remains relatively low, e.g., 25%–30% for IVF and around 50% for other techniques such as zygote and frozen-embryo intrafallopian transfer. These figures have fueled research into potential causes of such failures, with a growing interest in novel ways to improve the culture and successful development of zygotes generated *in vitro*.

Advances in techniques such as IVF (Casper et al., 2017) that require culture of fertilized eggs *in vitro*, have provided researchers with the ability to observe a critical period of early pre-implantation human development (Gerri et al., 2020). Observation of these early stages of human development has revealed unexpected features of early human embryogenesis such as a high frequency of genetic mosaicism and chromosomal abnormalities (Ambartsumyan and Clark, 2008; van Echten-Arends et al., 2011), species-specific signaling requirements (Kuijk et al., 2012; Roode et al., 2012), human-specific transcription factor network organization (Fogarty et al., 2017), and the ability to use early morphological features to predict successful outcomes of blastocyst-formation (Wong et al., 2010). These studies use surplus embryos from IVF and can suffer from low numbers of available embryos as well as the difficulty of following them through implantation. These problems could possibly be circumvented with the establishment and use of PSC-based models of pre-implantation development. Such systems exist in mouse, where *in vitro* aggregation of embryonic stem cells (ESCs) and trophoblast stem cells (TSCs) under well-defined culture conditions leads to the formation of structures—blastoids—resembling the pre-implantation conceptus (Rivron et al., 2018b). The discovery of human TSCs has opened up the possibility of creating similar structures for human cells, i.e., humanized blastoids, which, when perfected, could be useful tools in the study of defects in early pregnancy arising from problems in the interaction between embryonic and extraembryonic tissues. Such techniques using murine cells are already beginning to reveal the role of communication between embryonic and extraembryonic tissues in the early mouse embryo and further highlight the requirement of signaling modulation in defined culture conditions suitable for pre-implantation development (Harrison et al., 2017; Rivron et al., 2018b; Sozen et al., 2018). The establishment of naive human ESCs, capable of giving rise to embryonic and all extraembryonic lineages, trophectoderm, and primitive endoderm, will likely further accelerate the development and analysis of human and non-human embryo-like model systems capable of reconstituting at least some aspects of pre- and peri-implantation development (Guo et al., 2020; Linneberg-Agerholm et al., 2019; Okae et al., 2018).

Each of the three initial lineages (epiblast, trophectoderm, and primitive endoderm) are specified during cleavage and blastocyst development and contribute to the establishment of an embryonic-maternal interface necessary for further development and maturation of the embryo proper. Effective *in vitro* systems that can mimic and

reconstitute implantation in humans are still missing, although some minor progress is being made with the use of artificial matrices (Xiang et al., 2020). The development of microfluidic-controlled conceptus-like structures is another important recent development in this emerging arena of research (Zheng et al., 2019). These and other similar tools, despite their lack of extraembryonic tissues, allow the interrogation of peri-gastrulation stage embryonic development and are thus ideally placed to enable large-scale screens and high-powered studies into this period of human development. The recent establishment of placental organoids (Turco et al., 2018) will likely further enhance peri- and post-implantation development research. *In vitro* embryo and placental models could conceivably be assembled and combined into more advanced models of implantation and peri-gastrulation human development.

In light of recent advances, it is probable that future studies will enable the identification of chemical cocktails promoting the efficient development of early embryos under IVF conditions, while also identifying new tools and parameters able to predict successful early embryo development and implantation. It may even be possible to derive methods that could be used to correct early defects during embryogenesis, which would be a major addition to the current suite of available ART tools. With increasing demand for reproductive technologies alongside an ongoing debate about the use of human embryos in research, *in vitro* embryo models could thus provide a solution and contribute to rapid advances in reproductive biology research while limiting the consumption of precious embryonic material. These tools could further contribute to the development of novel infertility treatments, improvement of existing IVF technologies, and the design of novel contraceptives.

Teratogenicity, drug discovery, and screening

While some early miscarriages can be correlated with lifestyle choices such as smoking, alcohol, diet, and maternal age (Nybo Andersen et al., 2000), others can be caused by exposure to drugs or medicines with secondary teratogenic effects and to chemicals present in foods, drinks, and the environment. A particularly well-documented study of such an effect is the case of thalidomide, a 1950s non-barbiturate sedative that came to be prescribed as a remedy for morning sickness. The compound had been deemed to be safe after studies in mice showed no adverse effects in this organism. However, in 1961 two clinicians, Widukind Lenz and William McBride, reported a large number of abnormalities in fetuses and newborns that had been exposed to thalidomide *in utero*. A well-publicized effect of thalidomide was phocomelia, a shortening and deformation of the limbs, although the substance had a very wide range of effects (Vargesson, 2015). This devastating case highlighted the need to use non-mouse models for developmental screening and suggested that some compounds might have potentially human-specific susceptibility during development. By 1962 thalidomide had been withdrawn from the market worldwide and the consequences of its use triggered new systematic studies of the effects of chemicals on embryo development, driving advances in the fields of toxicology and teratology.

A central lesson from the thalidomide case was the need to find appropriate tests for suspect compounds, often known as DART (development and reproductive toxicology) tests. A wide range of studies suggests that compound exposure during peri-gastrulation stages of development is critical for the emerging phenotype of the organism as, during this time, the principal germ layers and from them the primordia of all organs and tissues emerge and become organized in time and space. Having identified the critical stage, the target is to identify an experimental model system that can provide information about the effects of various compounds within this developmental window. Mice, rabbits, and other non-rodent mammals have for a long time provided a standard reference for toxicological studies in human development and disease but, as well as species-specific differences in mechanisms and overall organization of the embryos, the low-throughput nature of such studies and push toward 3Rs principles limits their suitability at early stages of drug-discovery pipelines.

PSCs, including ESCs and iPSCs, open up the possibility of doing screens and tests with high throughput on human-derived cells. Two systems have proved popular in this context. In one of them PSCs are differentiated in adherent (two-dimensional [2D]) culture and exposed to defined substances of interest, and their possible phenotypic effects recorded to assess changes in gene or protein expression at the end of the assay. While informative, these cultures are only differentiated to a single lineage at a time, and they lack the three-dimensional (3D) organization of the cells in an embryo. To circumvent these problems, embryoid bodies (EBs) (Brickman and Serup, 2017) have become a workhorse of toxicology and teratology research. However, both systems have problems when compared with an embryo. The most obvious limitation is that while both cases allow the directed differentiation of PSCs into specific cell types and thus provide a substrate to test the effects of certain compounds, the cells are not in the relative proportions and arrangements characteristic of embryos and, in the case of EBs, the timing of the differentiation bears little relationship to that of embryos. Furthermore, in many instances a compound will affect the growth, morphogenesis, and relative proportions of specific tissues and organs in a specific manner *in vivo*, something that cannot be mimicked by these simplistic PSC-based models,

adherent 2D cultures, or disorganized EBs. Overall, these existing simplistic 2D and 3D model systems are not adequately suited for testing the diverse and potentially harmful effects of compounds on human embryonic development.

While there is a possibility of growing embryos *in vitro* until or beyond gastrulation for such studies, the numbers of embryos required for such experiments are often too high to be feasible in a screening context (only about 20% of blastocysts in Xiang et al., 2020). Embryo-like models, which can be established easily and in large quantities, represent potentially useful alternatives for these high-throughput experimental requirements, and have already begun to show promise toward this end.

Micropatterns, also called 2D-gastruloids, are arrays of PSCs growing on printed adhesive substrates of defined size and shape whose geometry triggers the processes of self-organization characteristic of early development (Warmflash et al., 2014). These structures have been shown to recapitulate the organization of the principal germ layers in a vertebrate embryo and can be used to study the interactions between signaling and transcriptional networks. In particular, their flat morphology facilitates easy imaging, and their regular shape enables computational averaging of replicates to provide quantitative and robust measurements for screening. Human PSC-derived micropatterns have already been used for teratogenicity assays and were able to reveal dose-dependent responses to species-specific compounds including thalidomide (Xing et al., 2015).

For compounds likely to affect earlier development, blastoids and PASEs probably make better models, since they capture pre-gastrulation events. Similarly, gastruloids, 3D aggregates of PSCs that recapitulate many of the events associated with gastrulation, emergence of the body plan, and axial extension (Fu et al., 2021; Veenvliet and Herrmann, 2020), might be able to provide an assay for later stages and, in contrast to micropatterned culture, allow assessment of development in three dimensions. Embryonal carcinoma cell-based gastruloids have already been shown to be a useful tool to the study of toxicology (Warkus and Marikawa, 2017), but the development of gastruloids from human PSCs is also likely to provide a potential assay (Marikawa et al., 2020; Moris et al., 2020). These studies, promising as they are, could be limited by our lack of understanding of pharmacokinetics and maternal-fetal interfaces, which remain to be fully explored.

Despite these considerations, embryo-like models are likely to become valuable tools for screening assays, with particular potential in the fields of teratogenicity and drug discovery because of their potential ability to recapitulate human-specific features in a high-throughput manner.

Disease modeling

The normal development of the embryo can fail because of various pathological conditions that lead to birth defects, and in some cases can even lead to the death of the embryo or fetus. In 2018 in the United Kingdom these affected almost 7,000 births, corresponding to 1 baby in 47 births being diagnosed with a congenital abnormality (Public Health England, 2020). Some of these cases result from defects in the maternal-fetal interface but many are associated with specific mutations that affect the development of the embryo, in particular during the establishment of the body plan around the process of gastrulation (Ferrer-Vaquer and Hadjantonakis, 2013). In addition, non-genetic causes (including environmental exposure) can often lead to developmental abnormalities. Most common among these are cardiac and limb defects, problems of neural tube closure (e.g., spina bifida), segmentation defects of the vertebrae, as well as orofacial clefts, and defects of the digestive tract (e.g., bowel malformations such as gastroschisis) and congenital diaphragmatic hernia. Understanding the origin of these pathologies represents a significant step toward their remedy. Historically, knowledge of these pathologies has been gained through clinical observations of individuals at birth, and while some conditions are fairly common or have familial patterns of heredity, many are rare and our knowledge relies largely on small case studies and anecdotal evidence generally lacking full etiology (Feldkamp et al., 2017).

Our limited knowledge of the mechanisms associated with human gastrulation and the challenges of obtaining material for studying this pivotal process create a barrier to our understanding and treatment of these conditions. When there is a clear association of a mutation in a specific gene with a syndrome, model organisms have been used as surrogates for the disease; *Drosophila* (Ugur et al., 2016), *Caenorhabditis elegans* (Markaki and Tavernarakis, 2020), and zebrafish (Adamson et al., 2018b) have been successfully used for this purpose, especially when the disease is associated with a specific molecular alteration. However, the physiological and, importantly, developmental differences between species (such as the differences in the development and function of the extraembryonic membranes) present a barrier to the modeling of developmental abnormalities. It is for these reasons that the mouse, closer phylogenetically and developmentally than other classical model organisms such as zebrafish, chick, or frog, has become the organism of choice for the modeling of human diseases (Raess et al., 2016; Rosenthal and Brown, 2007). Despite their relatedness, there are substantial differences between mouse and humans, and, on several occasions, the mouse model has failed to capture important features of human disease, for example cystic fibrosis or Cornelia de Lange syndrome (Lavelle et al., 2016). However, similar

to the case of toxicology studies, the emergence of PSCs, and in particular iPSCs, has opened the door to modeling human diseases and performing related drug screens using human cellular systems (Grskovic et al., 2011).

Adherent cultures recapitulate normal embryonic specification and differentiation pathways and have also been shown to recapitulate many aspects of disease phenotypes, for example, insulin-producing β cells and diabetes or dopamine-producing neurons and Parkinson's disease. A remarkable feature of these *in vitro* differentiation processes is that they occur in the absence of morphogenesis, i.e., they suggest a decoupling of the genetic programs from their organization in space. A surprising example of this is the ability of paraxial mesoderm derived from PSCs to reproduce the segmentation clock, thought to contribute to the generation of somites and the vertebral column, and to differentiate into somitic mesoderm without a context of tissue organization (Diaz-Cuadros et al., 2020; Matsuda et al., 2020). Such properties have been exploited to begin to understand the contribution of oscillations in gene expression to vertebral column pathologies such as spondylocostal dysostosis by the introduction of specific mutations into iPSCs and the use of iPSC lines from patients suffering from this disease (Matsuda et al., 2020). These and related experiments open up the way for the study of pathologies with an origin in the early embryo. These studies, using adherent cultures and micropatterns, have uncovered previously unseen phenotypes associated with human diseases.

Such studies could be enhanced by the addition of a 3D organization and relative positioning of tissues and organ primordia as seen in early embryos, which are likely to play a role in the development of these particular diseases and related pathologies. This could encompass both 3D embryo-like models as well as tissue- or organ-specific organoids. In both cases, models are able to reproduce the cellular and tissue-level organization of *in vitro*-derived cells in space and time and may therefore represent more realistic disease models. For instance, the development of endodermal organoids is very advanced in this regard (Kechele and Wells, 2019) and provides examples of the significance of cell interactions for the development of specific organs (Koike et al., 2019). Further advances with these and other multi-germ layer-derived organoids will prove to be very useful in modeling a variety of neonatal and congenital diseases (Aurora and Spence, 2016). Others have used *in vitro* embryo models to study late-onset diseases, including the use of PSCs with allelic series of CAG repeats associated with Huntington's disease (Haremaki et al., 2019). In terms of embryo-like models, gastruloids can give rise to the primordia of most tissues and organs (Beccari et al., 2018; van den Brink et al., 2020; van den Brink et al., 2014) and might provide a useful tool for disease modeling. The recent development of human gastruloids from human ESCs (Moris et al., 2020) is encouraging in this regard. Especially in combination with patient-derived iPSCs and CRISPR/Cas9-based genome-editing technology, such models are likely to provide novel and valuable insights into human development and disease.

Wider context: ethical aspects

The nature of *in vitro* embryo-like models, in particular their relationship with embryos and their biomedical potential, make it critical that researchers are acutely cognizant of the wider social context and potential impacts of their research. This includes not only ethical considerations but also the general social, political, and regulatory context in which this research takes place. It furthermore demands an acknowledgment of the obligation to engage in an open discussion across fields to define and establish the boundaries of such research. This is particularly true in the case of the generation of human embryo-like model systems, which, although at an early stage of development, have the potential to raise questions about the nature of the structures they represent (Hyun et al., 2020; Rivron et al., 2018a). Such discussions should encompass the regulation surrounding new technologies and technical advances of embryo-like models themselves, as well as the applications of these techniques in the future. Importantly, they should avoid misrepresentations and hype that can lead to negative interpretations of the work (Huch et al., 2017), as is the case in the United States where there is lack of clarity around whether researchers can use federal funds for research with human ESCs that leads to synthetic embryo-like structures (Subbaraman, 2020).

The extent to which human embryo-like models exactly mirror events in the *in vivo* embryo is an important question that remains to be conclusively answered. Early attempts have begun to try to address this question with pre-implantation concepti (Blakeley et al., 2015), transcriptomic comparison of single-cell datasets from human embryonic material (Tyser et al., 2020), and using multi-species *in vitro* embryo models in comparison with model organisms as a means to triangulate with human embryos (Moris et al., 2020). These techniques raise a paradoxical dilemma: embryo-like models should be as similar as possible to human embryos in order to support their utility as a research substitute, while remaining sufficiently different to preserve distinctions that ethically permit research. A major issue for this fledging field is therefore to define the ethically acceptable limit of embryo-like models themselves. This decision needs to be based on sense rather than hype and should be informed by contributions from biologists, clinicians, ethicists, philosophers, and, wherever possible, public opinion.

Recent discussions of ethical guidelines by the International Society for Stem Cell Research (Hyun et al., 2020; Taniguchi et al., 2019) have sought to clarify the regulatory landscape of this discussion by establishing the boundaries of definitions for conceptus and embryo. One key point is a distinction between structures with "full organismal potential" and model systems that are unable to develop or manifest into a full organism. Despite differences in the definition of the term "embryo" across jurisdictions (Hyun et al., 2020; Pera, 2017; Pera et al., 2015), it has been argued that ethical distinctions should be based on the organismal potential of the system rather than on a simplistic distinction between *in vivo* human embryos and *in vitro* embryo-like models. Currently described human embryo-like models appear to fall well within the boundaries of this definition: PASEs and gastruloids are limited to specific aspects of development and do not develop beyond a specific temporal stage of embryogenesis. Many models also lack specific cell types that preclude further development, such as a lack of extraembryonic cell types that prevent implantation and would prevent successful uterine transfers. As such, no existing human embryo-like model has predicted full organismal potential, so these models currently fall within the ethical limits of existing guidelines. However, it is difficult to imagine how a new technique in the future could be rigorously assessed for "full organismal potential" without severely compromising the very ethical boundary that the rule attempts to protect. Further research may well focus on adaptations to extend or improve these techniques to make them more representative of their *in vivo* counterparts. In particular, the very recent development of human blastoids (Liu et al., 2021; Yu et al., 2021) will raise questions since, even though mouse blastoids have been shown to lack the ability to fully implant and develop, it is conceivable that protocol optimizations could overcome these technical barriers and they might therefore adopt full organismal potential and the ability to implant. At this point we would suggest that these structures should be subject to the 14-day rule and that their consideration should evolve with changes related to this rule.

Further complications could also arise in assessing the ethical status of embryo-like structures because they do not necessarily follow "canonical embryogenesis" (Aach et al., 2017). The term "potential" also raises various ethical questions around exactly what support might be included to fulfill that potential. Even a naturally conceived embryo cannot support life unless it implants into the uterus, so would an *in vitro*-derived human blastoid-type model satisfy the requirement for "full organismal potential" even if it was never implanted? Similarly, reprogramming experiments with iPSCs have shown that theoretically any cell has to potential to give rise to a "full organism." Such definitions therefore require further clarification, and concrete limits should be set to prevent misunderstandings as the field continues to move forward.

While the exact boundaries of ethical acceptability for embryo-like models and their design have begun to receive attention, there has been little consideration of the consequences of the likely subsequent application of these techniques to various clinical and commercial applications. It seems to be generally accepted that research on human embryo-like models and *in vitro* structures should not be used for the purposes of assisted human reproduction directly, i.e., implantation and gestation of *in vitro*-derived embryos (Hyun et al., 2020), but we should be cognizant of the theoretical potential for this approach in future. There are various reasons for this limitation, including technical considerations and safety issues as well as the ethical ambiguity surrounding direct human reproduction.

One major benefit to *in vitro* embryo models is that they could reduce the number of human embryos required for research, thus contributing toward a "human 3Rs" approach. If the wider community did indeed agree that the number of human embryos used for research should be minimized, in a similar way to those of non-human primate and mouse embryos (and this is not necessarily the case, given the wide availability of human blastocysts, for example), then human *in vitro* embryo models could represent an alternative option that might be less ethically loaded. Wider public and ethical engagement on this point might well be important for future discussions.

Overall, it is becoming clear that embryo-like model systems represent a huge potential for applications beyond basic research and toward a host of biomedical applications. Their utility in providing experimentally tractable, high-throughput, and human-specific developmental insights makes them amenable to many different approaches, and their further advancement holds considerable promise for the future. Beyond the fields discussed here, they also have the potential to play a role in developing specific cell types for research, regenerative medicine, and perhaps even therapy. But we must also be aware that we, as scientists and developers of these systems, need to remain vocal in disseminating the current limitations and further considerations of such technology. Additionally, as a fledgling field, the community must consider wider implications of embryo-like techniques within the context of our social, political, and regulatory landscape beyond the confines of the laboratory.

ACKNOWLEDGMENTS

The work of A.M.-A. and N.M. is supported by grants from the MRC (MR/R017190/1 and MR/V005367/1) and the Leverhulme Trust (RPG-2018-356), of C.A. by grants from the Japan Agency for Medical Research and Development (AMED) under grant number

JP20bm0704050, the ASHBi Fusion Research Program, and ISHIZUE 2020 of Kyoto University Research Development Program, and M.P. is supported by The Jackson laboratory.

CONFLICTS OF INTEREST

N.M. and A.M.-A. have a patent application covering the generation and use of human gastruloids (PCT/GB2019/052670) filed by Cambridge Enterprise on behalf of the University of Cambridge. The other authors declare no competing interests.

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