Developmental biology

From stem cells to embryo models in the laboratory

Neal D. Amin & Sergiu P. Pașca

Two groups have grown self-organizing models of mouse embryos from stem cells *in vitro*. The models mimic mid-gestation embryos, providing an unparalleled opportunity to study early embryonic development. **See p.143**

For more than 100 years, researchers have been attempting to grow mammalian embryos in the laboratory[1], to study the mysterious forces and factors that transform a fertilized egg into a mature organism. Now, two groups, one[2] writing on page 143 and the other in *Cell*[3], have used advances in cell-culture techniques and stem-cell biology[4–6] to progress towards this goal. The groups grew structures resembling mouse embryos *in vitro* without the need for sperm, eggs or a uterus. These 'embryoids' developed to a stage equivalent to mouse mid-gestation, marking an impressive advance in our ability to study early mammalian development.

In the first steps of embryonic development *in vivo*, a fertilized egg undergoes multiple cell divisions. It then self-organizes into a hollow ball of cells called a blastocyst, which subsequently implants into the uterine wall. The blastocyst contains three cell types – the epiblast, which will give rise to the embryo, along with the supporting trophectoderm and primitive endoderm (Fig. 1a). Mouse embryonic stem cells (mESCs) isolated from blastocysts can be maintained in culture[7,8] and coaxed to generate specific cell lineages. However, mESCs cannot generate a complete embryo *in vitro*. Generating and culturing all three blastocyst cell types together could theoretically enable embryonic development.

In the current studies, Amadei *et al.*[2] and Tarazi *et al.*[3] set out to do just that using an 'assembloid-like' approach[9]. Both groups cultured mESCs along with an mESC line that transiently overexpressed Gata4 – a transcription factor specific to primitive endoderm. To supply the trophectoderm, Amadei and colleagues added a cell line similar to trophectoderm, whereas Tarazi and colleagues used mESCs that transiently overexpressed the trophectoderm-specific transcription factor Cdx2. Remarkably, three days after mixing, the cells had organized into an embryonic structure called an egg cylinder (Fig. 1b). Continued culture in an optimized culture medium, together with use of a rotating chamber (known as dynamic culture), enabled the embryoids to develop further than had ever been achieved before. The structures resembled mouse embryos that had been developing for 8.5 days (a stage dubbed E8.5).

Both groups used single-cell RNA sequencing and detailed cellular atlases to benchmark embryoid development, demonstrating that growth stopped at the initial outset of organ differentiation. Further analysis of tissue and cell markers revealed that the developing tissues of embryoids were organized in a similar manner to their *in vivo* counterparts. For example, the mouse embryoids contained structures that resembled the primitive gut, a beating heart and the bilateral, paired somites that would give rise to ribs and skeletal muscle. The groups also identified structures resembling precursors of the forebrain, midbrain and spinal cord, although neural development had not yet begun.

Each group then performed experiments that demonstrated the potential of this model system. For instance, Amadei *et al.* generated mouse embryoids from stem cells in which *Pax6* (a gene involved in brain and eye development[10]) was deleted. These embryoids showed similar defects to *Pax6*-deficient embryos grown *in vivo*, demonstrating that embryoids could be used to study gene–trait relationships. Tarazi *et al.* grew embryoids from a stem-cell line in which primordial germ cells (which will become sperm or eggs) fluoresced, to identify the timing and location of their genesis.

The development of mouse embryoids is a major advance on previous efforts to model post-implantation development *ex vivo*. Mouse embryoids could in future be used to study how environmental factors and genetic abnormalities contribute to pregnancy failure. Furthermore, comparing embryoids with *in vivo* embryos will reveal aspects of development that rely on maternal influences from the placenta or other extrinsic developmental cues.

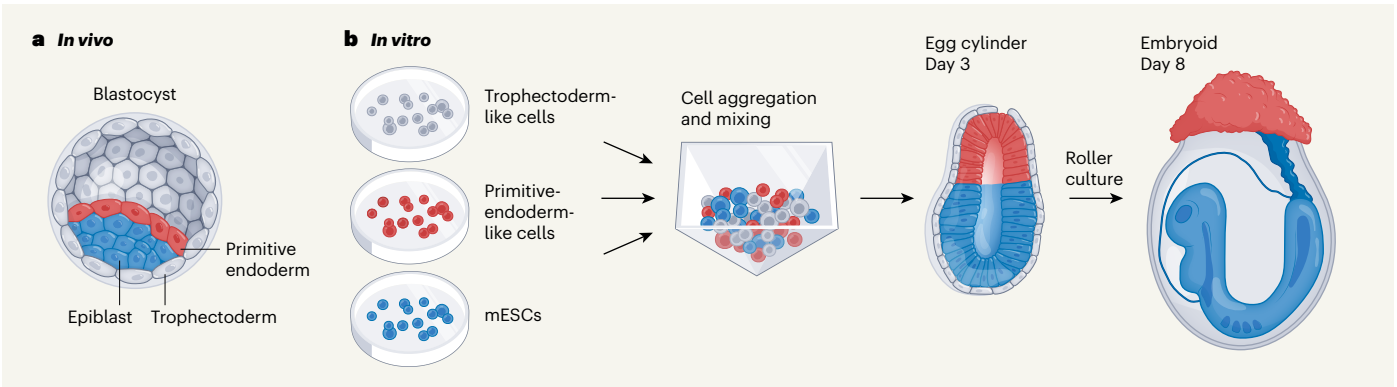

Figure 1 | Recapitulating mouse embryo development from stem cells *in vitro*. a, Early in development, a mammalian embryo forms a structure, called a blastocyst, that contains three cell types: trophectoderm and primitive endoderm, which provide support for the developing embryo, and epiblast cells, which will give rise to the embryo. **b**, Two groups[2,3] have now cultured trophectoderm-like and primitive-endoderm-like cells *in vitro*, in addition to mouse embryonic stem cells (mESCs), which have the capacity to give rise to embryonic cell types. The researchers then aggregated and mixed the cells in a culture well. The cells have innate principles of self-organization, which, together with an optimized culture medium, leads them to form a structure called an egg cylinder after three days. After five more days of culture and use of a rotating culture chamber, 'embryoids' develop, growing to resemble mid-gestation mouse embryos. (The embryoid shown is not an exact match for those described in ref. 2, but is adapted from Fig. 2 of ref. 3.)

Several limitations could prevent the use of embryoids for most immediate research and therapeutic questions, however. The failure rate is very high – the groups estimate that less than 1% of starting cell clusters successfully develop into embryoids. Tarazi *et al.* showed that some mESC lines produce embryoids that could not develop for more than six days in culture, indicating that embryoid development depends on the state of the starting cells. Furthermore, embryoids are highly variable in size and shape. Importantly, a lack of development past E8.5, noted by both groups, prevents investigation into the development of most organs and neural tissue.

Optimization of the three starting cell populations and culture conditions will be key to overcoming these limitations. For instance, embryoid culture media contain serum from the blood of rat and human umbilical cords; these sera are poorly characterized and can vary between batches, which might have a major effect on embryoid development. Changes to how carbon dioxide and oxygen are mixed, controlled and pressurized in the media, and tweaks to dynamic culture conditions, might allow longer *in vitro* growth, because these factors have been hypothesized to enable proper development[11,12]. Incorporation of other components, such as stem-cell-derived placental cell types, could extend embryoid development and reduce variability.

It is worth highlighting that these are not actual mouse embryos, but stem-cell-derived models of early mouse embryonic development. The use of terms such as 'synthetic embryos' for cell-based models of development has been generally discouraged by the International Society for Stem Cell Research (ISSCR)[13]. Consensus around a unified nomenclature is needed to accurately represent embryoid-based approaches to scientists and the public, as is currently being done for other cell-based models of development[14].

Eventually, researchers will want to apply these findings to human stem cells. The larger size of human embryos might mean that embryos depend more heavily than do mouse embryos on the placenta and vascular system – these properties could inherently limit *in vitro* development, which depends on diffusion for nutrient and gas exchange. Because human development takes several times as long to reach a stage equivalent to E8.5, generating human embryoids to late stages will be more costly and less feasible. And the use of human cells poses unique ethical challenges. The ISSCR has updated its guidelines to balance ethical concerns with the need for more research. For example, a '14-day rule' that limits *in vitro* studies of human embryos to the first two weeks of development has been replaced with oversight guidelines[10]. Specific consent for human stem cells to be used for embryoid research, and other legal issues, will need to be revisited before human-embryoid experiments can proceed.

The current studies elevate mouse stem-cell biology to the next level. The next frontiers – pushing the limits of *in vitro* development of mouse embryoids and applying these findings to human cells – are evident. Progress in these areas will undoubtedly advance our understanding of human embryonic development, improve our ability to generate disease-relevant cell types from stem cells and study the factors that affect pregnancy.

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Organic chemistry

A stable alternative to an explosive reaction

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The ozonolysis reaction is a classic of organic synthesis, but involves the formation of potentially explosive reaction intermediates. A modern, safer spin on this process makes use of previously overlooked chemistry. **See p.81**

In organic chemistry, a core set of fundamental mechanisms can be used to describe what happens in numerous individual reactions. But some reactions stand out because their mechanisms are unique. One such example is ozonolysis – an oxidation reaction widely used to cleave carbon–carbon double bonds. On page 81, Ruffoni *et al.*[1] present a modern riff on this mechanism, opening up a strategy for oxidative cleavage that overcomes the practical limitations of the classic ozonolysis reaction.

"The substitution of an oxygen for a nitrogen atom transforms a transiently formed, explosive species into an isolable intermediate."

Alkenes are the family of organic compounds that contain carbon–carbon double bonds (C=C bonds), and include many chemicals used as feedstocks for industrial processes, such as terpenes and fatty acids. Ozonolysis cuts alkenes into two, incorporating oxygen atoms into the resulting fragments. Discovered[2] in the 1840s, ozonolysis was widely used as an analytical tool for determining whether C=C bonds were present in organic molecules, and the position of such bonds in a molecule, before the advent of modern spectroscopic techniques.

Today, chemists use ozonolysis to convert alkenes into compounds that contain carbonyl (C=O) groups (Fig. 1a), thereby converting one group that has many uses in organic synthesis (the C=C bond) into another synthetically useful group. This invaluable transformation has few alternatives, and has been used in many important applications – including the syntheses of the antimalarial compound artemisinin and of the antibiotics ceftibuten and cefaclor[3].

One big limitation of ozonolysis is that the key reagent – ozone (O_3) – is a lethal, highly unstable and potent oxidizing agent. Ozone is generated in nature from molecular oxygen (O_2) during lightning strikes and through irradiation with ultraviolet radiation in the stratosphere. But it can't be stored in a bottle, because it rapidly decomposes back to O_2 under ambient conditions. A specialized