

## Synthetic Models of Human Embryonic Development

**W**hen it comes to human development, our knowledge of the earliest stages of embryonic development—especially peri- and post-implantation in the maternal uterus—is sorely limited. Model systems that do exist center not on humans but on non-primate systems that differ markedly. Associate Professor **Jianping Fu**, who directs the Integrated Biosystems and Biomechanics Laboratory at U-M, is changing that.

In a series of papers published recently (*Nature Materials*, vol. 16, pages 419–425, 2017; *Nature Communications*, vol. 8, article number 208, 2017), Fu's research group has successfully developed novel synthetic embryological platforms that can open up previously inaccessible phases of human development to experimental study, helping advance human embryology and reproductive medicine.

Fu's research on modeling human development has laid the foundation for the emerging field of "synthetic embryo," which has been selected by the *MIT Technology Review* as "10 Breakthrough Technologies of 2018."

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Fu's investigations into this emerging research area could be described as serendipitous. A few years ago, the lab of Dr. Deborah L. Gumucio, a professor in the Department of Cell and Developmental Biology, observed that human pluripotent stem cells (hPSCs), a stem cell type equivalent to embryonic cells in the implanting human embryo, possess intrinsic properties to form hollow structures with a central cavity or lumen. But how do hPSCs self-organize and form such three-dimensional structures? With Fu's longstanding interest in mechanobiology and hPSCs, he and Gumucio began to collaborate and study how different mechanical signals and forces might affect the function and behavior of hPSCs.

### NOVEL MICROSCALE 3D CULTURE SYSTEM

The research team developed a bioengineered three-dimensional cell culture system that mimics the implantation environment with properly controlled mechanical properties. As in earlier observations, within one day, hPSCs formed hollow spheres. But soon after, hPSCs in the implantation-like culture environment began to look strikingly different from control cells cultured in standard culture: On one side of the hollow cyst, the cells appeared flattened and squamous, while on the other side they appeared columnar.

Given the embryonic origin and developmental potential of hPSCs, the research team decided to investigate in greater detail the identities of the columnar and squamous cells by comparing their molecular features with data in the scientific literature from other primates, since little to no human data exists. The team soon realized—with great excitement, Fu recalled—that what it was observing in the implantation-like culture was a self-organized asymmetrical embryonic

structure, containing columnar pluripotent epiblast cells on one side and flattened, squamous amniotic cells on the other. *In vivo*, the epiblast or the embryonic disc would eventually develop into the fetus, while the amniotic cells would eventually develop into the amnion, the fluid-filled membrane sac in which the embryos develop.

The team has thus demonstrated for the first time that without maternal or extraembryonic tissues, hPSCs can self-organize into an asymmetric structure with amnion-epiblast patterning that resembles the core of the implanting human embryo. The researchers refer to the asymmetrical tissue as PASE, post-implantation amniotic sac embryoid.

### CONTROLLING THE PROCESS USING MICROFLUIDICS

The team further identified the involvement of signaling molecules, bone morphogenic proteins (BMPs), in driving amniotic differentiation of hPSCs. To further control

symmetry breaking of hPSC cysts, Fu and his co-workers developed a microfluidic system to expose half of the hPSC cysts to BMP stimulation. The cells exposed to BMP differentiated; the cells not exposed retained pluripotency and remained as the epiblast.

### FUNDAMENTAL SCIENCE AND FIRST APPLICATIONS

Fu and collaborators already have begun investigating fundamental questions that underlie the symmetry-breaking process. What are the signals that trigger the symmetry-breaking process? How is the tissue boundary between the amnion and the epiblast determined and maintained?

The group also is investigating the role of the amnion in controlling further embryonic development, including into the embryonic disc.

"We already know from follow-up studies that the amnion serves as a signaling center and triggers continuous

development," Fu said. "These are incredibly intricate intercellular interactions among the amniotic cells and between the amnion and the epiblast, and we need to better understand the amnion's functional role."

The microfluidic device Fu's team developed now is also about to be used to screen for drug toxicity and prenatal formation of birth defects, or teratogenesis. "Many compounds have unknown effects on pregnancy and human development, and our system is compatible with screening assays so it can help us identify them," he said.

"I should stress that we're not trying to grow a complete embryo," Fu added. "Our goal is to leverage our synthetic models to advance fundamental understanding of human development, which remains largely mysterious. Such efforts will be valuable for preventing pregnancy loss, birth defects and teratogenesis and improving stem cell-based research and therapies. There are many important questions about human development that we can study in the near future. I am very excited."

**BACKGROUND IMAGE:** Bright field image showing many individual cysts containing central cavities developed in 3D culture from human embryonic stem cells. Note that most cysts have uniformly squamous morphology. All cells contained in these cysts are amnion cells. The one cyst at the center (above), shows an asymmetrical morphology, with one side squamous (the amnion) and the other side columnar (the epiblast). The cavities in all cysts are the pro-amniotic cavity.

**CIRCULAR IMAGES:** Immunofluorescence images showing an asymmetrical cyst, with the columnar side (the epiblast) stained positive for OCT4 (pink; pluripotency marker), and the squamous side (the amnion) negative for OCT4. The green fluorescence is from staining for some central cavity marker.

