BioSpotlight

Bust a Gut

From the attachment and growth of microorganisms to the rapid renewal of epithelial tissues by stem cells, the intestine represents a unique biological environment for scientists to explore. But that uniqueness also presents challenges for researchers when it comes to studying intestinal epithelial cells and their interactions in the controlled environment of the lab. Protocols for culturing organoids that contain the crypts and villi characteristic of intestinal epithelium have been developed using intestines extracted from adult mice. The problem is that these organoids require serum-free media supplemented with a range of growth factors, agonists, and protein inhibitors for sustained culture. However, as reported in this issue of *BioTechniques*, a team of researchers led by M. Pierzchalska (Krakow, Poland) have now developed a new chicken embryo intestinal organoid culturing system that does not require extensive media supplementation for successful long-term culture. Similar to the mouse system, the authors were able to form chicken spheroid structures in Matrigel using defined media. Although exhibiting a

different growth pattern than mouse organoids, biochemical and molecular experiments demonstrated that cells in the chicken-derived organoids expressed cytokeratin, villin, and Sox-9, all markers for various intestinal epithelial cell types. Interestingly, the authors also discovered that prostaglandin E₂ was as effective as the combination of agonist R-spondin 1 and the protein inhibitor Noggin in promoting long-term growth of the chicken embryo organoids, providing a cost-effective alternative. Although the effectiveness of this new approach and prostaglandin E₂ supplementation remains to be determined for mammals, the method developed by Pierzchalska and colleagues could immediately be used as a cost-effective source for differentiated epithelial cells and will open new opportunities in the study of avian intestinal physiology and gut immunity.

See “Prostaglandin E₂ supports growth of chicken embryo intestinal organoids in Matrigel matrix” on page 307.

2D or Not 2D?

Since their development in the late 1800s, cell culture techniques have been refined and advanced to the point where 3-dimensional (3D) cultures can now simulate the mechanics and physiological behaviors of intact organs. While it is generally accepted that 3D cultures better mimic the *in vivo* conditions of cells from multicellular organisms, 2D cultures, where cells adhere to glass or plastic surfaces, are still more commonly employed in research labs since cells growing in a single plane tend to offer better imaging opportunities as well as more even gas exchange, nutrient access, and waste removal. “On-top” culturing methods, where cells attached to a gel surface are cultured in liquid media, mimic 3D culture conditions and improve imaging capabilities, but the cell progeny generated in the liquid media are not firmly immobilized and therefore occasionally migrate, aggregate, or spread unevenly across the gel surface. Writing in the current issue of *BioTechniques*, Gordeev et al. (Russian Academy of Sciences, Moscow) present a new method for generating planar cell cultures in a 3D environment. Starting with a dry polyacrylamide (PAA) gel covalently attached to a microscope slide, a cell suspension prepared in low-gelling temperature agarose is poured on top of the PAA gel and immediately covered with a

A single layer of cells in a merged gel culture.

cover slip. Centrifugation of the slide causes cells to settle in a single plane on the PAA surface as the PAA absorbs the liquid from the agarose, pushing the cell layer towards the cover slip. When the agarose hardens, it merges with the PAA, and both cells and their resulting progeny are secured in specific locations within the gel. Experiments demonstrated that cells grown under these conditions formed spheroid colonies

characteristic of those seen in 3D cultures and remained firmly anchored in the gel matrix, allowing bathing with experimental solutions without affecting cell density or morphology. Immobilization also facilitated rapid microscopic screening and subsequent removal of individual colonies for cloning. The authors clearly demonstrated this advantage by generating stable GFP-expressing cell lines using only two rounds of selection and no antibiotics. This new method provides the benefits of 3D culture while offering ease of imaging alongside the ability to clone cells growing at high densities.

See “Planar arrangement of eukaryotic cells in merged hydrogels combines the advantages of 3D and 2D cultures” on page 325.

Written by Kristie Nybo, Ph.D. and Nathan Blow, Ph.D.

BioTechniques 52:293 (May 2012)
doi 10.2144/000113844

To purchase reprints of this article, contact:
biotechniques@fosterprinting.com