BioTechniques
The International Journal of Life Science Methods

Development in a Dish

08/13/2014

Janelle Weaver, PhD

Simply changing the geometry of a culture surface can drastically change the differentiation of embryonic stem cells, even resulting in different fates for cells in the same culture dish. How will this discovery be harnessed to answer important questions about early embryo development? Find out...

In mammalian embryos, a process known as gastrulation results in germ layers called the ectoderm, mesoderm, and endoderm. While researchers can differentiate human embryonic stem cells (hESCs) to form cells of these three germ layers, until now, they have been unable to recapitulate their spatial patterning in vitro.

“We were interested in understanding how cells acquire their fates and how embryonic tissues are spatially patterned during embryonic development in a quantitative manner,” said Benoit Sorre at The Rockefeller University. By growing hESCs on micropatterend coverslips, Sorre and his colleagues recently discovered that simple geometric confinement of these cells to a disk-shaped region is sufficient to trigger self-organized germ layer patterning.

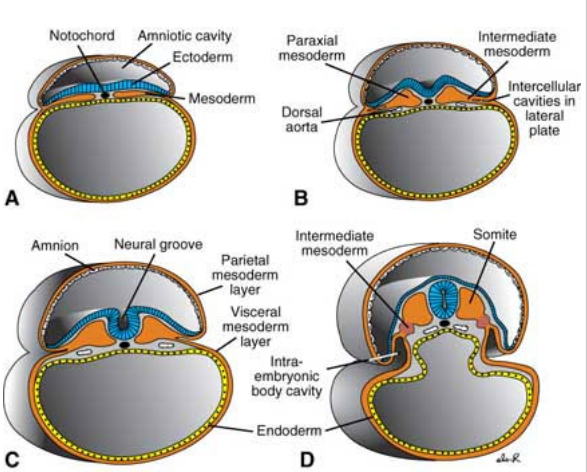


Diagram of mammalian gastrulation

“This is, to our knowledge, the first in vitro recapitulation of spontaneous spatial patterning with human embryonic stem cells,” Sorre said. “It allows us to ask and answer questions about spatial patterning during human development that were not possible before.”

More on Embryonic Stem Cells:

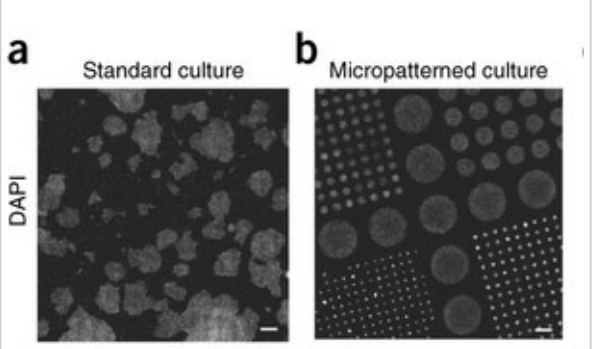
- Stem Cells Go Head-to-Head
- Send in the Clones
- Reprogramming Without Oocytes

Geometry Generates Germ Layers

In the new study, the researchers focused on differentiating hESCs with BMP4 because it is involved in the early embryonic signaling cascade that initiates gastrulation. “One of our first striking observations was that in regular cell culture conditions, the response of human embryonic stem cells to morphogens—BMP4 in this case—was spatially heterogeneous,” Sorre said, explaining that the hESC colonies exhibited a wide range of sizes and shapes. “This fact is usually not reported, probably because most of the studies in the field of stem cell research focus on making a pure population of one cell type.”

The researchers suspected that the heterogeneity in colony geometries could affect signaling between cells, resulting in a loss of reproducible spatial order upon differentiation. “We were aware of previous work where micropatterning technology had been used to standardize a single cell shape,” Sorre said. “We hypothesized that this could also be used to control the size and shape of human embryonic stem cell colonies and that it would give us some insight about the origin of spatial heterogeneity in the cell response to BMP4.”

The researchers found that cells confined to circular micropatterns and incubated with BMP4 differentiated into all three germ layers. But the road to discovery was a little bumpy, Sorre said. One challenging aspect was the surface treatment to make hESCs adhere to the glass of the micropatterned coverslips. “Human embryonic stem cells are a bit more picky for their adhesion to substrates than other cell lines, so it took us a little while to find the combination of adhesion proteins that make the cells adhere in a reliable and reproducible way,” he said.



Human ESCs grown under standard and micropatterned conditions (1)

Another challenge was the imaging protocol and image analysis. On each micropatterned coverslip, there were about 1000 patterns with sizes ranging from 80 to 1000 microns in diameter. Imaging the full coverslip took 12 hours with an automated microscope and generated 10,000 pictures, or 20 Gb of data. Then these pictures were stitched together, and the colonies were identified. “That's a huge amount of work that couldn't be done manually,” Sorre said.

Too Close for Comfort?

What surprised Sorre the most was seeing the germ layers emerge in the first place. “These patterns are so reproducible, and it takes so little to have human embryonic stem cells make them,” Sorre said. “The BMP stimulation is spatially homogenous—it was present in the culture medium—yet confining the cells to little disks is enough to let them reveal their patterning potential. Looking back, it was amusing to realize that what we did was actually to mimic the in vivo situation: The human epiblast is also a confined disk of cells in the embryo.”

But according to Michael Roberts of the University of Missouri, who was not involved with the study, the cells did not recapitulate all features of the epiblast. “Although the colonies demonstrate some analogy to the differentiating epiblast, under the culture conditions employed, they remain essentially as a flattened disc,” he said. “The next step will be to culture the cells in a format that more resembles the geometry of the epiblast at gastrulation.”

In the future, the authors believe that geometrically controlled cell culture should become standard practice for embryonic stem cell differentiation. Micropatterned differentiation can be used for interspecies comparisons, as well as comparisons between human induced pluripotent stem cells and hESCs under similar assay conditions. “One clear advantage is the standardization of the colonies’ shape,” Sorre said, explaining that it would allow researchers to average gene expression profiles across several colonies. “Because human embryonic stem cells can easily be engineered and visualized, this allows us to see things that are usually impossible to see in mammalian embryos as they develop in utero. The next step is clearly to use live reporters of cell fates, and we expect to learn a lot about early mammalian or human development.”

Reference

Warmflash A, Sorre B, Etoc F, Siggia ED, Brivanlou AH. A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. Nat Methods. 2014 Aug;11(8):847-54. doi: 10.1038/nmeth.3016. Epub 2014 Jun 29.

Keywords: gastrulation cell culture culture geometry human embryonic stem cells development differentiation epiblast