Cell Stem Cell

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Spotlight

Prolonged 3D culture unlocks black box of primate embryogenesis

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https://doi.org/10.1016/j.stem.2023.06.004

By developing *in vitro* 3D culture systems and identifying morphological and molecular events of early organogenesis, two recent studies[1,2] reported exciting research advances in non-human primate embryo development.

During the past decades, *in vitro* fertilization (IVF) technology has greatly facilitated the study of early primate development, including humans. Through observation, measurement and molecular manipulation, biologists and physician-scientists have been trying to understand the fundamental biological events at the very beginning of life. In primates, post-implantation embryonic development has long been a black box of research due to ethical issues and inaccessibility of samples. Given the evolutionary relationship between non-human primates and humans, monkey embryo models could provide valuable perspectives on human development. Previous studies have developed a promising induction of non-human primate embryos into 20 days post-fertilization (d.p.f.), enabling the observation of peri-implantation to gastrulation transition.[3,4] However, whether an advanced culture system could be promoted for studying early organogenesis in non-human primates remained a challenge. Using a newly developed, three-dimensional culture system, two recent studies in *Cell* successfully induced cynomolgus monkey embryos into early organogenesis without maternal tissue (Figure 1). With morphological observation and omics analysis, the authors found that non-human primate embryos underwent unique cellular and molecular dynamics, largely filling a gap in the study of this developmental stage.

Zhai et al. established a 3D culture system termed "prolonged *in vitro* culture (pIVC)". In brief, IVF-derived pre-implantation cynomolgus monkey blastocysts were cultured in 3D IVC medium in the low-adhesion plate. With additional rat serum[3] and Matrigel,[5] pIVC restricted attachment and growth of the trophoblast, maintained the 3D morphology, and mimicked interactions between maternal extracellular matrix and embryos. This protocol could support the embryos to grow to 25 d.p.f. with a 33.7% survival rate. Twelve cell types identified by single-cell atlas revealed that monkey pIVC embryos formed three germ layers. Multi-omics profiling further mirrored that the embryonic cells from pIVC embryos revealed higher global DNA methylation levels (~75%) than that in extra-embryonic cells (~50%). Moreover, the accessibility of chromatin exhibited no significant differences between embryonic and extra-embryonic cells. These findings indicated that the monkey pIVC embryos established similar epigenetic patterns to embryos in mice and humans.[6,7]

Combining lineage-marker patterns and immunofluorescence, multiple neural-related cell types and spatial distribution were identified and further uncovered. At 16–20 d.p.f., neural ectoderm appeared in the middle dorsal embryonic disc. At 22 d.p.f., the neural tube ($SOX2^+/PAX3^+/PAX6^+$) emerged on the dorsal region of the embryo, exhibiting an anterior open and posterior closed morphology. Both open and closed regions of the neural tube were $N\text{-}CAD^+/E\text{-}CAD^-$, indicating the completion of E-N cadherin transition before neural tube closure. The neural tube underwent regionalization, with progenitors of motor neurons and progenitors of ventral interneuron 3 cells distributed from the dorsal to ventral axis. At 24 d.p.f., neural crest cells were observed within the folded neural ectoderm and neural tube-like structures. At 25 d.p.f., the putative forebrain, midbrain, and hindbrain occurred sequentially from anterior to posterior axis of embryos.

Additionally, at 22–24 d.p.f., the authors observed primordial germ cell (PGC)-like cells, primitive streak-like cells, and presomitic mesoderm and definitive endoderm (DE). Summarily, the cynomolgus monkey pIVC embryos recapitulated the advanced gastrulation and early neurulation, exhibiting similarity to *in vivo* cynomolgus monkey embryos at both morphological and molecular levels.

Gong et al. developed a 3D *in vitro* culture system called "Enhanced Monkey *Ex Utero* Culture (EMEUC)" to support cynomolgus monkey embryos up to 25 d.p.f. The sandwich-like EMEUC system comprises three layers: 4% (v/v) Matrigel as the upper layer, undiluted Matrigel as the lower layer, and the middle layer containing 10% (v/v) Geltrex and glucose-supplemented IVC medium. 6 d.p.f. blastocysts were seeded into EMEUC and cultured to 25 d.p.f. with 20.61% of the embryos displaying visible embryonic structures. 25 d.p.f. embryos revealed a similar morphology with *in vivo* Carnegie stage 8 human embryos. Single-cell RNA sequencing revealed that 18–25 d.p.f. EMEUC embryos contained 25 major cell clusters, including most cell types of *in vivo* embryos.[8]

The authors next mapped lineage trajectories and gene-expression patterns in EMEUC embryos during neuroectoderm specialization at 18–23 d.p.f. Epiblast developed into neural plate progenitors, further differentiating into neural-crest-progenitor-like cells and

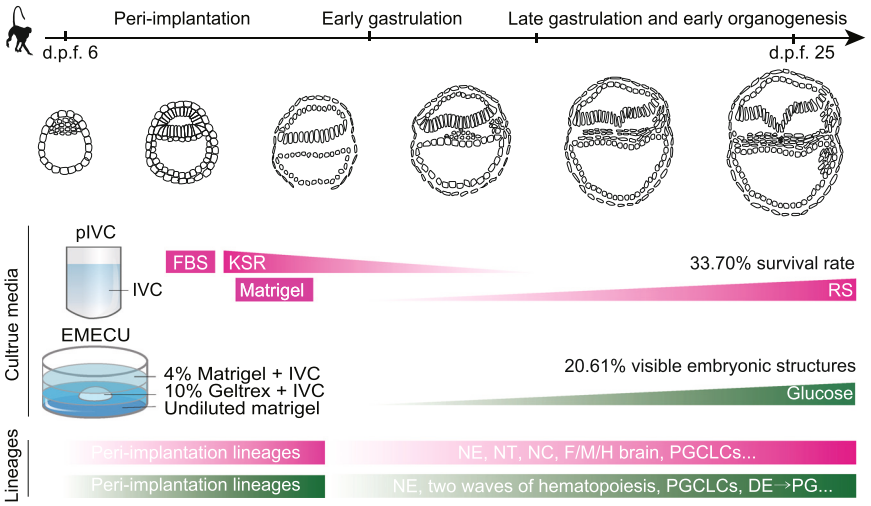

Figure 1. Schematic of *in vitro* culture system of monkey embryo into early organogenesis stages as Zhai et al. and Gong et al. reported

IVC, prolonged *in vitro* culture; KSR, knockout serum replacement; RS, rat serum; EMECU, Enhanced Monkey Ex *Utero* Culture; NE, neural ectoderm; NT, neural tube; NC, neural crest; F/M/H brain, forebrain, midbrain, and hindbrain; PGCLC, primordial germ-cell-like cell; DE, definitive endoderm; PG, primitive gut.

placode-progenitor-like cells with activation of NODAL and WNT signaling pathways. Compared to the stable axial mesoderm and paraxial mesoderm, the lateral plate mesoderm further gave rise to cardiomyocytes and hematopoietic cells. A subgroup within the DE could generate primitive gut cells, showing a conserved emerging mode of the primitive gut across monkeys and mice.[9]

Interestingly, two types of hematopoietic progenitors with expression of erythrocyte and myeloid related genes sequentially emerged from 18 to 23 d.p.f., indicating two waves of yolk sac hematopoiesis. During the first wave of hematopoiesis, monkeys and mice shared the expression of typical mesoderm-related genes and key transcription factors for erythrocytes and megakaryocytes but with different expression patterns of *FGF3* and *IGF2*. PGCs could undergo a metabolism switch from glycolysis to oxidative phosphorylation. In female embryos, the biallelic ratio of X-linked genes consistently decreased from pre-implantation (9 d.p.f.) to organogenesis (20 d.p.f.), indicating X chromosome inactivation. The EMEUC system also enabled monkey embryos to be cultured up to early organogenesis around 25 d.p.f., shedding light on the cellular and molecular dynamics underpinning primate development.

Balancing ethical concerns and material limitations, the establishment of *in vitro* culture systems for non-human primate embryos has greatly accelerated the research of human embryo development and regeneration. The two papers presented encouraging tools and databases for uncovering cell fate trajectories, and gene-expression and epigenetic patterns during early primate organogenesis. Combined with *in vitro* culture, stem cell-based reconstructed embryo-like models,[10] and omics profiling, *in vitro* embryo culture offers the opportunity to delve deeper into the molecular expression and potential regulatory characteristics of conserved developmental events among species. With further genetic and epigenetic manipulation in ethically permissible and sample-accessible embryo models, a new era to explore early primate development from a perspective of molecular function is launching.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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