News & views

Embryology

First complete model of the human embryo

Yi Zheng & Jianping Fu

Early in development, human embryos form a structure called the blastocyst. Two research groups have now generated human blastocyst-like structures from cells in a dish, providing a valuable model for advancing human embryology. **See p.620 & p.627**

A proper understanding of early human development is crucial if we are to improve assisted reproductive technologies and prevent pregnancy loss and birth defects. However, studying early development is a challenge – few human embryos are available, and research is subject to considerable ethical and legal constraints. The emergence of techniques that use cells cultured *in vitro* to construct models of mammalian embryos therefore opens up exciting opportunities[1]. Two papers in *Nature* now make key advances in this field, showing that human embryonic stem cells[2] or cells reprogrammed from adult tissues[2,3] can be induced to self-organize in a dish, forming structures that resemble early human embryos. This is the first integrated human embryo model containing cell types related to all the founding cell lineages of the fetus and its supporting tissues.

In mammals, a fertilized egg undergoes a series of cell divisions over the first days of development, leading to the formation of a structure called the blastocyst. The blastocyst contains an outer cell layer called the trophectoderm, which surrounds a cavity containing a cell cluster called the inner cell mass (ICM). As the blastocyst develops, the ICM becomes segregated into two adjacent cell populations – the epiblast and the hypoblast (known as the primitive endoderm in mouse embryos). The blastocyst then implants into the uterine tissue, setting the stage for an event called gastrulation, in which epiblast cells give rise to the three basic cell layers that will form the entire fetus. The trophectoderm goes on to form most of the placenta, and the hypoblast forms some layers of a structure called the yolk sac, which is required for early fetal blood supply.

The first *in vitro* models to recapitulate blastocyst formation using cultured cells (structures known as blastoids) used mouse stem cells corresponding to the stem cells found in the epiblast, trophoblast and primitive endoderm in the mouse blastocyst[4–6]. However, the generation of similar blastoids from human cells has not been achieved until now[1]. Previous models of early human development used human stem cells developmentally similar to post-implantation, pre-gastrulation epiblast cells[7–9]. As such, although they could recapitulate some stages of post-implantation human development, they lacked lineages associated with the trophectoderm, hypoblast or both.

In the current papers, Yu *et al.*[2] (page 620) and Liu *et al.*[3] (page 627) describe human blastoids. The key to these technological breakthroughs seems to have been twofold: first, the use of cells representative of lineages in the human blastocyst; and second, optimization of culture protocols.

Yu *et al.* started with either human embryonic stem cells, which are derived from human blastocysts, or induced pluripotent stem cells, which are generated from adult cells. Importantly, both of these types of stem cell are developmentally similar to epiblast cells in the blastocyst, and can also give rise to lineages related to the trophectoderm and hypoblast. By contrast, Liu *et al.* reprogrammed adult skin cells called fibroblasts to form a mixed cell population that contained cells with gene-expression profiles similar to those of cells of the epiblast, trophectoderm and hypoblast. As in the mouse blastoid protocols[4–6], both approaches involved seeding the cells in 3D culture dishes called Aggrewell plates, and

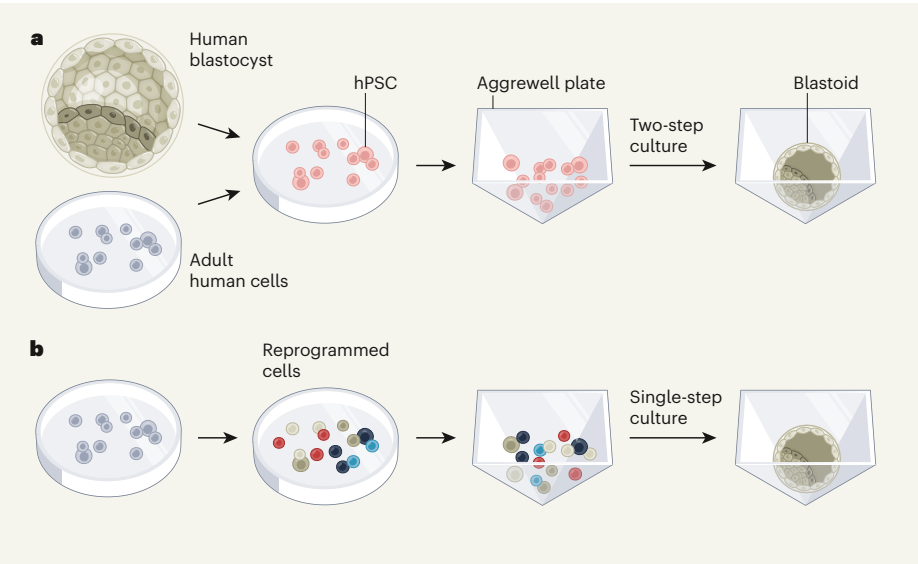

Figure 1 | Generating human blastoids. Blastocysts are structures formed early in mammalian development that comprise three cell types that will give rise to the embryo, placenta and a supporting tissue called the yolk sac. Two groups report *in vitro* methods to generate human blastoids, which closely model blastocysts. **a**, Yu *et al.*[2] used human pluripotent stem cells (hPSCs), which can give rise to lineages related to all cell types in the blastocyst. The hPSCs were either isolated from human blastocysts or generated by reprogramming adult human cells. The authors placed hPSCs in 3D culture dishes called Aggrewell plates, and used a two-step culture process to trigger the formation of human blastoids. **b**, By contrast, Liu *et al.*[3] reprogrammed adult human cells into cell types that had gene-expression profiles matching the three cell types found in blastocysts (some cells of unknown types were also generated, indicated by different colours). They used a single-step 3D culture process to generate the blastoid. (In both protocols, the blastoids contained some cells of unknown type, not shown).

treating them with liquid growth medium that contained chemical factors to control the signalling activities needed for blastocyst development (Fig. 1). Yu and colleagues treated the cells with two different types of culture medium in sequence, to promote differentiation of the cells into lineages representative of the trophectoderm and hypoblast.

Both groups found that human blastoids emerged after 6–8 days of culture, with a formation efficiency of up to almost 20%, comparable to the efficiencies of the mouse blastoid protocols[4–6]. The human blastoids were of a similar size and shape to natural blastocysts, with a similar total number of cells. They contained a cavity and an ICM-like cluster.

Detailed characterization of the blastoids (including genome-wide expression analysis and comparisons with human embryo data) showed that their cell lineages share molecular similarities with those of the pre-implantation human blastocyst. The spatial organization of the epiblast-, trophectoderm- and hypoblast-related lineages is consistent with that found in pre-implantation human embryos. The groups also demonstrated that the blastoid cells have key properties of blastocyst lineages — cells isolated from the blastoids could be used to generate various stem-cell types. Yu *et al*. showed that, if these stem cells were transplanted into mouse blastocysts, they gave rise to cells that could integrate with the corresponding mouse lineages in the mouse embryo.

Next, the researchers analysed further development of the blastoids using an established assay that mimics implantation into the uterus in culture dishes. Like blastocysts, when blastoids were grown in this assay for four to five days, some attached to the culture dish and continued to develop. In a portion of these attached blastoids, the cell lineage representative of the epiblast became reorganized into a structure enclosing a central cavity — reminiscent of the pro-amniotic cavity, which forms in the epiblast of post-implantation blastocysts. And in some blastoids, the trophectoderm-related cell lineage spread out and showed signs of differentiation into specialized placental cell types. Yu *et al*. also observed a second cavity in the hypoblast-related cell lineage in some blastoids, akin to the yolk-sac cavity.

Together, the groups' data demonstrate that human blastoids are promising *in vitro* models of pre-implantation and early post-implantation blastocyst development. However, there are notable limitations to overcome. For example, development of the blastoids is inefficient, and varies between cell lines produced from different donors, and between experimental batches. In addition, the three lineages seem to develop at slightly different rates in single blastoids, and development of blastoids in the same dish seems unsynchronized. Spatial organization of the hypoblast-related lineage in blastoids remains to be improved. Furthermore, the blastoids contain unidentified cell populations that do not have counterparts in natural human blastocysts.

Another challenge is that development of the blastoids is limited in post-implantation stages, unlike in mouse blastoids[4–6]. Further optimization of culture and experimental conditions will be needed to improve post-implantation-stage culturing of human blastoids *in vitro*, up to the equivalent of 14 days *in vivo*. Strict ethical rules prevent the culturing of human embryos past this stage, when structures associated with gastrulation begin to appear. Three-dimensional systems for culturing human blastocysts[10], which effectively promote post-implantation development, might help to improve our ability to culture blastoids up to this limit, by maintaining the normal 3D tissue architecture and spatial relationships between the different cell lineages in the blastoids.

Human blastoids are the first human embryo models that are derived from cells cultured *in vitro* and that have all the founding cell lineages of the fetus and its supporting tissues. As protocols are optimized, these blastoids will more-closely mimic human blastocysts. This will inevitably lead to bioethical questions. What should the ethical status of the human blastoids be, and how should they be regulated? Should the 14-day rule be applicable? These questions will need to be answered before research on human blastoids can proceed with due caution. To many people, the study of human blastoids will be less ethically challenging than the study of natural human blastocysts. However, others might view human blastoid research as a path towards engineering human embryos. Thus, the continuous development of human embryo models, including human blastoids, calls for public conversations on the scientific significance of such research, as well as on the societal and ethical issues it raises.

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Biogeochemistry

Fungi are key to CO_2 response of soil

Ana Bastos & Katrin Fleischer

An analysis of experiments in which the air around terrestrial plants or plant communities was enriched with carbon dioxide reveals a coordination between the resulting changes in soil carbon stocks and above-ground plant biomass. **See p.599**

On page 599, Terrer *et al*.[1] reveal an unexpected trade-off between the effects of rising atmospheric carbon dioxide levels on plant biomass and on stocks of soil carbon. Contrary to the assumptions encoded in most computational models of terrestrial ecosystems, the accrual of soil carbon is not positively related to the amount of carbon taken up by plants for biomass growth when CO_2 concentrations increase. Instead, the authors show that carbon accumulates in soils when there is a small boost in plant biomass growth in response to CO_2, and declines when the growth of biomass is high. Terrer *et al*. propose that associations of plants with mycorrhizal soil fungi are a key factor in this relationship between the above- and below-ground responses to elevated CO_2 levels.

Rising levels of atmospheric CO_2 are thought to have driven an increase in the amount of carbon absorbed globally by land ecosystems over the past few decades, a phenomenon known as the CO_2 fertilization effect[2]. This occurs because, at the scale of leaves, higher CO_2 levels enhance photosynthesis and the efficiency with which resources (water, light