

How Fibregum™ reinforces the gut barrier at the cellular level

Introduction

With the rise of some diseases like diabetes, obesity, high cholesterol, high blood pressure, Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD), consumers are increasingly attentive to their diet.

Consumers are reaching for more natural products. One of the food industry's answers is the "free from" products. Free from sugar, hydrogenated fat, salt, gluten, lactose, FODMAPs etc. Another answer is the product origin and its way of production (organic, GMO-free). Consumers are also looking for shorter and cleaner ingredient list (no synthetic ingredient, no additives...).

The other orientation is to adapt their diet and to consume more beneficial ingredients, such as fibers.

Nutrition, Fibers and healthy general wellbeing

The American Diabetes Association and WHO agree that dietary fiber intake should be near 25-30 g/day for a healthy life style. A study showed that mean daily dietary fiber intake for 2007-2008 was 15.9 g/day¹.

The advantage of a diet rich in fiber is not only related to the digestive process but also to a healthier intestinal tract. People who suffer from an unhealthy digestive tract experience uncomfortable conditions including heart burn, gas, constipation, diarrhea, nausea,

abdominal pain and flatulence. Digestive discomfort can be highly debilitating with a strong impact on overall well-being and quality of life. Fibers are considered to have some prevention effects on colorectal cancer and they also increase satiety sensation. Moreover fibers can enable additional positive health activities including colonic fermentation stimulation, reduction of cholesterolemia and reduction of postprandial glycaemia, as recently stated by the European Commission (Regulation (EU) n°2016/854).

Fibregum™



Fibregum™ is an all-natural source of soluble dietary fiber (able to dissolve in water). Fibregum™ is the only Non-GMO project verified acacia fiber.

It is obtained from carefully selected acacia gum sap exuded from the stems and branches of acacia trees. Fibregum™ offers a guaranteed minimum of 90% soluble fiber on a dry weight

basis, using the traditional testing method AOAC 985.29. It is a non-digestible, high molecular weight polysaccharide. It is composed of a proteinaceous core and associated polysaccharidic fractions. It is made up of neutral sugars and uronic acids (95 % of the dry matter), protein (1% to 2%, depending on the species), polyphenols (catechins, epicatechins, etc.) and minerals (magnesium, potassium, calcium, sodium). It has a very complex structure with an average molecular weight varying from 300 to 800 kDa.

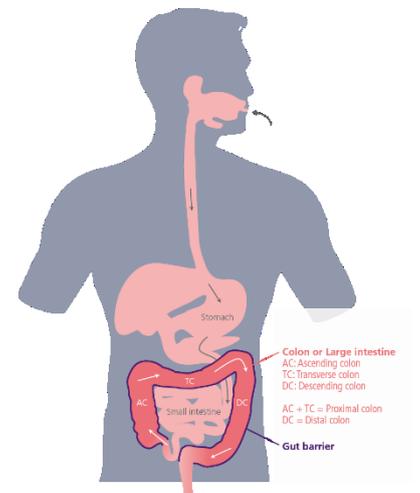


Demonstration of the comfortable digestibility of Fibregum™

In a study carried out in 2003 (Cherbut et al²) it was reported that Fibregum™ did not induce adverse gastrointestinal effects even when consumed at high doses, and demonstrated a bifidogenic activity. The study also showed a high digestive tolerance of Fibregum™. In this study, Fibregum™ did not induce flatulence below the dose of 30 g/day and daily doses higher than 50 g/day did not provoke any abdominal cramps or diarrhea. The study also demonstrated that Fibregum™ stimulates the growth of lactic acid bacteria from 10 g/day.

Progressive fermentation of Fibregum™

To further understand the intestinal tolerance of Fibregum™, Nexira conducted an *in vitro* experiment whose results were published in 2013³. Fibregum™ has been demonstrated to be gradually fermented in the distal colon (ie transverse and descending colon). It has been confirmed that Fibregum™ is only fermented by lactic bacteria in the large bowel. The study also showed that Fibregum™ was fermented in the transverse colon and that a residual part was still available for fermentation in the descending colon. It was also observed that an adaptation of the metabolism of bacteria increased their capability in fermenting Fibregum™ during the 3 weeks of the experiment.



See our previous White paper Fibregum™, a healthy, natural and gentle fiber with comfortable digestibility.

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. Our intestine is composed of epithelial cells sitting on a matrix (intestinal wall). Our intestine is colonized by trillions of bacteria (microbiota).

To summarize one can consider that:

Gut microbiota + Intestinal wall = Gut barrier

The incidence of impaired and increased intestinal wall permeability, also known as

leaky gut syndrome (LGS), is now closely studied because of its potential involvement in many health issues and diseases.

What are the health implications of the leaky gut syndrome?

Different conditions, such as infection, trauma from burns and surgery, and the use or overuse of many medications, can be at the origin of the LGS. Those conditions cause inflammation and damage to the intestinal lining.

LGS is associated with a wide range of general symptoms, such as fatigue, fevers of unknown origin, abdominal pain, bloating and diarrhea, memory problems, concentration difficulties, and poor tolerance to exercise. Some recent studies and experiments have considered the involvement of LGS in chronic heart failure⁴, irritable bowel syndrome⁵, inflammatory bowel diseases⁶, alcoholic dependence⁷, diabetes mellitus⁸, depression⁹, “sickness behavior”, chronic fatigue syndrome (CFS)¹⁰, and other autoimmune diseases.

See our previous White paper [How Fibregum™ reinforces the gut barrier](#).

Reinforcement of the gut barrier by Fibregum™, 1st experiment

In 2015, Nexira conducted new and ambitious experiments to demonstrate innovative physiological properties of Fibregum™ on gut permeability.

Effect of Fibregum™ on gut 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*¹¹.

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

Fibregum™ has been shown to induce an increase in total SCFA production and 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 indicated that Fibregum™ reduces the pro-inflammatory cytokines and, in a complementary way, increases the anti-inflammatory cytokines.

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 cytokines¹².

Intestinal gut permeability modulation

Samples collected during this experiment were used in a cell line model to assess potential gut wall modulation, specifically impermeability. Fibregum™ demonstrated a protective effect on barrier integrity as shown by an enhanced cell impermeability^{13,14}

Reinforcement of the gut barrier by Fibregum™, new experiments

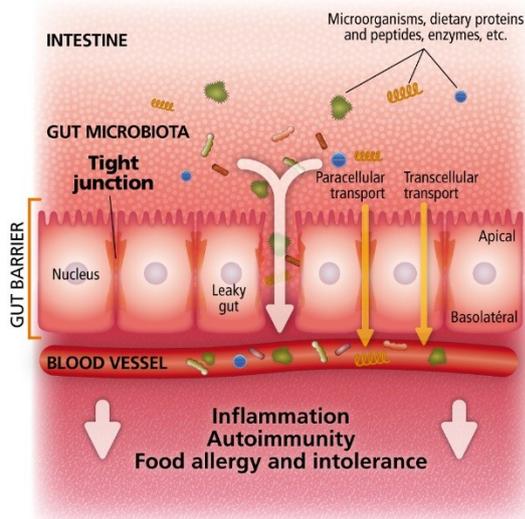
In 2016, in order to complete its scientific knowledge on the gut impermeability, Nexira worked with the INSERM (Institut National de la Santé Et de la Recherche Médicale) to carry out new experiments. This recognized scientific organization is the French national institute specialized in health and medical research.

These new experiments were dedicated to research the mechanisms of the gut impermeability restoration, at a cell level.

This second step was scheduled in Nexira's R&D program. Samples were made during the first experiment so results can be linked. Donors of this experiment were suffering of IBS and IBD.

Tight junctions

Tight junctions are different types of proteins (Claudin-1, Zonula Occludens (ZO-1) and Occludin).

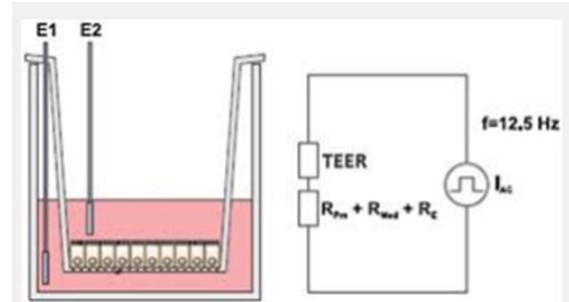


They consolidate the paracellular barrier that controls the flow of molecules in the intercellular space between the epithelium cells.

They are the closely associated areas of two cells whose membranes join together forming a virtually impermeable barrier to fluid.

Tight junctions are widely studied for their implication in the gut permeability in the IBD¹⁵ and for the involvement of the gut microbiota in their permeability¹⁶.

How to assess gut permeability

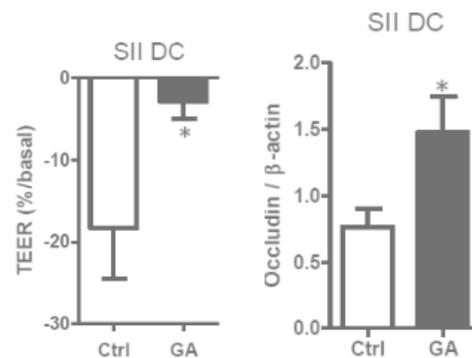


Trans Epithelial Electrical Resistance (TEER) measures electrical resistance through a membrane to assess the global quantitative impermeability. The higher the electrical resistance is, the more the cell line is impermeable.

Effects of Fibregum™ on gut permeability on IBS subjects

During the previous experiment, Fibregum™ fermentation products were sampled on the descending part of the artificial colon. By using two complementary technologies, TEER and FITC-dextran, it has been confirmed that these samples can significantly reduce the cell line permeability.

This activity was measured and confirmed by the two technologies.



During this experiment, the activity at the tight junction level has been assessed with the protein expression. The Claudin-1 and ZO-1

revealed a trend of increase. The Occludin increased significantly.

Furthermore, we measured an increase of the ARNm coding for the Occludin.

In conclusion, Fibregum™ has a positive effect by increasing the number of Occludin tight junctions and thus by improving the gut impermeability.

Tight Junctions	Increase (x)	P value
Claudin-1	1.8	NS
ZO-1	2.1	NS
Occludin	1.9	0.0381

NS: Non Significant

Effects of Fibregum™ on the inflammatory response on IBS subjects

The anti-inflammatory effect of the Fibregum™ fermentation has been tested both on the apical and basolateral sites of the cells.

The cytokines concentration at the basolateral level were too low to be measured.

At the apical level, IL-6 and TNF α , two pro-inflammatory cytokines, showed similar profiles. The IL-6 concentration has been reduced in the three part of the colon. The TNF α concentration has only decreased in the transverse and descending parts (see table).

In conclusion, the Fibregum™ fermentation, occurring in the transverse and descending colon, has a positive effect by reducing the pro-inflammatory cytokines.

	Pro-inflammatory cytokine reduction	
	IL-6	TNF α
Ascending colon	0.53 (p<0.05)	-
Transverse colon	0.48(p<0.05)	0.41 (p<0.05)
Descending colon	0.55(p<0.05)	0.53(p<0.05)

Effects of Fibregum™ on gut permeability on IBD subjects

By using two complementary technologies, TEER and FITC-dextran, it has been confirmed that the fermentation of Fibregum™ is able to significantly reduce the cell line permeability. This activity, measured and confirmed by the two technologies, has statistically significant results (p<0.05). However, for the IBD donors, the improvement of the gut permeability was only observed in the distal part of the colon.

Effects of Fibregum™ on the inflammatory response on IBD subjects

The anti-inflammatory effects of the Fibregum™ fermentation has been tested both on the apical and basolateral sites of the cells.

The IL-8, IL1 β and TNF α , all pro-inflammatory cytokines, showed a significant reduction (see table) in the distal part of the colon, at the apical level of the cells. Only a trend was observed for the IL-6. The anti-inflammatory cytokine IL-10 was increased in the distal colon.

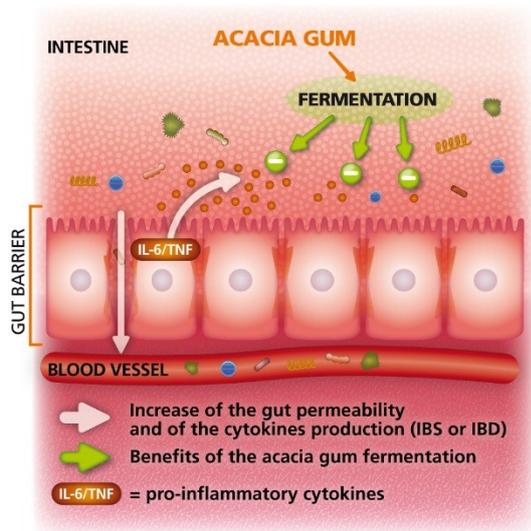
Fibregum™ has a positive effect on the inflammation by reducing the pro-inflammatory cytokines and by increasing the anti-inflammatory cytokines.

Cytokines	Expression (x)
IL-8	0.78 (p<0.05)
IL1- β	0.15 (p<0.05)
TNF α	0.36 (p<0.05)
IL-6	0.78
IL-10	12.7 (p<0.05)

Conclusion on the new experiments

This new experiment confirmed that the Fibregum™ fermentation in the distal part of the colon exerts beneficial effects on the improvement of the gut impermeability and inflammation. This experiment provided new

information on the mechanism involved at the cell level. It indicates that the Fibregum™ fermentation may exert its activity by reinforcing the tight junctions and by providing its anti-inflammatory effect at the apical level of the cells.



General conclusion

Nexira has conducted experiments on the acacia gum fermentation in the colon. The **Fibregum™** fermentation has positive activities by reinforcing the tight junctions and by providing anti-inflammatory effect at the apical level of the cells. These results bring new hypothesis. The acacia gum fermentation provides well-known products such as butyrate, acetate etc. This fermentation should also provide other products whose exact nature is still unknown. These other products are supposed to act directly at the cells level and on the tight junctions.

It has also been demonstrated a positive effect on the microbiota and the reinforcement of the gut impermeability. Hence, acacia gum is a very beneficial fiber with a high digestive tolerance.

To summarize, Fibregum™ presents powerful health benefits associated with a high digestive comfort that will allow its consumption by a wider range of consumers.

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Table of abbreviations

IBS: Irritable Bowel Syndrome

IBD: Inflammatory Bowel Disease

FODMAPs: Fermentable oligo, di- and monosaccharides and polyols

LGS: Leaky Gut Syndrome

CFS: Chronic Fatigue Syndrome

SCFA: Short Chain Fatty Acid

LPS: lipopolysaccharides

INSERM: Institut National de la Santé Et de la Recherche Médicale

TEER: Trans Epithelial Electrical Resistance

FITC: Fluorescein Isothiocyanate

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