nexira
Innovation Inspired by Nature

How Fibregum™ reinforces the gut barrier

The effect of the gut barrier on overall health

The gut barrier is one of the most important components of the immune system. Its main role is to absorb nutrients and to serve as one of our body's most important barrier. It protects us from potential allergic reactions, as well as microbiological and chemical threats.

With the worldwide growth of bowel pathologies such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), the overall interest in gut health and more specifically the gut barrier has grown. Today, IBS and IBD are common digestive problems affecting both women and men. Nearly 20% of the world's population is impacted by IBS[1,2] and millions of people are suffering from IBD.

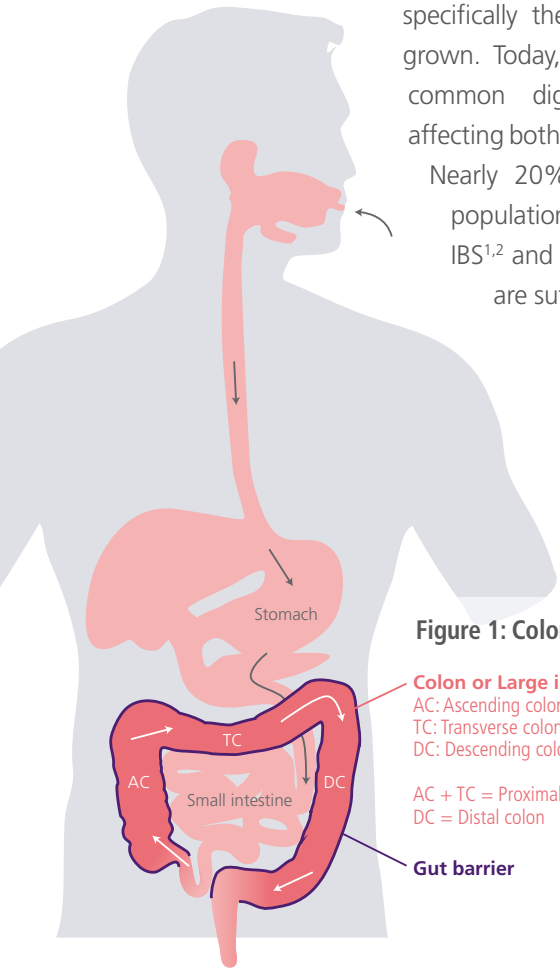


Figure 1: Colon and gut barrier

Colon or Large intestine
AC: Ascending colon
TC: Transverse colon
DC: Descending colon

AC + TC = Proximal colon
DC = Distal colon

Gut barrier

The incidence of impaired and increased gut permeability, also known as leaky gut syndrome (LGS), is now closely studied because of its potential involvement in many health issues and diseases, such as IBS, IBD and others (see appendix A).
The possibility of modulating the composition and activity of gut microbiota is considered to be a way to improve LGS and thus improve overall health and wellbeing.

Gut barrier functions

Our body is colonized by commensal microorganisms that interact with organs to maintain our natural barriers against external factors.

Our body disposes of two main natural barriers:

- Our first line of defense is our skin (our biggest organ). Here reside several microorganism communities that contribute to our immune protection.
- The second barrier is our intestine (figure 1), which is composed of a monolayer of epithelial cells sitting on a specialized extracellular matrix (intestinal wall). Our intestine is colonized by trillions of bacteria (microbiota). This barrier is involved in both innate and adaptive immune responses and is able to control the passage of nutrients, water, ions and macromolecules. The intestinal mucosa is constantly exposed to a vast array of microbes, food antigens and

Appendix A

What are the health implications of leaky gut syndrome (LGS)?

Much of the work in the area dealing with host-microbial interactions with host physiological processes are of great interest. Several studies have shown gut microbiota support energy metabolism and immune functions in the host. Furthermore, the concept of a "super-organism" has emerged.

This concept reflects the physiological importance of mutually advantageous host-microbe interactions. When gut microbiota equilibrium is altered, several gastrointestinal and extra intestinal diseases can occur. This may be explained because gut microbiota interacts with the immune system.

LGS can be caused by a number of different conditions that cause inflammation and damage to the intestinal lining, including infection, trauma from burns and surgery, and the use or overuse of many medications. LGS is associated with a wide range of general symptoms, such as fatigue, fevers of unknown origin, abdominal pain, bloating, and diarrhea, feelings of toxicity, memory problems, difficulty concentrating, and poor tolerance to exercise.

Some recent studies and experiments have considered the involvement of LGS in chronic heart failure[I], irritable bowel syndrome[II], inflammatory bowel diseases[III], alcoholic dependence[IV], diabetes mellitus[V], depression[VI], "sickness behavior", chronic fatigue syndrome (CFS)[VII], and other autoimmune diseases.

I. Krack A, Sharma R, Figulla HR, Anker SD., The importance of the gastrointestinal system in the pathogenesis of heart failure., Eur Heart J. 2005 Nov;26(22):2368-74.

II. Zhen Zhang Y. and Li Y.Y., Inflammatory bowel disease: Pathogenesis. World J Gastroenterol. 2014 January 7; 20(1):91-99.

III. Nahidi L, Day AS, Lemberg DA, Leach ST., Differential effects of nutritional and non-nutritional therapies on intestinal barrier function in an in vitro model., J Gastroenterol. 2012 Feb; 47(2):107-17.

IV. Leclercq S, Cani PD, Neyrinck AM, Stärkel P, Jamar F, Mikolajczak M, Delzenne NM, de Timary P., Role of intestinal permeability and inflammation in the biological and behavioural control of alcohol-dependent subjects., Brain Behav Immun. 2012 Aug; 26(6):911-8.

V. Vaarala O, Atkinson MA, Neu J., The "perfect storm" for type 1 diabetes: The complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. Diabetes. 2008 Oct; 57(10):2555-62.

VI. Maes M, Kubera M, Leunis JC., The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. Neuro Endocrinol Lett. 2008 Feb; 29(1):117-24.

VII. Maes M, Coucke F, Leunis JC., Normalization of the increased translocation of endotoxin from gram negative enterobacteria (leaky gut) is accompanied by a remission of chronic fatigue syndrome., Neuro Endocrinol Lett. 2007 Dec; 28(6):739-44.

toxins. Gut microbiota contributes to our immune system by allowing us to tolerate a large amount of antigens.

Gut microbiota + Intestinal wall
= **Gut barrier**

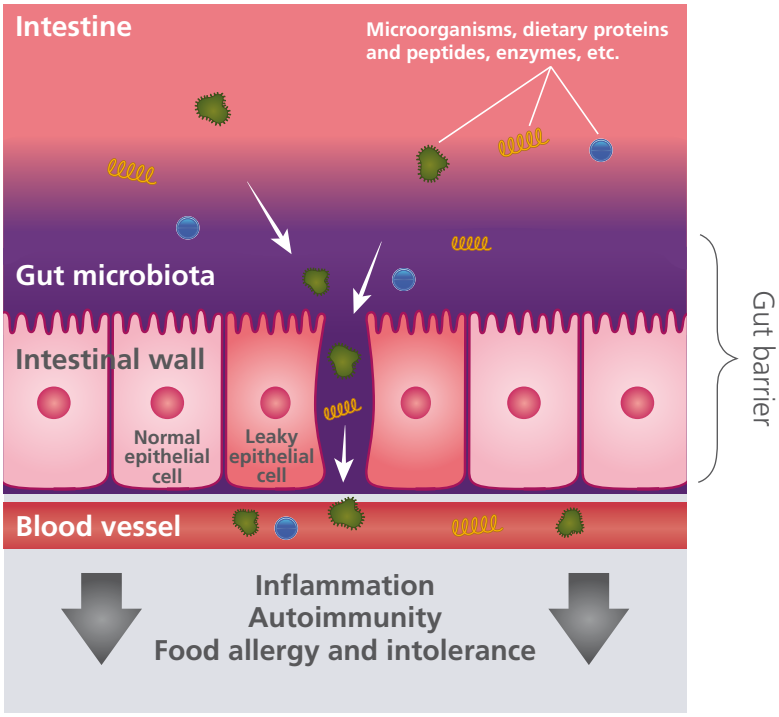
Day after day, one of the most important roles of this barrier is to discriminate between pathogenic and non-pathogenic compounds. Intestinal inflammation compromises the gut barrier and alters gut impermeability. Alteration of gut permeability, or impermeability, is known as leaky gut syndrome (LGS, figure 2). In this case, not all the "unauthorized" invasive molecules are blocked by the intestinal barrier, and some penetrate our organism.

As we have to take care of our skin, we have to take care of our intestine.

Benefits of a fiber rich diet and the benefits of acacia fiber

Fibers play a significant role in the digestive process. Amongst other multiple health benefits, fiber increases stool production, prevents constipation, and may have some preventive effects on colorectal cancer. Some fibers have shown a prebiotic effect.

Figure 2: Leaky gut mechanism



Fibregum™

Fibregum™ is an all-natural, GMO-free source of soluble dietary fiber, carefully obtained from selected acacia gum sap exuded from the stems and branches of acacia trees. Fibregum™ provides a guaranteed minimum of 90% soluble dietary fiber on a dry weight basis (AOAC 985.29 method). Fibregum™ is a non-digestible, high molecular weight carbohydrate composed of a proteinaceous core (1-3% of the dry matter), a polysaccharidic fraction (95% of the dry matter), and minerals (magnesium, potassium, calcium, and sodium). Fibregum™ has a very complex structure with an average molecular weight ranging from 300 to 800 kDa (kilodalton).

To understand the effect of Fibregum™ on the intestinal tract, Nexira has conducted an *in vitro* experiment on healthy donors. This study performed with the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®)[3] demonstrated the specific and progressive gut fermentation of Fibregum™ versus fructooligosaccharides (FOS). This study, as well as the clinical trial already performed by Cherbut *et al.* 2003[4], confirmed the prebiotic performance of Fibregum™. Most particularly the slow fermentation of Fibregum™ by the gut microbiota provides a significantly and interesting lower incidence of intestinal discomfort even at high doses (50g/day).

This gradual fermentation of Fibregum™ makes it highly tolerable and a very potent tool to increase the fiber content of diets, even diets for people that normally experience high levels of digestive discomfort.

Reinforcement of the intestinal barrier by Fibregum™

Based on the positive results obtained by those previous studies on healthy donors, Nexira, world leader in acacia gum, recently conducted new and ambitious experiments to demonstrate innovative physiological properties of Fibregum™ on gut permeability.
FOS was again used as the comparative reference and the same results with FOS supported the coherence with the previous experiments.

IBS and IBD subjects

The causes of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) and their subsequent development (see appendix B) involves intestinal hyper-permeability, inflammation, and the incidence of specific microbiota. This is the reason why donors with IBS and IBD were included in this study to investigate the loss of intestinal epithelial barrier function.

Appendix B

IBS and IBD are characterized by the following 3 main factors:

- Suspected factors impacting gut permeability

Chemicals, mechanical shock and pathogens are believed to disrupt intestinal barrier function resulting in activation of the immune system and leading to pathological inflammation.

- Inflammatory conditions

Pro and anti-inflammatory cytokines are important modulators of the immune response system and play a crucial role in intestinal inflammation. The most studied pro-inflammatory cytokines, with respect to gut inflammation, are TNF-α, IL-1β, IL-6, IL-8 and NF-KB/AP1. In a healthy body, to maintain homeostasis, inflammatory activation is counteracted by anti-inflammatory cytokines such as IL-10.

- Microbial conditions

In 2009, Lyra A. *et al.*, showed that It is possible to distinguish between IBS and IBD subjects ad control subjects by analyzing their intestinal flora.

As mentioned by Bixquert Jimenez M. in 2009, disturbance of intestinal flora may occur in patients with IBS justifying probiotic health treatments.

Those studies indicate the need to positively influence the microbiota.

SHIME & M-SHIME experiments

The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) is a device that has been used for experiments to mimic the entire gastrointestinal tract (GIT), including simulating the stomach, small intestine and the three regions of the large intestine (ascending, transverse, descending). Furthermore, plastic beads covered with a layer of mucin agar can be added to each of the compartments simulating the colon, as recently validated[7], to mimic the microbial processes that occur in the gastrointestinal tract of patients with ulcerative colitis (IBD). In this way, the device allows investigation of both the luminal and mucus associated microbial communities.

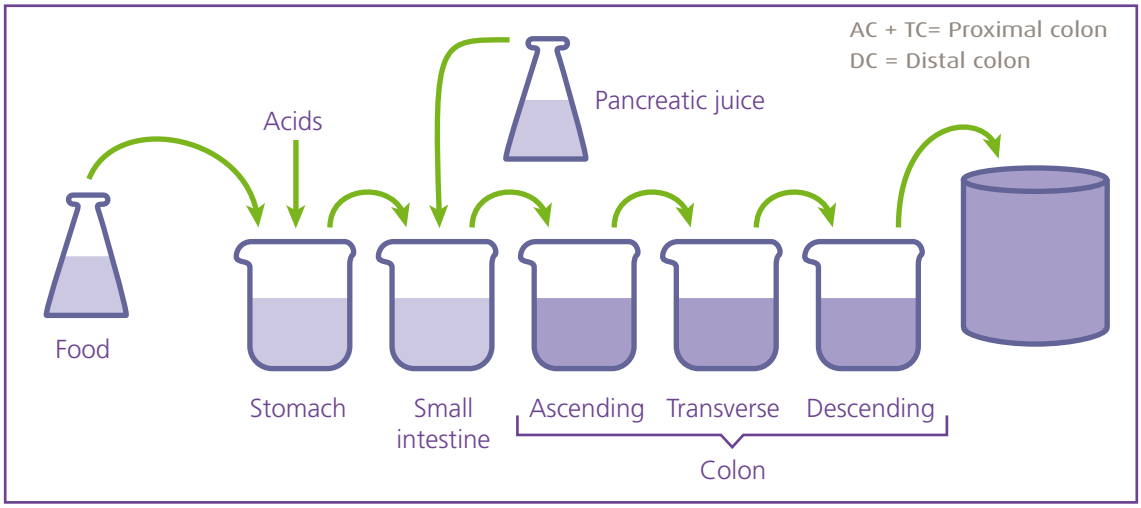
Effect of Fibregum™ on intestinal microbiota

Fibregum™ has been shown to increase the intestinal population of *Bifidobacteria* and *Bacteroidetes*, known to be commensal healthy *bacteria*, and more specifically the anti-inflammatory bacterium, *Faecalibacterium prausnitzii*[8].

Prebiotic dietary fibers have been shown to positively affect the intestinal bacteria responsible for beneficial short chain fatty acid (SCFA) production, specifically butyrate and propionate. The prebiotic effect of dietary fiber increases the growth of specific bacteria that have important physiological and health benefits.

- **Studies on luminal microbial communities** that do not adhere to the intestinal mucosa using the SHIME device:
  - In experiments with IBS patient donors, Fibregum™ has been shown to slightly increase total bacteria populations in each of the colon compartments of the SHIME device.
  - In experiments with IBD patient donors, Fibregum™ induced an immediate increase in the *Bifidobacteria* population in the distal colon compared to the control.
- **Studies on mucosal microbial communities** that adhere to and interact with the intestinal mucosa using the

Figure 3: Overview of SHIME® device*



**This device is a continuous model that allows the in-depth study of the biological activity of selected molecules in the gut, under representative environmental conditions and under long-term repeated administration conditions. This technology platform allows validation of the functional properties of food products in the human gastrointestinal tract, and the performing of mechanistic studies in areas of the gut that are not easily accessible in in-vivo trials, therefore providing useful complementary data.*

M-SHIME device:

- The population of *Roseburia* bacteria, which produces beneficial SCFA butyrate is increased in the proximal colon.
- More interestingly, the mucosa-associated and anti-inflammatory bacteria *Faecalibacterium prausnitzii* population is increased in the proximal and distal colon compared to the control.

Effect of Fibregum™ on short chain fatty acid (SCFA) production

SCFAs include acetate, propionate and butyrate and small amounts of other acids. Fibregum™ has been shown to induce an increase in total SCFA production, in experiments with both IBS and IBD patient donors. Fibregum™ mainly exerted a butyrogenic effect in the distal colon. These results support the conclusion that the distal colon is the main area of bacterial fermentation of acacia fiber.

Effect of Fibregum™ on inflammation

Under lipopolysaccharides (LPS) stimulation, experiments on pro-inflammatory cytokines involving IBS patient donors indicate that Fibregum™ completely inhibited NF-kB/AP1 activity by the end of the treatment. For TNF-α the trend was more fluctuating, and IL-8 secretion was mildly reduced. In a complementary way, the anti-inflammatory cytokine IL-10 was increased by Fibregum™.
Regarding the pro-inflammatory cytokines involving IBD patient donors, Fibregum™ produced the strongest decrease in NF-kB/AP-1 activity in the proximal and distal colon. Secretion of IL-8 and IL-6 were reduced in both the proximal and distal colon. IL-10 was increased in the distal colon.
In conclusion, these results demonstrate that the anti-inflammatory effect of Fibregum™ is based on two combined actions: inhibition of pro-inflammatory cytokines and stimulation of anti-inflammatory ones.

For values please refer to the following tables.

Table 1: Modulation assessed at the end of experiment

IBS	Ascending Colon	Transverse Colon	Descending Colon
NF-kB/AP1		-100%	-100%
TNF alpha	-62%	+29%	-40%
IL-8		-85%	-31%
IL-10	Induction	Slight decrease	

IBD	Proximal Colon	Distal Colon
NF-kB/AP1	-16%	-24%
TNF alpha	No induction or reduction	
IL-8	-12%	
IL-6	-32%	
IL-10	+32%	

Pro-inflammatory cytokines
Anti-inflammatory cytokines

Intestinal gut wall barrier modulation

Samples collected from the above study were used in a cell line model assay to assess potential gut wall modulation, specifically impermeability. Cell impermeability was assessed using two complementary methods: TransEpithelial Electrical Resistance (TEER) and Lucifer Yellow (LY) paracellular transport.

- For the IBS treatment, Fibregum™ had a protective effect on barrier integrity in the descending part of the device (mimic of colon descending part), as shown by an increase in TEER by 40% (figure 4). The LY paracellular transport method confirmed this result.
- For the IBD treatment, Fibregum™ enhanced cell impermeability, as shown by significant TEER increases of 65% in the proximal colon and 75% in the distal colon compared to the control (figure 5). The LY paracellular transport method confirmed this result.

Figure 4: IBS impermeability (TEER)

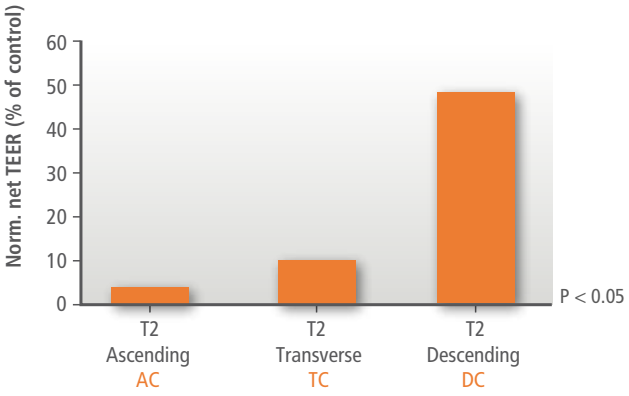
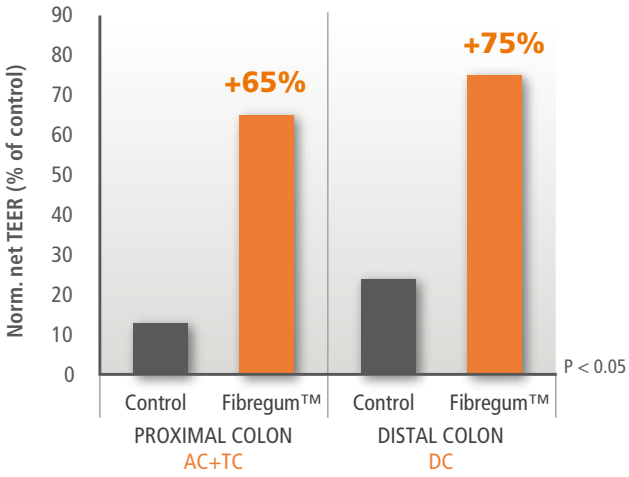


Figure 5: IBD impermeability (TEER)



Conclusion:

Fibregum™ reinforces the gut barrier

The study results demonstrated the innovative health benefits of Fibregum™.

It has been shown that Fibregum™'s acacia fiber is progressively and gently fermented in the intestinal tract[3,4].

The M-SHIME study confirmed the prebiotic properties of Fibregum™. It also showed positive effects by reducing inflammatory mechanisms and increasing gut barrier impermeability.

Fibregum™ has demonstrated that the unique combination of these activities reinforces the gut barrier.

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