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Criteria for the standardization of stem-cell-based embryo models

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Pluripotent stem cells are being used to generate models of early embryogenesis that are promising for discovery and translational research. To be useful, these models require critical consideration of their level of efficiency and fidelity to natural embryos. Here we propose criteria with which to raise the standards of stem-cell-based embryo models of human embryogenesis.

The term 'model' has different contextual meanings in science. In physics and engineering, a model is a simplified, often mathematical, representation of a reality that enables the exploration of interactions between the component parts of a system to understand the behaviour of the whole. In developmental biology, the term model refers to the use of an organism to help elucidate general principles of development across many organisms. The conservation of many processes across species has lent legitimacy to the use of model organisms in research[1]. In the case of mammals, the laboratory mouse is the conventional model organism. However, given the species differences in morphogenetic processes, architecture and chronology, it remains unclear to what extent knowledge gained from the mouse can be extrapolated to other mammals, in particular to humans.

Studies of human embryos go back over 100 years but have been mainly descriptive rather than mechanistic in nature[2]. Recently, interest in studying early human development has been rekindled by the increasing availability of surplus human embryos created by in vitro fertilization and donated for research. This has led to some new insights into the development of the human embryo up to gastrulation (around day 14)[3,4]. However, experimentation with human embryos in vitro beyond this stage remains technically difficult and is not even permitted in many jurisdictions. Therefore, mechanistic studies of human embryos remain a considerable challenge. Although some countries, such as China and Israel, are less restrictive for human embryo research, the current regulatory framework of human embryo research has constrained advances in the understanding of human embryogenesis in health and disease.

In the past two decades, pluripotent stem cells (PSCs), such as embryonic stem cells and induced PSCs, have emerged as a tool for studying mammalian development[2]. Adherent PSC cultures and three-dimensional (3D) embryoid bodies derived from PSCs can delineate genetic and epigenetic programs, such as in response to cytokines, that model aspects of cell lineage specification, determination and differentiation seen in natural embryonic development. These cultures, commonly referred to as 'cellular models of development', have proven useful for the production of relevant cell types for scientific studies and therapeutic purposes, such as drug discovery and cellular therapy. However, these models lack the complete representation, proportionality and organization of cell types that characterize the 3D development of natural embryos.

Pioneering studies over the past decade have revealed a previously unrecognized ability of PSC aggregates to self-organize and form patterned structures, called organoids, that mimic in vitro the architecture of organ primordia[5,6]. Since then, numerous protocols using PSCs and precise signalling regimens have been shown to recapitulate cell lineage trajectories observed in 'cellular models' and further incorporate the morphogenetic and tissue-patterning processes characteristic of natural mammalian organogenesis. The self-organizing ability of PSCs has further been leveraged recently for the formation of structures that resemble early mammalian embryos[7], including human embryos, which has ushered in a reductionist and 'bottom-up' approach to simulating human embryogenesis. These models of mammalian embryogenesis need not generate an exact replica of natural embryos to warrant their utility in research. However, they should be sufficiently close to their in vivo counterparts, such as comprising the correct constituent cell types and displaying the structural organization of the natural embryo, as well as being amenable to experimentation, to provide informative and actionable new knowledge of development. Building on this paradox, embryo models can be particularly useful if they form only a particular ensemble of tissues in isolation, thereby revealing autonomous processes that may be masked in complex integrated environment of the natural embryo. Embryo models might also take a route different from canonical paths of development, as their cellular function attributes may be less constrained than those in the embryo; this can be informative. Thus, the main goal of embryo modelling is not necessarily to generate a full replica of the embryo or its component structures but to allow elucidation of specific aspects of development by leveraging their scalability, accessibility, modularity and amenability.

For the study of human development, a particularly useful class of embryo models is those derived from human PSCs[8]. However, because of ethical sensitivities due to their resemblance to natural embryos, questions have been raised about whether scientific enquiry justifies the use of these human embryo models in research. We believe that their relevance for use should be judged by weighing their potential benefits against those of ethically less burdened models, such as cellular models or organoids[9,10]. Furthermore, their utility depends on their ability to efficiently recapitulate developmental events, such as tissue patterning and morphogenesis. Modelling a complete embryo could be

BOX 1

Attributes of an embryo model

Generation of an embryo model

1. Starting materials:
   a. The pluripotency state and genome integrity of stem cell lines should be reported.
   b. Ideally multiple different cell lines should be used and compared.
   c. Report requisite initial cell number for embryo modelling and other cell types incorporated, where appropriate.
2. Protocol:
   a. Method of assembling the starting cell colonies to kick-start model generation should be described in detail.
   b. Stepwise in vitro culture conditions to attain the modelling end point should be described in detail.
3. Quantitative measures of the efficiency of model generation and the reproducibility of the end point features of the model should be reported in a statistically accurate manner.

Characterization of an embryo model

Preamble: define the target of modelling; for example, blastocyst, bilaminar embryonic disc, day 14 embryo, gastrula, body axis pattern, etc.

1. Identify cellular composition and spatial organization of cells and tissues in the model.
2. Assess morphological features of the resultant cellular structures.
3. Determine the fidelity level of modelling of the specific target in the context of the findings of points 1 and 2 above, using the benchmarking criteria described below.
4. Quantitatively measure the intra- and inter-experimental variation of the modelling outcome.
5. Make the limitations of the model explicit.

Benchmarking criteria

1. The cellular composition and cellular states, as determined by transcriptome and, where appropriate, additional modalities (for example, proteome, metabolome).
2. The spatial organization of cell types in the modelled structure and, where relevant, sub-structures (for example, somites, neural tube).
3. The morphology of the complete structures and, where relevant, its components (for example, individual organ primordia).
4. The spatiotemporal sequence of morphogenetic events.
5. The matching of developmental stages to the target on the basis of points 1–4 above.

Further reporting standards

1. Both commonalities of and differences between the embryo model and its target structure of the natural embryo should be assessed and reported. For classifier-powered annotation, confidence scores should be reported.
2. The limitations of the model, such as off-target cell types and morphological variation, should be made explicit.
3. For embryo models that require scientific and regulatory oversight, provide a statement of research ethics and regulatory governance of the generation and use of the embryo model for research.

justified in situations in which the outcomes and positive impact of the scientific understanding that can be gained outweigh the ethical and legal concerns, as long as regulatory guidelines are adhered to. In such cases, oversight of the generation and use of embryo models should ensure that these models avoid features that, for ethical reasons, are excluded from research of natural embryos. However, we surmise that if at some point the embryo model becomes indistinguishable from a natural embryo, it should be subject to the same rules and legislative windows that apply to embryo research[9].

Criteria for defining a stem-cell-based embryo model

Embryo models can display varying efficiency of generation, cellular composition and structural organization with a low level of fidelity to embryos. This is problematic, as it can result in misrepresentation of a model, which runs the risk of misleading scientific and medical discoveries. For example, over-reliance on insufficiently characterized models could lead to erroneous findings in embryology and misleading use in disease modelling or drug testing. For this reason, we believe that it is important to set out some basic attributes that should be applied in the characterization of embryo models (Box 1). A critical requisite of a model is that its features should reflect those of the whole or part of the embryo structure being modelled, and that this should be clearly stated in the report of the findings.

In order to make the experiments and the findings reproducible, the report should respond to the following questions. What is the pluripotency state of the initial PSC population? To what degree do the component cells of embryo model structures reflect the cell states and spatial organization of the embryo? What is the frequency with which the embryonic target is successfully modelled? If the final model structure deviates from the target, it is important to acknowledge the differences and to ascertain the point at which it deviates from the ground truth of embryonic development. In addition, the path of development taken by an embryo model should be documented, as there are some important questions that need to be considered, such as to what extent cells progress through the same sequence and pace of cellular states and morphometries of the embryo, which developmental stages cells and tissues traverse over time, the degree of synchrony within the model, and do the models skip events, accelerate or decelerate, compared with progression of the embryo.

The efficiency, reproducibility and robustness of the model are key requisites for adopting an embryo model as a useful experimental tool for elucidating embryogenesis or for translational research. Therefore, these variables should be reported by quantifying relevant characteristics with robust statistical determination, and publications must be accompanied by reports of this information.

Glossary

Axial elongation
The process whereby an embryo extends its body plan in an anteroposterior direction.

Cardiogenesis
The process of cardiac structure generation.

Embryogenesis
The generation of an embryo from the zygote of an organism.

Efficiency
In an experiment, the ratio of successful end products from initial attempts.

Embryoid bodies
3D aggregates of pluripotent stem cells in which cell differentiation occurs.

Fidelity
The degree of similarity of two entities; for example, how similar the embryo model is to the natural embryo or part thereof.

Functionality
The ability of components (for example, cells, molecules) to perform their roles effectively, contributing to the formation and organization of tissues, organs and/or their primordia.

Gastrulation
A developmental event whereby a population of embryonic cells are allocated to a multitude of cell types in the primary germ layers of the early embryo.

Germ layers
The primary sources of cell types (ectoderm, mesoderm, endoderm) formed during gastrulation that give rise to all tissues and organs.

Granularity
The degree of detail with which a system or process is analysed.

Morphogenesis
The process(es) by which multicellular systems acquire their shape.

Patterning
The spatial organization of cells and tissues into morphologically distinct structures.

Pseudoembryo
An embryo model with some features of an embryo but otherwise incomplete.

Robustness
A reproducible pattern of a process and resilience to perturbations.

Reproducibility
A feature of a manufacturing process that results in making similar or identical copies of a unit in a consistent manner.

Stembryo
An embryo model derived from stem cells.

Embryoid
An embryo model derived from stem cells.

Somitogenesis
The process for generating somites, which are progenitors of dermis of the skin, skeletal muscles and bones of the spine.

Trunk-like structure
An embryo model representing the trunk region of an embryo comprising the spinal cord and somites.

Methods for characterizing embryo models

The fidelity of an embryo model can be characterized by benchmarking its cellular constitution and transcriptional states against reference datasets, usually the transcriptomes of various cell types in the natural embryo or its body parts and organs at defined developmental stages. Such analysis, supported by improved visualization methods, is key to understanding the similarities and differences between embryo models and the natural embryo. When the model transcriptome data are projected onto the reference transcriptome dataset, cells that are atypical of those in the targeted tissues, or residual pluripotent cells or progenitor cells, should be included to ensure that projection-based label transfers are not unduly overfitted.

Gene-expression profiles are a useful proxy for characterizing models of preimplantation stage development, during which the number of lineages is small and their organization is relatively simple[11]. However, as development proceeds, the value of gene-expression descriptors decreases. For example, the range of cell types expands substantially during gastrulation and, on its own, the catalogue of cell types alone becomes a less important criterion than their spatial arrangement and relative proportions. For example, two-dimensional culture models of gastrulation produce arrays of cell types similar to those present in the gastrula. Thus, their topological organization and functionality should be a main criterion for comparing the model and the embryo. Future efforts should be devoted to increasing the granularity of reference datasets by integrating, where possible, additional layers of stage-specific multi-omics information — for example, spatial organization of cell types, and features of the epigenome, proteome and metabolome.

Overall, an accurate and quantitative description of specification, differentiation, patterning and morphogenetic events in space and time is needed to assess the degree of fidelity and reproducibility of a model, and therefore its capacity to model embryogenesis. This is crucial for comparing models, defining the scope of scientific and medical questions that can be addressed with them, and selecting the most useful ones for specific questions. We acknowledge that such a detailed characterization would not be accomplished in a single study. Instead, a collective community effort is needed to deliver a comprehensive characterization of the model of interest.

Crafting a terminology

The name of a model is an important descriptor that can affect the perception of the research work and influence the attitude of funding bodies such those as in the USA, where funding for research into structures that resemble embryos, composed of three germ layers or derivatives, is under close federal scrutiny and is currently decided on a case-by-case basis by the US National Institutes of Health. The 'generic' name of an embryo model should reflect the organization of the system, as well as the identity of what it aims to model. We suggest that, first and foremost, the model should use a vocabulary that is universally understood. Second, it should reflect the stage and tissues being modelled, ideally while also hinting, as necessary, at the imperfect nature of the modelling. This is often the intention behind the use of the suffix '-oid', to indicate that something is similar but not equivalent.

Accurate terminology will improve clarity for the work performed, both within the field and, notably, for adequate public perception. An example of a problem with the current situation has arisen from the different names given to models that recapitulate aspects of tissue patterning that are associated with the primary body axis in the paraxial mesoderm: somitoids, segmentoids, axioloids and trunk-like structures. Although each of these models exhibits specific features, and some of them that represent somites without axial organization could be deemed organoids, most attempt to represent the same process of formation of somites and the associated embryonic structures. However, the diverse names for similar or related objects are potentially confusing, and a united terminology should be decided upon to facilitate not only dialogue between scientists but also, most importantly, understanding of the field by the media and society. We suggest that this be done by consensus by those who develop the models.

As for an umbrella term, several names have been suggested, for example, stembryos, embryoids and pseudoembryos, each with its own value and shortcomings. All have the merit of suggesting embryo models as an imperfect replica of the natural embryo, but all can be misconstrued as being close to embryos. We acknowledge that matters of nomenclature require a consensus that should be reached

by discussions within the scientific community that lie beyond the scope of this Comment. However, we note that at present, the term 'stem-cell-based embryo model' is gaining traction, and we propose adhering to this. We further suggest that specific features of embryogenesis being modelled (gastrulation, cardiogenesis, axial elongation and patterning) can be added to clearly define the attributes of the particular model as a 'suffix' where appropriate; for example, stem-cell-based embryo model of gastrulation, and stem-cell-based embryo model of somitogenesis.

Concluding remarks

Our aim here is to highlight the need for clear and consistent descriptors of the efficiency and fidelity of embryo models that define their utility, and to reach a consensus on terminology to improve communication. To this end, we have made some suggestions for experimental standards and reporting. Appropriate characterization of embryo models will guide the deliberation of funding bodies on the value of the research and the scrutiny of regulatory authorities so that the field can progress with public trust[12].

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Author contributions

All authors contributed equally to the manuscript through discussions, writing and editing.

Competing interests

A.M.A. and N.M. are inventors on the patent Human Polarised Three-Dimensional Cellular Aggregates PCT/GB2019/052670. A.W. is a cofounder of and holds equity in Simbryo Technologies. N.R. is an inventor on the patents 'Blastoid, cell line based artificial blastocyst' (EP2986711) and 'Blastocyst-like cell aggregate and methods' (EP21151455.9), which are both licensed to dawn-bio, a company he co-founded. J.F. has filed patents or patent applications describing devices and methods for the development of human embryo models (U.S. Patent Application numbers 63/553,448, US2020049721, US20190321415 and WO2018106997). J.H.H. was granted (through the Yeda–Weizmann Institute of Science) patents relevant to the findings and technologies discussed here (naive and naive-like pluripotency and mouse and human structure-complete embryo models), and is a co-founder and chief scientific advisor of Renewal Bio, which has licensed technologies mentioned above. J.V.V. declares that part of the work in his laboratory is funded by an EIC Pathfinder grant (Horizon-EIC-2021-PathfinderChallenges-01 101071203, SUMO), which includes support of ELSA activities. The remaining authors declare no competing interests.

Additional information

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