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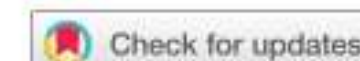


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REVIEW



A spotlight on intestinal permeability and inflammatory bowel diseases

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ABSTRACT

Introduction: The intestinal barrier is a multi-faced structure lining the surface of the intestinal mucosa of the GI tract. To exert its main functions as a physical and immunological defense barrier, several components of the intestinal barrier act in a concerted and cooperative manner.

Areas covered: Herein, we first introduce to the basic organization of the intestinal barrier and then summarize different methods to assess barrier function in and ex vivo. Finally, we provide an in-depth overview of the relevance of intestinal barrier dysfunction in inflammatory bowel diseases.

Expert opinion: In parallel to a more fundamental understanding of the intestinal barrier as a key component for intestinal integrity is the notion that intestinal barrier defects are associated with a variety of diseases such as inflammatory bowel diseases. Recent research has fueled and perpetuated the concept that barrier defects are critical components of disease development, disease behavior, and potentially also an area of therapeutic intervention in IBD patients. Although being far away from standard, new technologies can be used to easily assess barrier healing in IBD and to derive clinical consequences from these findings such as more accurate forecasting of future disease behavior or the identification of novel therapeutic targets.

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Intestinal barrier; intestinal permeability; inflammatory bowel diseases; confocal laser endomicroscopy; Crohn's disease; ulcerative colitis; leaky gut

1. Introduction

The intestinal barrier encompasses a multi-faced structure that lines the surface of the entire gastrointestinal tract and, apart from skin and airways, provides our major separation between 'foreign' and 'self.' The association between defects in intestinal barrier function, or increased intestinal permeability, and the development of manifest intestinal inflammation such as inflammatory bowel diseases (IBD) has been noted already more than 30 years ago [1]. Increased intestinal permeability has recently gained much attention in the daily and popular press and is then commonly referred to as 'leaky gut.' Within these public press reports, several associations are frequently drawn between 'leaky gut' and several diseases or systemic symptoms leading a perception that a 'leaky gut' is the root or cause for various multi-faced syndromes. In light of this folklore about 'leaky gut' [2], this review aims to provide an up-to-date overview of the basic organization of the intestinal barrier and to summarize current state-of-the-art technologies and parameters for the assessment of intestinal barrier function and increased intestinal permeability. Finally, we aim to discuss and reflect the relevance of increase intestinal permeability in patients suffering from inflammatory bowel diseases.

2. Basic organization of the intestinal barrier

The intestinal barrier lines the luminal surface of the intestinal mucosa and is of central importance for maintaining intestinal

integrity and homeostasis [3]. Structurally, the intestinal barrier is formed by several components that act concerted and cooperatively for providing a mechanical, functional, and immunological barrier as its main functions. Among these, the following components can be found: (i) a mucus layer that, apart from mucus itself, contains antimicrobial peptides (AMP) and secretory Immunoglobulin A and is the habitat for commensal bacteria, (ii) a single layered line of epithelial cells or enterocytes, (iii) the lamina propria with a variety of immune cells such as T cells, B cells, macrophages and dendritic cells, orchestrating intestinal adaptive and innate immune responses [3,4], (iv) finally, the gut microbiota is increasingly recognized as a critical component for maintaining intestinal homeostasis and integrity of the intestinal barrier [5].

By preventing bacteria from direct interaction with the epithelial cells underneath, the **mucus layer** provides the first defense mechanism of the intestinal barrier [4]. The mucus layer shows remarkable differences along the GI tract. In the colon, the mucus layer on the outer surface consists of two layers. The outer layer is thick and loose and a natural habitat of commensal bacteria and their metabolites whereas as the inner dense mucus layer is firm and adherent with comparatively low bacterial density. In contrast, in the small intestine, the mucus layer is single and fluid and contains abundant antimicrobial substances [6]. Apart from water, the major component of the intestinal mucus is the MUC2 mucin [7] produced by Goblet cells. Several lines of evidence suggest

Article highlights

- The intestinal barrier is a multi-faced structure lining the surface of the intestinal mucosa of the GI tract that acts as a physical and immunological defense barrier
- Increasing evidence suggests that defects in the intestinal barrier are associated with a variety of diseases such as inflammatory bowel diseases (IBD)
- As such recent research has fueled and perpetuated the concept that barrier defects are critical components of disease development, disease behavior and potentially also an area of therapeutic intervention in IBD patients
- In this article, we introduce to the basic organization of the intestinal barrier and summarize different methods to assess barrier function in and ex vivo
- Finally, we provide an in-depth overview on the relevance of intestinal barrier dysfunction in inflammatory bowel diseases

that MUC2 is critical for colonic protection: Patients with ulcerative colitis often exhibit depletion of recognizable goblet cells in the colonic epithelium [8] and the inflammatory activity in UC has been shown to correlate with a decrease in MUC2 synthesis and secretion [9,10]. Furthermore, MUC2-deficient mice spontaneously develop colitis, thereby further corroborating that MUC2 deficiency contributes to the onset and perpetuation of experimental colitis [11].

The **epithelium** is located underneath the mucus layer and forms a physical or mechanical barrier that is constituted by single layer of intestinal cells organized into crypts and villi. Among these, enterocytes are the most abundant intestinal cell type. Enterocytes have a columnar shape with a specialized apical membrane domain and in their cellular cohesion, enterocytes form a continuous and polarized monolayer that is, in the absence of specific transporters, impermeable to hydrophilic metabolites. In contrast, the uptake of lipophilic or large molecules is mostly dependent on diffusion and endocytosis. Besides enterocytes, the epithelium contains several other cell types such as Goblet cells or Paneth cells in the small intestine. The latter are located at the bottom of the Lieberkühn crypts and are thought to have a dual role: (i) local secretion of trophic factor such as epidermal growth factor, transforming growth factor α and Wnt3 and (ii) secretion of antibacterial peptides such as α -defensin 5 and 6 as the most abundant peptides that serve to maintain crypt sterility and control of the local microbiome [12]. Overall the intestinal epithelium is a highly dynamic systems that is continually renewed by pluripotent stem cells which reside in the base of crypts and differentiation patterns along the crypt-villous axis play an important role for integrity of the intestinal barrier [13,14].

The transport of molecules between the IECs, the so-called paracellular transported, is regulated by a specialized apical junctional complex consisting of tight junctions (TJs), adherens junctions (AJs), and desmosomes [15].

TJs are the most apical sealing complex that are further composed of transmembrane proteins such as claudins and occludins, intracellular plaque proteins such as zonula occludens (ZO) and regulatory proteins [16,17]. TJs are the main contributors of the regulation of paracellular transport and therefore define paracellular permeability [18]. Increased

paracellular permeability across the tight junction can occur via two routes: the pore pathway and the leak pathway [18]. The pore pathway, which is structurally based on claudins which generate size- and charge-selective channels, is mainly regulated by IL-13 and IL-2, leading to increased claudin-2 expression, which then increases intestinal permeability by the pore pathway [18].

The leak pathway allows molecules with a diameter of up to 12.5 nm to cross the epithelial barrier and in contrast to the pore pathway is not charge-selective. Regarding their transport capacity, the pore pathway is considered to be a high-capacity pathway whereas the leak pathway has low transport capacity but allows transport of molecules 20-fold large than those that cross the epithelium via the pore pathway [18].

As further structural components of the apical junctional complex, AJ and desmosomes exert their role mainly in cell-to-cell adhesion and in the maintenance of integrity of the epithelium. Specifically, AJs are multiprotein complexes that are usually more basal than TJs and form an element of cell-cell interaction in which cadherin receptors bridge the neighboring plasma membrane via homophilic interactions [19]. AJs and TJs provide strong cellular adhesion and therefore serve to maintain cellular proximity and epithelial association. Importantly, cadherins within the AJs act as sensors of intra- and extracellular mechanical signals and can respond with a modulation of actinomyosin connections and therefore are able to modulate the strengths of AJs [20]. Overall, AJs have a dual role: apart from maintaining epithelial integrity, AJs serve to facilitate cell movement during developmental regenerative or renewal processes [21]. Alterations or loss of AJs leads to loss of cells-cell contacts and cellular polarization, and, as shown in animal models and in vitro studies, results in increased and premature apoptosis [3,15,22]. Similar to AJ, desmosomes are cadherin-based multiprotein complexes that mediate intercellular adhesion but unlike AJ, desmosomes are tethered to intermediate filaments instead of the actin cytoskeleton [20]. Abundant expression of desmosomes can be found in tissue that are subject to high mechanical stress such as epithelia, heart or skin. Apart from their major role in strengthening cell-cell-interactions, desmosomes exhibit also functions in other central processes such as cellular growth and differentiation as well as immune homeostasis. Furthermore, emerging evidence suggests that desmosomes are critically involved in the regulation of the intestinal barrier and that components of desmosomes are emerging targets to prevent loss of intestinal barrier function [23].

The **gut microbiota** comprises approximately 10^{13} bacterial cells [24] from more than 250 different bacterial species. Among them, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria phylum are most abundantly found [25,26]. Remarkably, although the composition of our gut bacteria can be quickly altered by external factors such as diet, drugs, and other environmental factors, the intestinal microbiome is relatively stable over time surpassing our genetic arsenal more than 150-fold [27,28]. Mechanistically, our gut microbiome can facilitate recruitment of immune cells to the mucosa, thereby promoting the generation and maturation of the gut-associated lymphatic tissue (GALT) [29]. As such, it has been noted that germ-free mice exhibit poorly differentiated GALTs

and that this deficiency can be corrected by restoration of conventional flora or Toll-like receptor (TLR) ligands, indicating that signaling from the commensal bacterial flora is a critical component for development and maturation of the mucosal immune system [30].

3. Assessment of intestinal barrier function

3.1. Experimental in and ex vivo assessment of intestinal permeability

One of the oldest techniques to assess intestinal permeability is based on the use of so-called Ussing chambers, named after the Danish scientist Hans Ussing who performed electrophysiologic experiment in skin frog more than half a century ago [31]. To date, different Ussing chamber equipment is available so that the transepithelial electrical resistance (TEER) and paracellular permeability can be studied in a variety of different epithelia such as cell cultures grown in membranes, animal tissue and human tissue from resection specimens or endoscopic biopsies [32]. The common principle of using Ussing chambers is that each side of a polarized epithelium, which exhibited a potential difference between the apical and basolateral membrane, is isolated in a hemi-chamber from which each chamber-half is filled with an equal amount of Ringer solution. Due to active ion transport, a transepithelial potential difference (PD) can be measured with both-sided electrodes. Generally spoken, the TEER measures the electrical resistance that the intestinal cells are exerting against an ion flow from the luminal to the basolateral chamber half. Therefore, low TEER values serve as an indicator of increased epithelial permeability to ions. Since TEER cannot discriminate between the pore from the leak pathway as a source of increased permeability, marker molecules (e.g. EDTA, mannitol, sucrose, inulin and polyethylene glycols (PEG) or dextran molecules) are frequently added to the apical chamber-half. Subsequently, samples from the basolateral side can be taken over time to assess passage of the respective marker molecules across the tissue. In addition to TEER measurement, Ussing chambers can be used to study transcellular transport ex vivo by utilizing large molecules or probes that are known to cross the epithelium transcellularly (such as Horseradish Peroxidase, molecular weight: 44 kDa) [32]. Transcellular transport is also used by bacteria during the process of bacterial translocation. By adding labeled live or inactivated bacteria such as *Escherichia coli* or *Staphylococcus aureus* to the luminal side of the Ussing chamber, the process of bacterial translocation can also be mimicked ex vivo [33,34]. Although commonly regarded as the gold standard for assessing intestinal permeability, Ussing chambers experiments are time- and labor-intensive and require dedicated expertise [32]. Hence, Ussing chambers are not largely available, thereby limiting their routine use for assessing epithelial integrity.

Other powerful methods for real-time measurement of barrier function are electrical cell-substrate impedance sensing (ECIS) or ECIS-based TEER [35] or the use of transwell systems with intestinal cells that grow as a monolayer [36]. Finally, the exteriorized intestinal loop model is a microsurgical in vivo method that utilizes a vascularized and exteriorized intestinal

segment (ileum or proximal colon) that can be used in rodent experiments to study intestinal barrier function in a more physiologic context and therefore may be used to corroborate or even expand findings from in vitro assays. This model is based on the generation of an isolated, yet vascularized intestinal segment that has blind ends on each side. When non-digestible markers (such as chemo- and cytokines, antibodies, marker molecules or even live bacteria) are injected into this isolated intestinal segment, translocation of these markers can then subsequently be measured in serum (or other target tissue) [37,38].

3.2. Biomarkers for assessing integrity of the intestinal barrier

Although the assessment of the integrity of the intestinal barrier with different biomarkers allows noninvasive evaluation and monitoring of intestinal permeability frequently without the need of prior administration of test molecules, they commonly exhibit rather low sensitivity and specificity. In the following, different biomarkers from urine, serum and feces that are available to date for noninvasive evaluation of the intestinal barrier are discussed.

Fatty acid-binding proteins (FABPs) are small proteins located intracellularly or within the plasma membrane. So far, nine isoforms of FABP with different organ-specific expression have been identified [39,40]. Among these, intestinal FABP (I-FABP) is expressed in enterocytes of the small intestine and, to a lesser extent, in the colon whereas I-FABP has not been found in other tissues [41,42]. While I-FABP is detectable in only low amounts systemically, I-FABP is released into the bloodstream upon damage to the intestinal barrier [43]. Due to its organ specificity, I-FABP has been studied as a biomarker in necrotizing enterocolitis [44,45], mesenteric ischemia [44,45], and celiac disease and the majority of studies found increased serological expression (as quantified by ELISA) of I-FABP in these diseases.

Lipopolysaccharides (LPS) are amphiphilic molecules of 10–20 kDa that constitute the major component of the outer membrane of gram-negative bacteria. Due to bacterial abundance, high LPS concentrations can be found in the intestinal lumen. Since LPS do not cross the intestinal barrier in a healthy state [46,47], LPS usually cannot be detected systemically in healthy individuals [48–51]. Increased serum levels of LPS have been described in a variety of diseases that are commonly associated with impaired intestinal barrier such as Crohn's disease, necrotizing enterocolitis or liver cirrhosis [48–52]. Since contamination of laboratory equipment with LPS is relatively common, LPS-binding protein (LBP), reflecting increased trans-epithelial uptake of LPS, has been proposed as alternative noninvasive marker [53]. In this regard, a significant correlation between LBP levels and the lactulose-to-mannitol ratio (LMR) in normal weight and obese subjects [54] has been described just recently [54].

Claudins constitute a family of transmembrane cellular adhesion molecules located at the TJs [55]. Among them, Claudin-2 forms paracellular ion and water channels, therefore claudin-2 is a pore-forming claudin. Claudin-2 cannot be detected in healthy colon [56–58] while in patients with

inflammatory bowel disease, cytokine triggered overexpression of claudin-2 in patients with inflammatory bowel diseases has been reported [59]. The role of claudin-2 in intestinal homeostasis and inflammatory bowel diseases appears to be complex, however. As such, as shown in transgenic mice, targeted colonic overexpression of claudin-2 increases mucosal permeability, but at the same time provides protection against experimental colitis [60]. Contrarily, recent experimental studies in transgenic mice suggests that claudin-2 inactivation can ameliorate immune-mediated colitis progression [61]. So far, relatively few studies have assessed a potential role of claudins as biomarkers for impaired intestinal barrier function. In this regard, increased urinary claudin-3 excretion and decreased claudin-3 staining in intestinal biopsies was noted in patients with active Crohn's disease [62]; however, validation of claudins as markers of impaired intestinal barrier function is still missing.

Recently, *Zonulin* has been utilized as a serum marker for intestinal permeability. Zonulin was identified in the year 2000 in non-human primates as a protein that induces disassembly of TJs [63]. Early studies in rodents found evidence that activation of zonulin can mediate cytoskeleton reorganization and tight junction opening, leading to increased intestinal permeability [64]. However, subsequently, zonulin was identified as prehaptoglobin-2 (preHO2) [65] and it is important to point out that preHP2 is not expressed in mice and that the HP genotype is unique to humans [66]. Increased serum zonulin concentrations have been found in a variety of diseases such as celiac disease, diabetes or inflammatory bowel diseases. As such, a pilot study has shown that IBD patients exhibit higher zonulin concentrations compared to healthy controls with no difference in zonulin levels between patients with CD and UC [67]. Furthermore, another recent study assessed fecal levels of zonulin-related proteins and found that these are associated with activity of Crohn's disease and strongly correlate to levels of fecal calprotectin [68]. However, recently, evidence from independent experiments suggest that most commercially available ELISA Kits do not detect human zonulin but concentrations of unknown proteins [66,69,70]

Another just recently published landmark study used serum proteome profiling to identify serum markers that are associated with barrier dysfunction as assessed by LMR in Crohn's disease. Using this approach, the authors found that MMP-12 and CXCL9 correlated with increased LMR. Importantly, as shown in this nested case-control study, these proteins were also predictive for future CD development, thereby indicating that they might be used as biomarkers for future disease development [71].

Although it has been shown already 10 years ago that *occludin* depletion in intestinal epithelial cells leads to an increase in macromolecule flux through the large-channel TJ pathway [72], studies investigating the potential of occludin as a biomarker for intestinal permeability are still lacking to date.

3.3. In vivo assessment of intestinal barrier permeability

As a common feature, the majority of methods to assess intestinal barrier permeability utilizes orally administered

markers that are renally excreted and can then be quantified in the urine. After oral administration of these markers, they cross the intestinal barrier in a defined period of time, then enter the systemic circulation and are finally renally excreted. In case of increased intestinal permeability, a higher amount of markers cross the intestinal barrier and consequently, higher urine levels of these markers can be found. Given this concept, ideal markers utilized for these in vivo tests should exhibit the following characteristics: (i) paracellular absorption (ii) metabolic inertness (iii) free renal excretion. Markers that are frequently used for those tests include Chromium-labeled EDTA (^{51}Cr -EDTA), polyethylene glycols (PEG) of different sizes and sugars or sugar alcohols such as lactulose, sucrose, rhamnose or mannitol [73]. However, it has to be mentioned that ^{51}Cr -EDTA is radioactive, thereby potentially limiting its wide utilization.

Furthermore, the use of two different markers and the determination of an excretion ratio can, at least partly, correct for confounding factors such as gastric emptying or intestinal transit time. To date, the most widely used method to assess small bowel permeability is the determination of the differential renal excretion of the disaccharide lactulose and the sugar alcohol mannitol. As a disaccharide, Lactulose (9.5 Å) is transported across the intestinal barrier via the paracellular pathway in a highly restricted manner whereas mannitol, as a smaller monosaccharide (6.7 Å), freely crosses the intestinal epithelium via transcellular and paracellular routes. Using these markers, the lactulose-to-mannitol ratio (LMR) in urine 0–2 h after ingestion serves as a marker of small bowel permeability [32,74]. However, it has to be pointed out that lactulose and mannitol are degraded by microbiota in the colon. Therefore, both markers cannot be used to evaluate colonic permeability [32,75] while other markers (e.g. sucralose, PEG, and ^{51}Cr -EDTA) are resistant to bacterial fermentation and can be used for evaluation of total gastrointestinal permeability [76]

3.3.1. Confocal laser endomicroscopy

Confocal laser endomicroscopy (CLE) is a microscopic imaging technology that was brought to the market almost 20 years ago. Since then, CLE has emerged as a technology allowing to perform real-time microscopy at 1000-fold magnification [77]. As a technology of confocal imaging, CLE is based on the emission of low-powered blue laser light that is directed through a pinhole onto a defined area within the tissue. The mucosa then produces a fluorescence signal that is reflected and refocused on the detection systems. The reflected light passes again a pinhole so that scattered light from areas outside of the imaging plane is not detected. This principal of confocal imaging with emission and detection of light that passed a pinhole results in increased lateral resolution (Figure 1).

Currently, commercially available CLE systems utilize confocal mini probes (probe-based CLE, pCLE) that can be advanced through the working channel of any standard endoscope. Images are obtained at a speed of 8 frames per second, thereby pCLE enables real-time microscopic videos of the

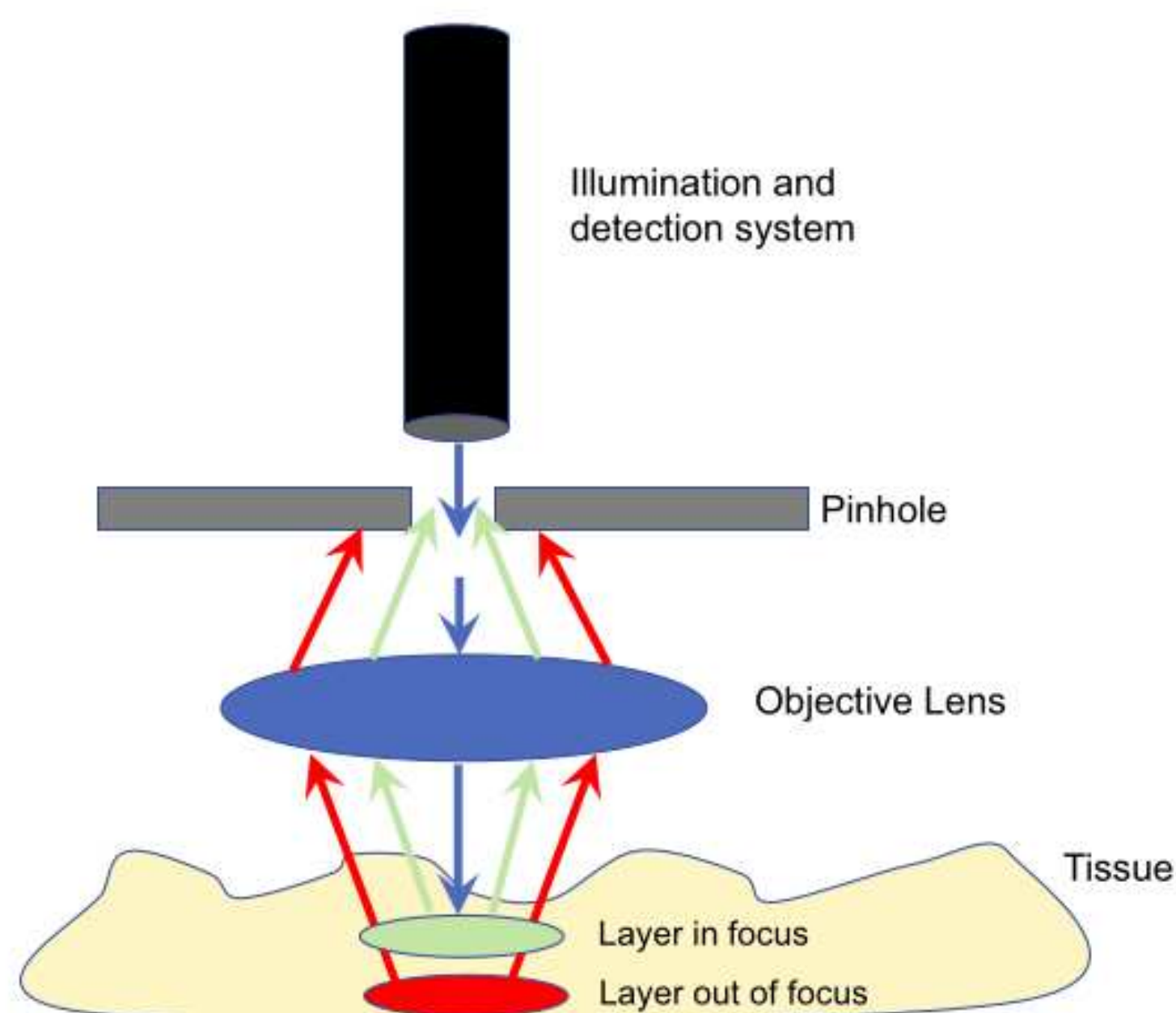


Figure 1. Principle of confocal laser endomicroscopy. Confocal laser endomicroscopy is based on the emission of low-powered blue laser light that is directed through a pinhole on a defined point within the tissue. The target tissue produces then a fluorescence signal and only returning light refocused through the pinhole is detected whereas reflected light from areas out of the imaging plane is excluded from detection.

mucosal surface. Further imaging properties of pCLE are the following: fixed imaging depth with a lateral resolution between 3.5 and 1 μm and a field of view between 600 μm and 240 μm (depending on the confocal probe) [78,79].

During pCLE imaging, contrast agents are usually administered systemically and in the vast majority of studies fluorescein was administered systemically for intravascular fluorescence. Apart from the possibility to perform *in vivo* microscopy, pCLE enables to functionally assess the integrity of the intestinal barrier, as shown in several studies [80–87] and the relevance of assessing intestinal barrier function with pCLE is further discussed below.

4. Intestinal permeability in inflammatory bowel diseases

Already more than 30 years ago, increased intestinal permeability has been noted in patients with inflammatory bowel disease. In one of the earliest studies in the field, Hollander and coworkers assessed intestinal permeability using the marker polyethylene glycol-400 ingested with a standard meal. Using this approach it was shown in this early trial that healthy volunteers had a mean absorption of 215 mg which was increased two-fold not only in patients with Crohn's disease but also in relatives of CD patients. Remarkably, the authors already speculated that the increased intestinal permeability is not secondary to clinically recognized inflammation but might be a primary defect that is etiologically involved in disease pathogenesis [1].

Subsequent studies were able to corroborate increased intestinal permeability in IBD, mainly by using orally administered marker molecules, above all the lactulose-mannitol-ratio

[1,88,89]. Importantly, these studies not only consistently showed that patients with Crohn's disease exhibit an increased intestinal permeability compared to controls, but also that this is potentially related to disease behavior. As such, a landmark paper by Wyatt and colleagues found not only a significant correlation between increased permeability and disease activity, but also that increased permeability is predictive of subsequent disease relapse [89]. Furthermore, the authors already speculated 'increased permeability may lead to the absorption of endotoxin and lipopolysaccharides from the lumen' and therefore might act as a pathogenic factor in IBD [89].

In addition to this almost historic evidence, a just recently published study was able to link increased intestinal barrier permeability measured by the lactulose-to-mannitol ratio to future development of CD [90]. During this study, barrier permeability was assessed in 1420 asymptomatic first-degree relatives of CD patients and during long-term follow up, increased intestinal permeability significantly predicted the risk of developing CD and acted as an independent risk factor for developing Crohn's disease in first degree relatives in the future [90].

In parallel to clinical studies as discussed above, basic science provided several lines of evidence on the relevance of increased intestinal permeability for the development of IBD. As such, it has been noted for a long time that $\text{IL10}^{-/-}$ mice not only develop chronic enterocolitis with focal distribution resembling human CD [91] but also that these mice develop increased intestinal permeability very early in life and before the occurrence of colitis [92]. In a more translational context, research on human specimens has revealed impairments in tight junction function and epithelial resistance in patients with inflammatory bowel disease. In an early seminal study, Schultzke and colleagues set off to characterize the epithelial barrier function in colectomy specimens without gross macroscopic inflammation from UC patients. In Ussing chamber experiments, it was demonstrated that colonic tissue from UC patients exhibited a reduction of the total electrical wall resistance (as assessed by transmural impedance analysis) by 50% compared to healthy subjects. Furthermore, this reduction in resistance was paralleled by an increase in paracellular permeability for mannitol and a decrease in the number of strands of the TJs, thereby suggesting that a change in epithelial cell TJ structure is responsible for increased permeability in UC [93]. A further study by the same group sheds light on the question whether increased epithelial permeability is caused predominantly by local leaks or increased homogeneous basal permeability. Using a microelectrode to record the spatial distribution of current clamped across viable epithelium, Gitter and colleagues showed that in early UC intact epithelium exhibits leaks at apoptotic foci whereas, in areas with macroscopically more intense inflammation, erosion and ulcerations were highly conductive [94]. Importantly, those local leaks as discovered in this report contributed 19% to the overall epithelial conductivity in mild and 65% in moderate-to-severe inflammation [94]. Consistent with these observations, a recent study

revealed an increased ^{51}Cr -EDTA permeability in inactive UC and patients with irritable bowel syndrome (IBS) with significantly higher permeability in UC as compared to IBS, thereby further strengthening the concept that increased permeability can be observed even in a state of disease remission and is not necessarily related to areas with active disease [95].

Similar to those observations in UC, early studies using freeze fracture electron microscopy were able to identify tight junction strand breaks in Crohn's disease as a potential cause of barrier dysfunction in CD [96,97]. However, although morphologic changes of tight junction strands often result from changes in tight junction protein expression, only a few tight junction proteins have been analyzed in Crohn's disease so far. As such, a recent study reported downregulation of occludin in Crohn's disease, whereas claudin-1 expression was unchanged [98]. Furthermore, Zeissig and coworkers showed that, in parallel to increased expression of the pore-forming claudin-2, patients with mild-to-moderately inflamed Crohn's disease exhibit a decreased expression and redistribution of the sealing claudins 5 and 8. Moreover, as already discussed for UC above, increased epithelial apoptosis contributed to barrier dysfunction in Crohn's disease in this report [58]. Consistent with these observations, Söderholm et al. showed that noninflamed ileum from CD patients exhibits an increased transcellular and paracellular permeability for large protein and increased transcellular uptake of nonpathogenic *E. coli* in the follicle-associated epithelium (FAE) in CD [33,99,100]. Consistent with these observations, the epithelium of the non-inflamed ileum is more susceptible to luminal contents that are present in the environments. As such, it has been shown that sodium caprate, which constitutes 2–3% of fatty acids in dairy products, can induce a rapid increase in paracellular permeability with dilation within the tight junctions and disassembly of perijunctional filamentous actin, indicating that TJs in the non-inflamed ileum of CD might be more reactive to luminal stimuli and can contribute to the development of mucosal inflammation [101].

In addition to that it has been shown that serum markers that are commonly associated with impaired barrier function

and bacterial translocation such as LPS, Lipopolysaccharide-binding protein (LBP), and soluble CD14 receptor (sCD14) are increased in patients with active UC and active or inactive CD and importantly, medical treatment led to significant decline of increased LBP, LPS, and sCD14 serum levels [102].

Originally described on CLE by Kiesslich et al., epithelial gaps are of central importance for evaluation of the barrier in IBD patients with CLE [85]. In subsequent work by the same group of authors, a semiquantitative scoring system for grading of the severity of intestinal barrier dysfunction by CLE was developed [84] and has been utilized in several studies and trials since then [80,83,84,86,87,103]. This so-called Watson-Score (Figure 2) grades the intestinal barrier function into three grades (I) *intact epithelial barrier*: single epithelial gaps but no fluorescein leakage into the lumen; (II) *functional barrier defect* with shedding of single epithelial cells and fluorescein leakage into the intestinal lumen; (III) *structural barrier defect* with shedding of multiple epithelial cells, exposure of the lamina propria to the lumen and fluorescein leakage into the lumen [84]. Importantly, a prospective study on IBD patients with clinical and endoscopic remission was able to show that grading of intestinal barrier dysfunction by CLE along the Watson Score can be used to predict the risk of disease relapse. As such, patients who subsequently experienced flaring of disease had significantly more fluorescein leakage and microerosions and thus a higher Watson grade than patients without flare. Furthermore, CLE-based grading of intestinal barrier dysfunction was highly specific for predicting subsequent disease flares [84] and additionally, mouse experiments demonstrated inward flow through some leakage-associated shedding events, thereby supporting the hypothesis that access of luminal antigens to the immune system in the lamina propria might play a major role in the pathogenesis of IBD [84]. Soon thereafter, these results were confirmed in another prospective trial in 50 IBD patients by Karstensen and coworkers. Herein, ileal fluorescein leakage and microerosions, as assessed by CLE, were significant risk factors for relapse in IBD patients in remission with high inter- and intraobserver reproducibility [83].

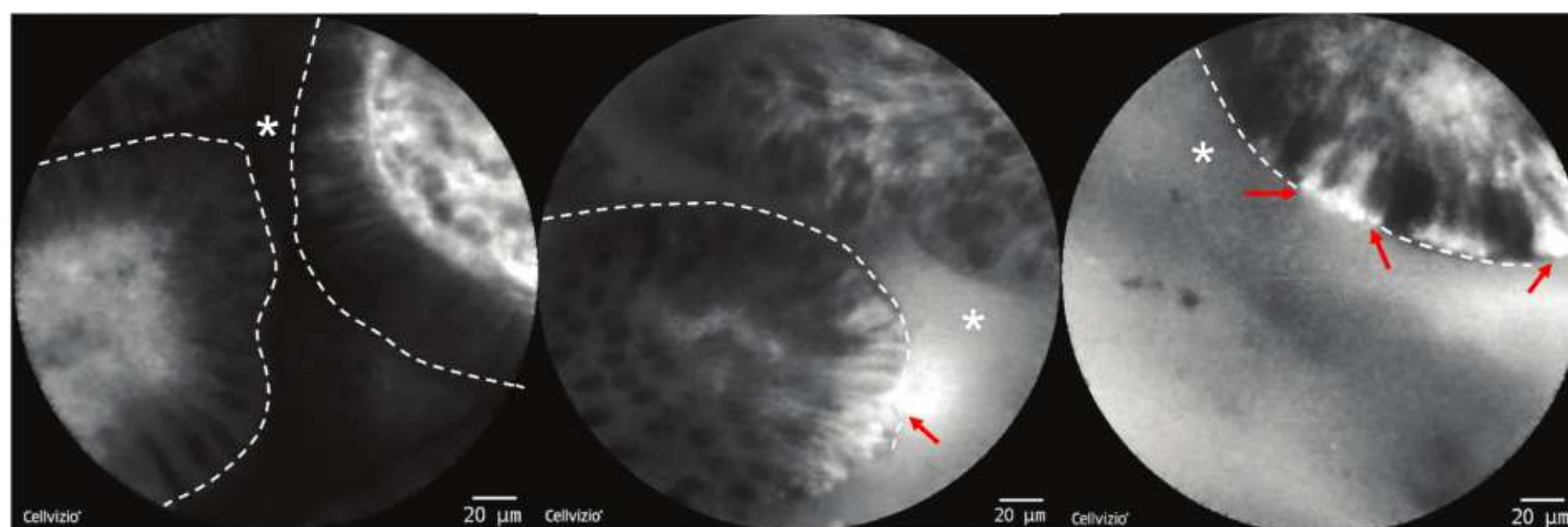


Figure 2. Assessment of intestinal barrier function with confocal laser endomicroscopy. Left: Intact intestinal barrier (Watson °I). Middle: functional barrier defect with an epithelial gap and fluorescein leakage through the gap (Watson °II). Right: structural barrier defect with shedding of neighboring epithelial cells. Dashed line: epithelial barrier, Arrows: epithelial barrier defect, Asterisk: intestinal lumen.

Another prospective trial in IBD patients with mucosal healing was able to directly establish a link between increased intestinal permeability and persistence of clinical symptoms: as such, in UC and CD patients with endoscopic remission, impaired barrier function, as evaluated by CLE, was significantly correlated with severity of diarrhea in both diseases, thereby raising the possibility that resolution of mucosal permeability beyond mucosal healing might improve outcomes of IBD patients UC and CD [80].

Just recently, results from the large prospective ERICa trial demonstrated not only that CLE-based assessment of an intact ileal and colonic barrier (i.e. barrier healing) is associated with decreased risk of disease progression in clinically remittent IBD patients but also that assessment of barrier dysfunction in the ileum and colon barrier with CLE exhibits a superior predictive performance compared to endoscopic and histologic remission [103]. With this, monitoring of the intestinal barrier during routine ileocolonoscopy by CLE might allow to risk-stratify IBD patients for the occurrence of complicated disease behavior in clinical practice. Apart from the possibility that CLE-based evaluation of the intestinal barrier might be used as a companion diagnostic in clinical practice, the cumulating evidence as reviewed above suggests that barrier healing might also be considered as future target for the treatment of inflammatory bowel diseases.

5. Expert opinion

In parallel to a more fundamental understanding of the intestinal barrier as a key component for maintaining intestinal homeostasis and integrity is the increased notion that intestinal barrier defects are associated with a variety of different diseases. As such, the association between inflammatory bowel diseases and increased intestinal permeability has been first recognized almost half a century ago and recent research has further fueled and perpetuated the concept that intestinal barrier defects are critical components of disease development, disease behavior, and potentially also an area of therapeutic intervention.

Although the data from basic and clinical research on the relevance of barrier dysfunction for various aspects of inflammatory bowel disease such as disease etiology, disease behavior in patients with known IBD, or the possibility to use the presence of intestinal barrier dysfunction as a screening test to identify individuals at risk for future disease development, are almost compelling, the assessment of intestinal barrier (dys)function is far away from being implemented into clinical practice. This has many reasons, among them, and from authors perspective the most limiting factor, the lack of a diagnostic standard for the evaluation of barrier function in IBD. Although commonly regarded as the gold standard for the evaluation of epithelial/intestinal permeability, Ussing chamber experiments are time and labor intense and require dedicated experience, thereby limiting their availability and their widespread and routine use. Furthermore, no serum marker has been proven to reliably assess barrier function, a fact that certainly, at least partly, can be made attributable

to the lack of a clear diagnostic gold standard. Although widely use, measurement of permeability by the LMR is limited by the bacterial fermentation of lactulose and mannitol in the colon, thereby assessing small bowel permeability only. From the authors perspective, the most promising technology for the evaluation of intestinal permeability both in the colon and in the small bowel is confocal laser endomicroscopy as CLE (i) can easily be incorporated into the existing examination (i.e. endoscopy) and (ii) it can directly visualize areas with barrier dysfunction. Clearly, CLE has many obstacles to overcome, among them the so far limited distribution of the system as well as the image interpretation that requires some expertise. Nevertheless, with the development of algorithms for automated CLE image analyses, it can be expected that times needed for CLE images analysis will be reduced with objective and operator-independent image analysis. In their togetherness, this might facilitate transferability of assessing barrier (dys)function in IBD to less experienced centers in the future.

Secondly, although data on the important relevance of barrier dysfunction in IBD has been described in a multitude of studies, these studies are very heterogenic in terms of patient population, their study design or the utilized technology for assessment of barrier function. In the future, large scale prospective multi-centric studies are highly warranted in which (i) barrier function is assessed in a multi-faced approach (e.g. CLE, LMR, serum markers, biopsies with histopathological staining) and (ii) broad approaches (e.g. Proteomics, Genomics, etc.) are incorporated.

If, in these approaches, a marker, a technology, or an algorithm can be identified that, also in subsequent studies, can reliably assess the presence of barrier dysfunction or barrier healing in inflammatory bowel disease, it is the authors view that this can significantly improve the clinical care of IBD patients in the following areas: (i) screening of individuals at risk for IBD development, (ii) accurate prediction of disease behavior in patients with known IBD and the (iii) identification of novel or additional therapeutic targets.

Finally, the authors note with some concerns that increasing numbers of associations are drawn between intestinal permeability and various diseases or systemic symptoms such as depression, fatigue or anxiety, for which the scientific proof is sometimes questionable or still pending. Furthermore, within the last years, the authors note that intestinal permeability is increasingly addressed in public press and nonscientific media in which 'leaky gut' serves as the root or cause for various multi-faced syndromes and symptoms. In light of this, it is the author's opinion that the scientific community needs to remain critical and reflective in that way (i) a clear and precise nomenclature should be consistently used (e.g. avoid the term 'leaky gut') (ii) only associations that withstand critical scientific standards should be taken into account when the contribution of intestinal permeability to certain diseases are discussed and presented.

If we, as a scientific community, do not manage to be precise, accurate and evidence-based in further exploring intestinal permeability, we risk to dilute our scientific approach with the consequence of watering down and blurring a phenomenon that we just begin to fully understand.

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Author contributions

T Rath wrote the manuscript. R Atreya and MF Neurath critically revised the manuscript.

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