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EDITORIAL

Microfabricated Crypt Scaffolds: A New Foundation for Evaluating Human Colon Stem Cells

The emergence of 3-dimensional organoid technology has significantly advanced the understanding of epithelial stem cell biology within the past decade. Stem cells of the gastrointestinal tract have served as a template for this technology, with initial propagation of mouse intestinal enteroids and colonoids (derived from adult small intestine or colon crypt units, respectively) and subsequent adaptation of these protocols for human tissue.[1–4] As such, researchers may now evaluate basic intestinal and/or colonic stem cell properties directly from patient tissue. In addition, understanding the molecular and cellular underpinnings of intestinal/colonic stem cell plasticity continues to fuel the rapidly growing field of regenerative medicine. The current, most widely used strategy is to evaluate enteroids or colonoids as 3-dimensional structures with the luminal surface located inside the "sphere"; however, there are limitations to this model. For example, such factors as crypt plating density and/or variable access to media (caused by localization within the Matrigel paddy) can lead to stochastic budding and thus heterogeneity within a sample. This can provide significant challenges, particularly when seeking to evaluate distinct proliferation/differentiation zones. In addition, accessing the luminal surface represents a technical challenge in studying epithelial-microbial interactions, currently requiring microinjection of individual enteroids/colonoids. Although generation of enteroid/colonoid-derived monolayers on flat, Transwell inserts may overcome some of the previously mentioned challenges, this is at the expense of maintaining *in vivo* crypt architecture (as reviewed in Ref.[5]).

In the current issue of *Cell and Molecular Gastroenterology and Hepatology*, Wang et al[6] describe the functional significance of using microfabricated, U-shaped collagen scaffolds together with defined chemical gradients to recapitulate a self-renewing monolayer of human colon epithelium with crypt-like morphology. This model exhibits remarkable similarity to *in vivo* crypt morphology, including proper localization of crypt/progenitor and differentiation zones achieved through the application of stem cell medium in the lower/basal chamber and differentiation medium in the upper/luminal chamber. In addition, this model exhibits unidirectional migration of cells from the progenitor to differentiation zones similar to *in vivo* crypts. To generate "crypt arrays," the authors first grew isolated crypts into monolayers over the course of 5–25 days. To evaluate the manipulability of these monolayers, the authors then tested the effect of adding previously defined differentiation factors. Addition of the γ-secretase inhibitor DAPT, for example, promoted mucin 2 expression, indicating increased goblet cell differentiation. In addition, monolayers expressed tight junction proteins including ZO-1 and occludin, suggesting appropriate localization of barrier proteins. The authors then propagated cells from monolayers atop micromolded collagen hydrogels on a hydrophilic 0.4-μM porous PTFE membrane. These collagen scaffolds each contain 245 microwells located in the center of a modified 12-well Transwell insert. *In vitro* crypts were polarized over the course of 4 days by providing stem cell media to the basal chamber and differentiation media to the upper chamber, creating a chemical gradient with high growth factor concentration at the crypt base. Staining for localization of differentiation, proliferation, and stem cell markers confirmed *in vitro* crypt polarization.

Finally, the authors introduced 2 separate perturbations into the system: luminal bacterial metabolites (short-chain fatty acids) or basal inflammatory cytokines (interferon-γ and tumor necrosis factor-α) and measured effects on colonic epithelial proliferation and differentiation. Colonic crypt arrays were treated with short-chain fatty acids acetate, propionate, or butyrate, which promoted enhanced alkaline phosphatase activity, suggesting increased absorptive colonocytes. With the addition of a butyrate gradient from the upper chamber, cell proliferation (assayed via EdU incorporation) was significantly decreased throughout the crypt, restricting proliferative cells to the crypt base. These findings support the hypothesis that butyrate may suppress stem cell activity, previously suggested in cell lines[7] and mice,[8] filling an important translational gap by demonstrating this phenomenon directly in nontransformed human cells.

Although traditional methods for establishing and growing colonoids as 3-dimensional spheres remain the fastest and most accessible means to studying stem cell phenomena in humans, this new technology will open the door to more sophisticated analyses. The ability to study distinct crypt/progenitor and differentiation zones in this culture system fills a critical gap between evaluating *in vivo* tissue samples and *in vitro* culture. It would be interesting to introduce finer zonation of the basal scaffold to understand, for example, the contribution of specific paracrine factors to stem cell division and migration. One could also imagine introducing 1 or multiple cell types (unaltered or genetically engineered) to the basal surface, including subepithelial myofibroblasts, immune cells, vasculature, and/or enteric nerves. Similarly, the accessibility of the luminal surface will expand the possibilities of studying host-microbe interactions. From a pathophysiology view, next steps may include evaluating genetically susceptible epithelium from patients with gastrointestinal diseases using this platform. Finally, it would be exciting to

incorporate this technology with live-cell imaging, akin to intravital imaging, to evaluate stem cell dynamics in real time. In summary, Wang et al[6] describe a novel, clinically relevant technology and demonstrate the ability to evaluate patient-derived colonoids with higher precision and depth than current methods.

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