# Engineered models of the human embryo

Popovic, M.; Azpiroz, F.; Lopes, S.C.D.M.

**Citation**
Popovic, M., Azpiroz, F., & Lopes, S. C. D. M. (2021). Engineered models of the human embryo. *Nature Biotechnology*, *39*, 918-920. doi:10.1038/s41587-021-01004-4

Version: Publisher's Version
License: Creative Commons CC BY 4.0 license
Downloaded from: https://hdl.handle.net/1887/3280156

**Note:** To cite this publication please use the final published version (if applicable).

STEM CELLS

# Engineered models of the human embryo

Embryo models built from stem cells promise to shed light on human development.

Mina Popovic, Felicitas Azpiroz and Susana M. Chuva de Sousa Lopes

The past decade has seen the rise of a new subfield in human developmental biology known as synthetic embryology. Starting from human pluripotent stem cells, researchers have begun to create cell structures that capture specific features or processes of the early embryo, such as the epiblast, trophoblast, amniotic cavity, axis development and gastrulation[1]. These models do not demonstrate sequential embryonic growth beginning with the zygote but mimic discrete stages of development. The first embryo model generated from stem cells was published in 2014 and captured aspects of early human gastrulation[2]. In a recent breakthrough, two papers in *Nature*, by Liu et al.[3] and Yu et al.[4], reported the first stem cell–based models of the human blastocyst, and several subsequent papers have described similar results[5–7]. The blastocyst-like structures, or blastoids, are already a useful research tool, but their value will ultimately depend on how closely they approximate natural blastocysts.

Studies of early human development have long relied on donated embryos generated by in vitro fertilization (IVF). This research has enabled tremendous progress in medically assisted reproduction

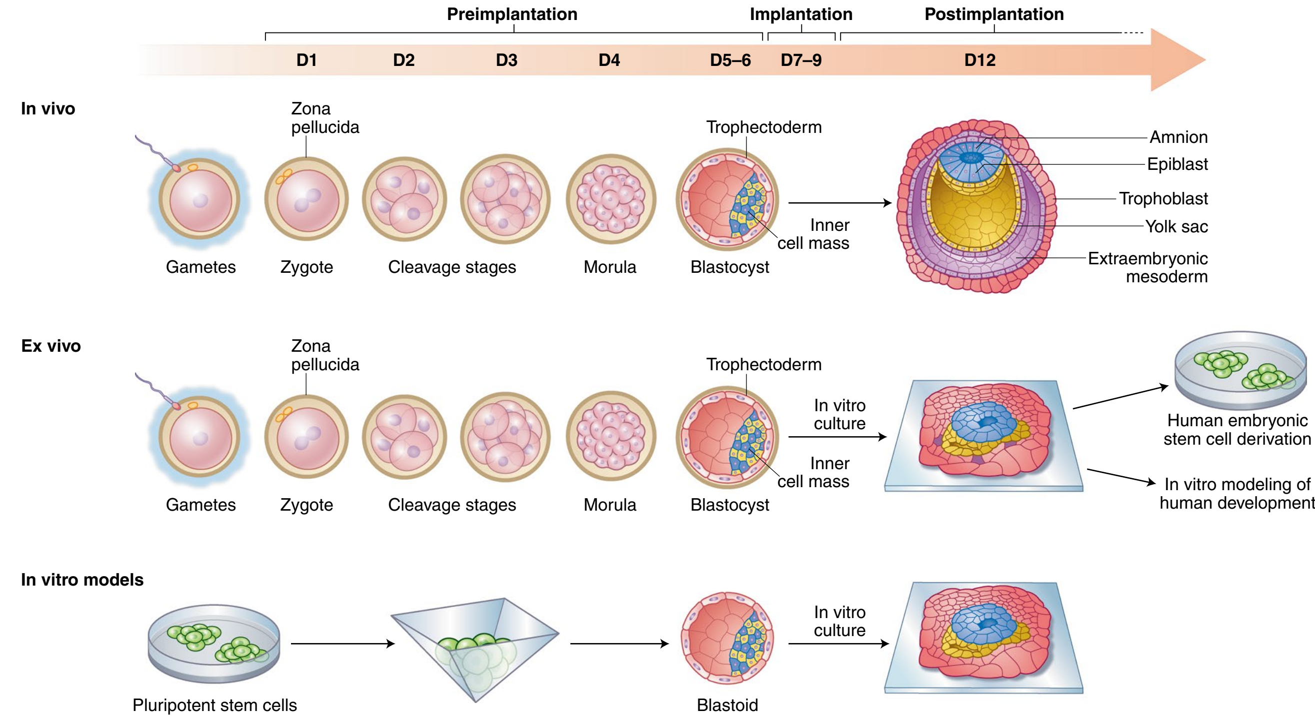


**Fig. 1 | Comparison of early human development in vivo, ex vivo and in blastoid models in vitro. a**, Early human development in vivo. Gametes unite at fertilization to form the zygote. The blastocyst is formed around day (D) 5 of development. Around D6, the inner cell mass segregates into epiblast and primitive endoderm. The blastocyst then hatches out of the zona pellucida and begins to implant by invading the uterine epithelium, around D7. After implantation, the epiblast forms the amniotic cavity while the primitive endoderm contributes to the hypoblast and the yolk sac endoderm. Around D12, the inner surface of the trophoblast and the outer region of the amniotic ectoderm and yolk sac endoderm become lined with extraembryonic mesoderm. **b**, Early human development ex vivo. Extended culture assays allow the culture of human embryos up to day 14 ex vivo. Human blastocysts attach to a culture dish and form a flattened outgrowth. Epiblast-like, amnion-like, yolk sac–like and trophoblast-like structures can be observed, but the cavities do not expand because the outgrowth is flattened. Extraembryonic mesoderm is not properly formed. Excising and plating the epiblast-like structure is a strategy used to generate human embryonic stem cells. **c**, Early human development in blastoid models in vitro. Liu et al. and Yu et al. generated blastoids from human pluripotent stem cells using a 3D microwell system. When allowed to develop in an extended culture assay, the blastoids formed similar structures to those of human embryos cultured ex vivo.

and continues to provide insights into developmental biology. Nevertheless, a holistic understanding of human embryogenesis requires multi-scale, multi-omics analyses that are not feasible given the limited availability of human embryos for research. To fill this gap, synthetic embryology aims to generate scalable in vitro embryo models. The challenge for the field is not only to faithfully mimic in vivo development but also to benchmark the models against in vivo processes that are difficult to study directly, particularly during the early postimplantation period.

The human zygote is totipotent—that is, having the potential to give rise to all embryonic and extraembryonic lineages. In the first few days after its formation, the one-cell zygote undergoes consecutive cleavage divisions to form progressively smaller blastomeres encased in the zona pellucida (Fig. 1). As development progresses, the potency of the blastomeres is gradually restricted. Around day 4, the originally round and loosely adherent cells flatten and develop a polarity that maximizes contact between them. Differential cell–cell adhesion of blastomeres leads to segregation of some cells to the center of the embryo. At the same time, the embryo starts to absorb fluid, establishing the blastocoel cavity as the hydrostatic pressure increases. This process culminates in the formation of the blastocyst, which contains an inner cell mass and outer trophectoderm cells, around day 5 of development (Fig. 1). The inner cell mass further segregates into epiblast and primitive endoderm cells around day 6 (Fig. 1).

Starting from pluripotent stem cells, Liu et al. and Yu et al. have now generated structures that resemble human blastocysts in their morphology, size and cell number. Both groups employed a 3D microwell culture system. Liu et al. used a mixed cell population generated during the reprogramming of adult fibroblasts to pluripotency in fibroblast medium whereas Yu et al. used conventional induced pluripotent stem cells and embryonic stem cells. In both studies, ~10% of the cell aggregates formed blastoids that contained inner cell mass and trophectoderm-like compartments and a visible cavity after 6–8 days of culture.

Despite these remarkable similarities, the blastoids differed from blastocysts in important ways. They lacked a zona pellucida, and although some primitive endoderm-like cells were present, a defined layer was not observed. Compared with day 5–6 blastocysts, blastoids had a higher ratio of epiblast cells to trophectoderm. Single-cell RNA sequencing further revealed that, in addition to the epiblast, primitive endoderm and trophectoderm lineages found in blastocysts, blastoids also contained many unidentified cell types with intermediate or undefined signatures. In addition, gene expression analysis using more comprehensive reference datasets that included human postimplantation embryos indicated that the trophectoderm cells identified by Liu et al. are more similar to postimplantation amniotic ectoderm cells[8]. Notably, blastoid formation was unsynchronized, with lineage specification occurring at differing rates.

To evaluate developmental potential, both groups cultured blastoids from day 6 until day 10 using published assays for extended culture[9–11] that mimic the early postimplantation period (Fig. 1). The blastoids attached to the culture dish and flattened out, like human embryos grown in the same conditions (Fig. 1). Interestingly, a small portion of outgrowths revealed phenotypes akin to the postimplantation epiblast and amniotic cavity. However, several functional characteristics remain to be evaluated, such as whether the blastoids show a normal karyotype and proper epigenetic regulation.

Although the blastoids reported in these papers do not recapitulate the full complexity of natural blastocysts, they hold great promise for embryology studies and biotechnological applications. The achievement of high-fidelity blastocyst models would enable applications in infertility studies, disease modeling, toxicity testing and drug target interrogation. Scalable blastoid generation would provide enough material for in-depth assays and high-throughput screens. Blastoids are more amenable to genetic modification than natural blastocysts and should therefore be useful for dissecting lineage commitment; unraveling these regulatory programs could enhance the efficiency and control of stem cell differentiation protocols for regenerative medicine. Blastoids may also allow examination of pathologies related to embryo implantation and placental function.

Blastoids, however, cannot replace human embryos for the study of the early preimplantation period (from zygote formation to the morula stage at day 4), when events such as pronuclear fusion, embryonic genome activation, cleavage divisions and epigenetic remodeling take place. To study these processes without using embryos would require a different type of model, generated, for example, from gametes derived from pluripotent stem cells. Blastoids cannot replace human or mouse embryos for embryotoxicity testing or for IVF media manufacturing and quality control.

Perhaps no single model will ever perfectly capture all aspects of embryogenesis. But creating an exact replica of a natural embryo is not necessarily the goal of synthetic embryology. Any differences between blastoids and blastocysts should be evaluated in the context of specific intended applications. For example, generating blastoids with a proper size and morphology alone could be useful for training programs in clinical embryology.

Looking ahead, a priority for the field is to develop a suite of tools to address complex biological questions. At present, low efficiency and poor reproducibility across experiments remain a concern, with a risk of culture artifacts that do not reflect true biological events. Future research will benefit from comparing blastoids generated by different methods to each other, as well as to ex vivo–cultured embryos at various developmental stages. However, we still lack good culture systems to support human early postimplantation development[12] (Fig. 1). Overcoming this technical challenge will be key to advancing the field, along with thorough characterization of blastoids using high-resolution genetic analysis, imaging technologies and genetic engineering. Another important direction is research to better define the in vivo uterine niche and to mimic it through bioengineering. Quantitative approaches are likely to rely on data-driven strategies, machine learning and mathematical modeling.

Finally, alongside the many scientific questions about blastoids, the nature of this research invites ethical reflection. Blastoids and other stem cell–based embryo models may reduce the need for human embryos in research, but as their genome is a genetic clone of the stem cells or donor cells of origin, it will be important to revise informed consent procedures accordingly. Another issue is whether blastoids fall within the remit of regulations governing human embryo research, such as the 14-day rule[13]. Ultimately, the full developmental potential of human blastoids remains unknown. Nevertheless, in the mouse system, blastoids generated from pluripotent stem cells have not developed beyond the early postimplantation stages in vivo[14]. ❐

Mina Popovic[1]✉, Felicitas Azpiroz[1,2] and Susana M. Chuva de Sousa Lopes[3,4]✉

*[1]Clínica Eugin, Barcelona, Spain. [2]CEGyR Medicina y Genética Reproductiva, Buenos Aires, Argentina. [3]Ghent Fertility and Stem cell Team (G-FaST), Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium. [4]Department of Anatomy and Embryology, Leiden University Medical*

Center, Leiden, the Netherlands.
✉e-mail: mpopovic@eugin.es; S.M.Chuva_de_Sousa_Lopes@lumc.nl

Published online: 2 August 2021
https://doi.org/10.1038/s41587-021-01004-4

References
1. Rossant, J. & Tam, P. P. L. *Stem Cell Rep.* **16**, 1031–1038 (2021).
2. Warmflash, A. et al. *Nat. Methods* **11**, 847–854 (2014).
3. Liu, X. et al. *Nature* **591**, 627–632 (2021).
4. Yu, L. et al. *Nature* **591**, 620–626 (2021).
5. Sozen, B., Jorgensen, V., Zhu, M., Cui, T. & Zernicka-Goetz, M. Preprint at *bioRxiv* https://doi.org/10.1101/2021.03.12.435175 (2021).
6. Fan, Y. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2021.03.09.434313 (2021).
7. Yanagida, A. et al. *Cell Stem Cell* **28**, 1016–1022.e4 (2021).
8. Zhao, C. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2021.05.07.442980 (2021).
9. Shahbazi, M. N. et al. *Nat. Cell Biol.* **18**, 700–708 (2016).
10. Deglincerti, A. et al. *Nature* **533**, 251–254 (2016).
11. O'Leary, T. et al. *Nat. Biotechnol.* **30**, 278–282 (2012).
12. Popovic, M. et al. *Mol. Hum. Reprod.* **25**, 519–526 (2019).
13. Pereira Daoud, A. M. et al. *Hum. Reprod. Update* **26**, 779–798 (2020).
14. Rivron, N. C. et al. *Nature* **557**, 106–111 (2018).

Competing interests
The authors declare no competing interests.

GENOME EDITING

# CRISPR base editing lowers cholesterol in monkeys

Targeting a gene linked to cardiovascular disease shows therapeutic promise.

Sebastiaan Johannes van Kampen and Eva van Rooij

CRISPR base editors are advancing rapidly toward the clinic with the publication of two studies demonstrating successful reduction of blood cholesterol in monkeys. The papers, by Rothgangl et al.[1] in *Nature Biotechnology* and Musunuru et al.[2] in *Nature*, show that transient expression of base editors to knock out a gene associated with cardiovascular disease is effective and safe in non-human primates. Delivery of the base editors to the liver in lipid nanoparticles led to efficient knockout of the target gene—proprotein convertase subtilisin/kexin type 9 (*PCSK9*)—and significant reductions of blood cholesterol, with no substantial off-target editing or serious immune responses. These impressive results suggest that the first cardiovascular gene-editing therapy could rapidly become a reality once the approach is fine-tuned for human testing.

The secreted enzyme PCSK9 is highly expressed in the liver and is important for cholesterol homeostasis. Binding of PCSK9 to the low-density lipoprotein (LDL) receptor results in endocytosis and degradation of the receptor, reducing the uptake of LDL cholesterol particles from the blood[3]. Gain-of-function variants in *PCSK9* have been linked to familial hypercholesterolemia, a condition marked by high circulating LDL cholesterol and high mortality[4]. In contrast, some naturally occurring mutations or knockouts of *PCSK9* lower the risk of cardiovascular disease[5].

Existing treatments for patients with familial hypercholesterolemia include statins, RNA interference (RNAi) therapeutics, and antibodies targeting PCSK9. However, these transient therapies must be taken frequently throughout life and have a high incidence of drug intolerance. Hepatocytes, the main cell type of the liver, are marked by a slow turnover rate and the ability to divide. A one-time administration of gene editing components to permanently inhibit PCSK9 function in the liver could therefore be effective for decades, improving quality of life and reducing healthcare costs.

Gene editing first entered the clinic with zinc finger nucleases[6]. But unlike zinc finger nucleases, CRISPR–Cas9 can be easily reprogrammed to target genomic loci of interest, enabling many academic and commercial laboratories to develop gene-editing therapies. Fusions of cytidine

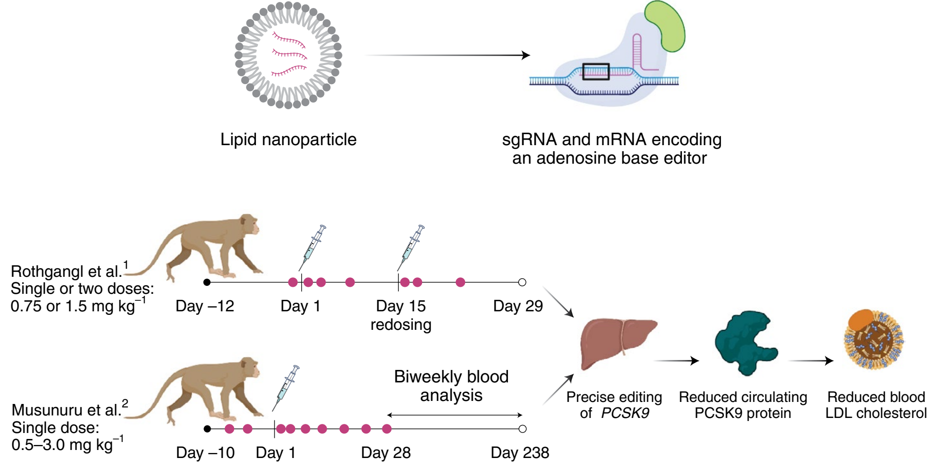


**Fig. 1 | Delivery of CRISPR base editing components in lipid nanoparticles for precise genome editing.** A single-guide RNA and mRNA encoding a CRISPR base editor are packaged into lipid nanoparticles and administered to cynomolgus macaques. After uptake by hepatocytes, the CRISPR base editor introduces a loss-of-function mutation in the disease-causing gene *PCSK9*, reducing PCSK9 protein levels and LDL cholesterol particles in the blood. In vivo base editing of *PCSK9* in monkeys[1,2] paves the way for precise genome editing in patients. Each dot in magenta represents a time point for sample collection and analysis. LNP, lipid nanoparticle; sgRNA, single-guide RNA; mRNA, messenger RNA; PCSK9, proprotein convertase subtilisin/kexin type 9; LDL, low-density lipoprotein. (Portions created with BioRender.com.)