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Novel approaches: Tissue engineering and stem cells – *In vitro* modelling of the gut

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A B S T R A C T

In many intestinal diseases, the function of the epithelial lining is impaired. In this review, we describe the recent developments of *in vitro* intestinal stem cell cultures. When these stem cells are grown in 3D structures (organoids), they provide a model of the intestinal epithelium, which is closely similar to the growth and development of the *in vivo* gut. This model provides a new tool to study various diseases of malabsorption in functional detail and therapeutic applications, which could not be achieved with traditional cell lines. First, we describe the organization and function of the healthy small intestinal epithelium. Then, we discuss the establishment of organoid cultures and how these structures represent the healthy epithelium. Finally, we discuss organoid cultures as a tool for studying intrinsic properties of the epithelium, as a model for intestinal disease, and as a possible source for stem cell transplantations.

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The intestinal epithelium

The small intestine is with approximately six meters the longest organ in our body. It has a tube shape and consists of different tissue layers: the serosa and the muscularis externa on the outside, the submucosa (stromal layer) in the middle, and the mucosa in the inside of the intestinal tube. The mucosa is composed of the muscularis mucosae (smooth muscle layer), the lamina propria (connective tissue), and a single layer of epithelial cells facing the lumen of the intestine. This epithelial sheet is responsible for digestion and nutrient uptake. The epithelium is highly organized and contains protrusions on several levels (called villi and microvilli), which allow for a tremendous increase of the surface. If flattened out, the epithelium of an adult person could cover ~30 m^2 area [1]. In many intestinal diseases (enteropathies), the function of this epithelial lining is impaired [2].

The epithelial cell layer in the small intestine is organized into proliferative compartments and differentiated compartments. The proliferative compartments, which also contain the stem cells, are known as the crypts of Lieberkühn, and are embedded in the submucosa [3]. Several crypts surround and fuel one differentiated compartment, known as a villus [4,5]. The villi protrude into the lumen of the small intestine.

The organization of the epithelium is the same throughout the whole small intestine, however the morphology varies a bit: the most proximal part of the small intestine - the duodenum - shows a high abundance of very long villi, whereas the number and lengths of the villi decrease towards the jejunum (the middle part) and the ileum (the most distal part of the small intestine) [6]. The colonic epithelium consists of a flat differentiated compartment rather than villi.

Intestinal epithelial cell types and homeostasis

The intestinal epithelium is constantly regenerating, with an enormous cellular turnover. New cells are generated in the crypts. They proliferate in the crypts, migrate and differentiate upwards on the villi and undergo anoikis (cell-detachment-induced apoptosis) at the tip of the villi, and are then shed into the lumen of the small intestine [7]. In humans, approximately 100 billion cells are estimated to be replaced every day [8].

The intestinal stem cells

The self-renewing capacity of the intestinal epithelium depends on the presence of intestinal stem cells. Multipotent adult intestinal stem cells are defined as cells that give rise to all differentiated intestinal epithelial cell types, and are at the same time capable of self-renewal. Actively cycling intestinal stem cells are located at the bottom of the crypts. Those cells were first mentioned in 1887, in a publication by Josef Paneth, who termed them “schmale Zellen” (=slender cells) [9]. In 1974, Cheng and Leblond characterized those cells as *crypt base columnar* cells (CBC cells), and proposed them as intestinal stem cells [10,11]. In 2007, lineage-tracing experiments showed that CBC cells are actively cycling, undergo self-renewal, and generate all mature intestinal epithelial cell types, proving they are multipotent stem cells [12]. The CBC stem cells express several unique marker genes, such as Lgr5 and olfactomedin-4. Upon injury and stem cell loss, cells from a quiescent stem cell pool higher up in the crypt (so-called +4 stem cells or label retaining cells), and secretory progenitor cells can dedifferentiate to replace the pool of active cycling Lgr5-positive CBC stem cells [13–17]. The CBC stem cells symmetrically divide once every 24 hours and produce new stem cells and daughter cells in a stochastic manner [18,19]. The daughter cells are termed transit amplifying (TA) cells, are highly proliferative and line the flanks of the crypts. They migrate, differentiate and give rise to the six differentiated cell types of the small intestinal epithelium.

Differentiated intestinal cells

The differentiated cell types of the small intestinal epithelium each have a specialized function in nutrient digestion and absorption, immune response or as supportive niche cells.