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REVIEW

Bioengineered Systems and Designer Matrices That Recapitulate the Intestinal Stem Cell Niche

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SUMMARY

Gradients of ligands, growth factors, receptors, extracellular matrix, metabolites, and gases along the crypt (colon) and crypt-villus (small intestine) axis drive maintenance of intestinal stem cells, orderly differentiation, and movement of epithelial cells from the intestinal stem cell niche to the luminal intestinal epithelium. Advances in biomaterials and microdevices enable reconstruction of this complex microenvironment, replicating the key architectural features and physiological functions of the in vivo intestinal epithelium.

The relationship between intestinal stem cells (ISCs) and the surrounding niche environment is complex and dynamic. Key factors localized at the base of the crypt are necessary to promote ISC self-renewal and proliferation, to ultimately provide a constant stream of differentiated cells to maintain the epithelial barrier. These factors diminish as epithelial cells divide, migrate away from the crypt base, differentiate into the postmitotic lineages, and end their life span in approximately 7 days when they are sloughed into the intestinal lumen. To facilitate the rapid and complex physiology of ISC-driven epithelial renewal, in vivo gradients of growth factors, extracellular matrix, bacterial products, gases, and stiffness are formed along the crypt-villus axis. New bioengineered tools and platforms are available to recapitulate various gradients and support the stereotypical cellular responses associated with these gradients. Many of these technologies have been paired with primary small intestinal and colonic epithelial cells to re-create select aspects of normal physiology or disease states. These biomimetic platforms are becoming increasingly sophisticated with the rapid discovery of new niche factors and gradients. These advancements are contributing to the development of high-fidelity tissue constructs for basic science applications, drug screening, and personalized medicine applications. Here, we discuss the direct and indirect evidence for many of the important gradients found in vivo and their successful application to date in bioengineered in vitro models, including organ-on-chip and microfluidic culture devices. *(Cell Mol Gastroenterol Hepatol 2018;5:440–453; https://doi.org/10.1016/j.jcmgh.2018.01.008)*

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The phrase *stem cell niche* refers to a specific anatomic tissue location that provides a microenvironment enabling intestinal stem cells (ISCs) to remain in an undifferentiated state and promote self-renewal.[1–3] The intestinal epithelium represents one of the most well-characterized stem cell niches, with recent studies that use fluorescent reporter genes, lineage tracing transgenic mouse models, and single-cell transcriptomics defining epithelial cell signatures, behaviors, and function at unprecedented cellular resolution.[1,2,4–6] The intestinal epithelium undergoes rapid and continuous stem cell–driven renewal during homeostasis, and the fine balance between ISC maintenance and lineage allocation must be finely regulated to maintain the epithelial barrier and intestinal health. In both the small intestine and colon, ISCs reside at the base of the crypts, which are microanatomic units of epithelial monolayers that invaginate into the luminal wall (Figure 1).[2] In the small intestine, crypts are present in tightly packed arrays that feed cells into luminal protrusions called *villi*, which increase the surface area for nutrient absorption. In the colon, crypts also are present in densely packed arrays, but feed cells onto a flat luminal surface. Although there are functional differences between the small intestine and colon, remarkable similarities exist in the ordered arrangement of crypts, for example, the location of the stem cell zone at the base of the crypt, and the differentiation and migration pattern of epithelial cells.

ISCs divide to produce progenitor cells known as transit-amplifying (TA) cells, which reside above the ISCs within the crypt. The TA cells undergo several additional cell divisions

Abbreviations used in this paper: 3D, 3-dimensional; BMP, Bone morphogenetic protein; ECM, extracellular matrix; Eph, erythropoietin-producing human hepatocellular receptor; Ephrin, Eph family receptor interacting proteins; IFN-γ, interferon-γ; ISC, intestinal stem cell; NO, nitric oxide; SFCA, short-chain fatty acids; TA, transit amplifying; Wnt, wingless-related integration site.

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as they migrate upward along the crypt axis and their progeny terminally differentiate into a variety of cell lineages. Absorptive enterocytes represent the majority of cells in the small intestine, while a host of secretory lineages including goblet, enteroendocrine, tuft, and M cells contribute to the functional epithelium. When these cells reach the villus tip in the small intestine or flat luminal surface in the colon, they undergo anoikis and exfoliate into the intestinal lumen to finish a self-renewal cycle that lasts approximately 3–5 days for mice and 5–7 days for human beings.[2,3] An exception to the upward migration of differentiated epithelial cells is the secretory Paneth cell in the small intestine and a Paneth-like cell ($cKit^{+}$) cell in the colon, which remains at the crypt base intercalated among ISCs.[7] These epithelial cells secrete growth factors and present ligands at the base of the crypt to support ISC maintenance-forming gradients of these molecules along the crypt long axis.[4] Additional gradients, including ligands, other growth factors, receptors, extracellular matrices, metabolites, and gases, along the epithelial axis drive the ordered differentiation and movement of cells from the proliferative niche at the base of the crypt to the differentiated epithelium in contact with the intestinal lumen (Figure 1, Table 1).[5,8–19]

Although rodent models laid the foundation for understanding ISC biology and the niche in vivo, 3-dimensional (3D) organoid and monolayer models of the small intestinal and colonic epithelium have fueled progress toward in vitro recapitulation of the ISC niche microenvironment to study both epithelial function and pathology.[5,20–27] However, conventional organoid and monolayer culture systems do not fully recapitulate the microarchitecture of gut epithelium and cannot support the formation of gradients across geometric structures because of the nature of conventional culture systems. To develop high-fidelity, physiologically relevant in vitro models, new culture systems need to incorporate these in vivo gradients. Although there is clear evidence for factor gradients that drive gut epithelial dynamics, visualizing, measuring, and recreating these gradients has historically been technically challenging. This review focuses on the direct and indirect evidence for in vivo gradients that impact ISC biology and gut epithelial dynamics, and then presents the current state-of-the-art technologies and platforms for the in vitro culture of gut epithelium, particularly as they relate to lab-on-chip and microfluidic culture devices.

In Vivo Factors and Gradients

Growth factor gradients commonly are associated with fundamental mechanisms that underlie ISC maintenance and differentiation. Paneth cells have been the focus of much attention as an ISC niche cell by secreting factors that set up gradients to regulate stemness. A number of studies have shown that Paneth cells are dispensable for ISC maintenance and suggest that other niche cells generate factor gradients that function similar to those set up by Paneth cells.[28,29] In this regard, the underlying mesenchyme releases diffusible factors and also deposits nondiffusible extracellular matrix (ECM) that can present ligands and bind factors to regulate ISC dynamics.[30,31] These studies have merely scratched the surface on a complex cellular and molecular balance that is required to maintain the ISC-driven renewal of the epithelium. The full complement of growth factors, ECM components, and cell types involved in regenerating the epithelial monolayer are not yet fully appreciated. Moreover, noncanonical gradients such as tissue stiffness, gases, and microbial metabolites likely play critical roles but are technically difficult to study in vivo. Understanding these physical properties is of substantial interest to those investigating the broad and diverse factors that regulate gut biology, and is essential for efforts to engineer functional intestinal and colonic tissues. The following section is a brief review of the current state of in vivo gradients and highlights gaps in knowledge as new avenues for investigation.

Key Pathways Regulating ISC Maintenance and Differentiation

Modern advances in understanding ISC biology are based largely on studies that define genetic pathways and mechanisms that govern ISC maintenance and differentiation. Among these are the wingless-related integration site (Wnt), bone morphogenetic protein (BMP), and Notch pathways, which classically are studied as key contributors to epithelial renewal in homeostasis, disease, and injury. Arguably, the Wnt/β-catenin signaling pathway has been a central focus of studies that have heavily influenced the current state in the field.[4,15,20] Wnts are secreted ligands that bind their cognate receptors and function to regulate ISC maintenance and differentiation. *Sox9* is largely a downstream Wnt target gene and shows a distinct expression gradient with higher expression at the base of the crypt in the ISC zone and lower expression through the TA zone, suggesting that Wnt signaling also is present in a gradient that mimics its downstream target genes.[32–35] In fact, 9 Wnts are expressed in the small intestine of mice and are regionally expressed along the crypt-villus axis.[30] Contrary to popular assumptions, it appears that Wnt3 gradients may be formed not by simple diffusion, but rather by "plasma membrane dilution" as cells divide.[36] A Wnt3-enhanced green fluorescent protein (EGFP) fusion transgenic mouse model enabled visualization of Wnt3 expression by proxy and showed high Wnt3 expression at Paneth cells, which produce Wnt3, and lower expression up the crypt axis (Table 1).[36] Paneth cell–derived Wnt transfer involves direct contact between Paneth cells, which previously was suggested by in vitro ISC-Paneth cell co-cultures.[37] However, it remains to be determined whether all 9 Wnts establish gradients from their cellular sources.[38,39] Complete understanding of Wnt gradient formation is challenging because there are many sources of Wnts and Wnt antagonists, including subepithelial myofibroblasts and non-myofibroblast mesenchymal cells expressing Foxl1, Gli1, and CD34 within the ISC niche.[13,14,40–42] In addition, potentiation of Wnt signaling by the R-spondin family of secreted factors recently was implicated as a major