THE GLOBAL GRANTS
FOR GUT HEALTH

Illuminating a neglected part of the microbiome

Studies on the small-intestine microbiota to shed new light on modulation of gastrointestinal diseases and glycemic responses and on host–microbiome interactions

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THE GLOBAL GRANTS FOR GUT HEALTH

2021 Call for Applications

A growing body of evidence has emerged in the last decade supporting the major role the human microbiota plays in health and disease. To encourage the development of this promising field, in 2018 Yakult and Nature Research launched a multi-year competitive grant programme for research into the human microbiota.

In 2021, the Global Grants for Gut Health will consider proposals for one-year research projects that advance understanding of the role of the early life microbiome in human health.

Applications close on **15th September 2021.**

Discover more at **guthealth-grants.com**

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LAYING THE FOUNDATIONS FOR TOMORROW'S SOLUTIONS

We warmly congratulate the three 2020 recipients of the Global Grants for Gut Health (GGGH), which support studies seeking to elucidate unexplored host–microbe communication pathways and develop new strategies for preventing metabolic diseases and functional gastrointestinal disorders.

For most of us, the past year was very different from others due to the COVID-19 pandemic. Labs were closed down, and researchers and students were obliged to work from home. Like many other meetings, the 2020 panel meeting for the Global Grants for Gut Health was held online. The panel was greatly reassured to discover that good ideas are still blooming despite the many challenges imposed on the global scientific community by the pandemic.

In 2020, we asked for applications that focused on the microbiome of the small intestine, which is less understood than the colonic and faecal microbiome since it is much harder to sample. We received a wealth of great proposals, and, as in the previous years, it was tough selecting the best ones. Here, we proudly present the three applicants and their projects that made it across the finishing line.

Marco Jost, member of the Faculty of Medicine, Harvard Medical School, USA, is seeking to achieve the ambitious goal of decoding the communication between gut microbes and the enteroendocrine system. The panel was highly impressed by this excellent proposal, which involves using a series of techniques that far exceed the present state of the art to generate knowledge that in the long term may help develop therapeutics that affect hormone-producing pathways and gut–brain signalling circuits.

Purna C. Kashyap, consultant at the Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine in the US sets out to investigate the role of the small-intestinal microbiome in modulating intestinal physiology and symptoms underlying functional gastrointestinal disorders. Such disorders, which include irritable bowel syndrome, are very common but are difficult to diagnose and treat. The proposal, which is original in its strategy to combine human work with rodent recolonization, seeks to alleviate an important but currently unmet clinical need.

Guido Hooiveld, assistant professor in Nutrition, Metabolism and Genomics at Wageningen University in the Netherlands aims to elucidate how the human small-intestine microbiota affects interpersonal differences in glycemic responses on consuming food. The panel found this an original and exciting proposal that could lead to new strategies for preventing metabolic diseases — a huge challenge in many countries where diabetes is approaching epidemic proportions.

Together with the rest of the panel, I am confident that these excellent projects will help to lay the foundations for future solutions in the battle against several diseases and conditions that are becoming increasingly prevalent and that are likely to be linked to features of our gut microbiome. I wish the three recipients the best of luck with their crucial work!

Finally, I'd like to say a heartfelt thank you to fellow panelists Eran Elinav, Paul W. O'Toole, Karen P. Scott, Kiyoshi Takeda and Liping Zhao for their excellent contributions to the evaluation process.

Tine Rask Licht
Chair of the independent evaluation panel for the Global Grants for Gut Health

Meet the panel

The independent panel is made up of internationally renowned researchers in human microbiota from across the world.

Tine Rask Licht
Chair of the independent evaluation panel for the Global Grants for Gut Health
Panel Chair

Eran Elinav
Department of Immunology, Weizmann Institute of Science, Israel

Paul W. O'Toole
School of Microbiology and APC Microbiome Ireland, University of Cork College, Ireland

Karen P. Scott
Rowett Institute, University of Aberdeen, United Kingdom

Kiyoshi Takeda
Graduate School of Medicine, Osaka University, Japan

Liping Zhao
Chair of Applied Microbiology at Rutgers University, United States; Distinguished Professor of Microbiology at Shanghai Jiao Tong University, China

DECIPHERING THE MOLECULAR LANGUAGE OF THE SMALL INTESTINE

Marco Jost will use small-intestine organoids and his expertise in RNA sequencing and CRISPR technologies to study host–microbiome molecular communication. He will examine how these interactions might influence the physiology of other organs.

Marco Jost is an assistant professor of microbiology at Harvard Medical School in Boston, US, where he set up the Jost Lab in April 2021. His postdoc with Jonathan Weissman and Carol Gross at the University of California San Francisco involved developing tailored CRISPR screening techniques, which he used to begin exploring the gut microbiome in collaboration with Michael Fischbach and colleagues. The Jost Lab will use single-cell RNA sequencing and CRISPR technologies to improve understanding of molecular communications in the gut and how this influences physiology throughout the body.

What will you explore in your Global Grants for Gut Health project?

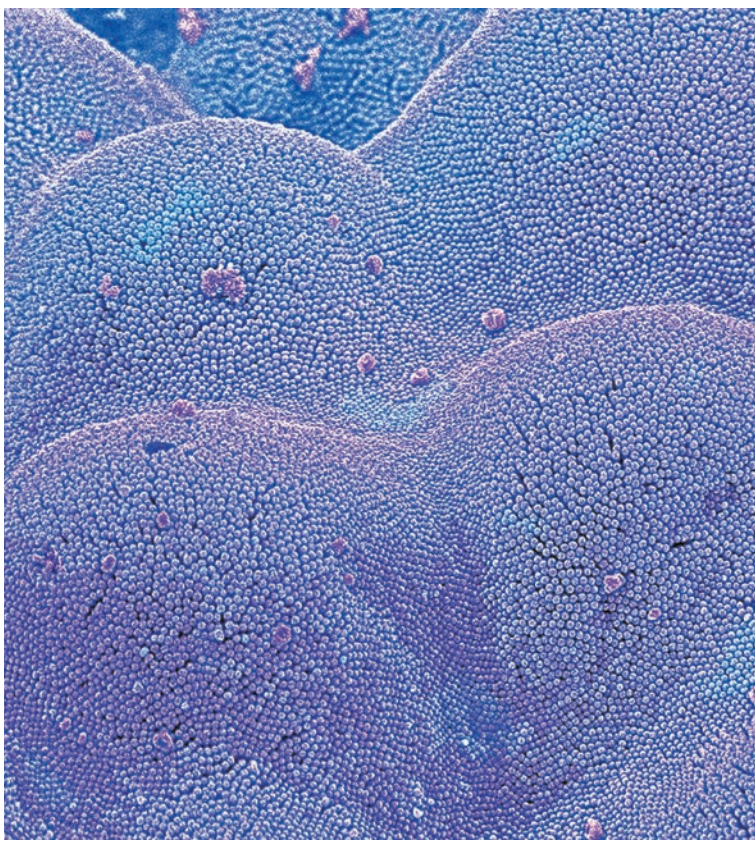
Our main goal is to begin deciphering the molecular communication between bacteria (and the small molecules they secrete) and host cells in the small intestine. We're particularly interested in enteroendocrine cells — cells found in the lining of the small intestine and throughout the intestinal tract — and how the small-intestine microbiome prompts them to release hormones. We also want to explore how this communication allows gut bacteria to alter physiology throughout the body. We know that gut bacteria affect biological processes in distal places such as the brain and skeletal muscles. I believe that bacterial communication with enteroendocrine cells is a major mechanism. These cells secrete hormones and neurotransmitters, and they also talk to neurons, sending signals across the body. We know there are physical interactions, where bacteria adhere to host cells. There are also chemical-signalling interactions, where intestinal cells sense and react to small molecules produced by microbes. We don't yet understand the full context or implications of this molecular language in the small intestine. We hope to begin probing which small molecules the enteroendocrine cells respond to, what produces these molecules, which receptors they bind to, and how that translates to changes in biological processes. Our hope is to provide valuable novel therapeutic targets, not just for metabolic disorders, but also for neuropsychiatric conditions, and disorders influenced by the microbiome.

What cells and molecules will you focus on?

The small intestine is an incredibly dynamic environment, and we know that diet is a key influence on small-intestine activity. We also know there is a rich diversity of enteroendocrine cells in the small intestine, and that the chemical landscape is very interesting. There are different types of enteroendocrine cells, each with its own specialized function. Most commonly, they each secrete a different hormone in response to external prompts such as diet. For this project, we're interested in two specific hormones secreted by small-intestine enteroendocrine cells: glucagon-like peptide 1 (GLP1) and peptide tyrosine tyrosine (PYY). Both hormones are released after eating, and mediate our sense of satiation, essentially telling us when we should stop eating. They also help regulate glucose homeostasis, and are key players in maintaining healthy metabolism. Disruption to these hormones has been implicated in metabolic diseases, and so it seems pertinent to start with these, given the considerable burden of metabolic disease on global health services at present. However, we don't know exactly what mechanisms are involved in inducing and regulating hormone secretion, which is what we will explore.

What steps will you take to achieve your aims?

We are starting at the molecular



Coloured scanning electron micrograph (SEM) of microvilli from the small intestine. These tiny structures help the cells lining the small intestine to absorb nutrients from digested food.

level, and from there we will add layers of complexity to learn more about the bigger picture. We will use a simplified version of the small intestine in the lab in the form of an organoid. An organoid is a three-dimensional, miniaturized model of an organ that mimics its activity and replicates the interactions that occur between and within cells. We will use small-intestine organoids to monitor interactions between host enteroendocrine cells and the small-intestine microbiota population. In particular, we will use single-cell RNA sequencing to probe how each individual cell type responds to bacteria and their associated small molecules. We will then use CRISPR screening to examine which receptors are responding to which signals, and define those factors involved in mediating host responses of interest — such as the secretion of the two hormones mentioned earlier.

Why is developing a small-intestine organoid helpful in this context?

Organoids are essential because it's impossible to grow most types of enteroendocrine cells in a standard culture. Organoids allow you to grow and enrich cells within their physiological context, and other groups have developed organoids models of all regions of the intestine. Organoids also allow us to look at responses of enteroendocrine cells at much higher throughput than mouse models, which many previous studies probing enteroendocrine cells have used. Organoids are constructed through the differentiation of human pluripotent stem cells — this is a targeted way of generating the main cell types of interest, which then assemble themselves into a functioning miniature organ. An important element to consider is that the small intestine is a rapidly changing environment inside humans, and it is influenced by a multiplicity of factors. My vision is to use our organoid *in vitro* model as a reductionist model — it's a starting point, but not a definitive end point. We can use it to identify key molecules and receptors before exploring them further in *in vivo* models in future.

How might the results inform future therapies?

We're designing our *in vitro* experiments to be as informative as possible, exploring a wide range of interactions, not just for therapeutics but also for improving our understanding of basic biology. But it's important that we also prioritize, which is why we're looking at GLP1 and PYY in this project. We might find specific molecules — bacterial or dietary — or specific receptors that trigger production of these hormones and we can then use classical drug-discovery techniques to explore ways of optimizing these interactions to improve glucose control, for example. Alternatively, we may find links between diet and host responses, which may lead to the design of dietary interventions. Longer term, there's the possibility of engineering microbes that could reduce or degrade unwanted substances, or optimize the activity of enteroendocrine cells in specific circumstances. This might give us more temporal and spatial control over the gut microbiome. Ultimately, we need big clinical trials on humans, but they require well-defined targets.

What are your hopes for future research?

Hopefully, this study will provide a blueprint for us to look at molecular communication in far greater detail, not just between gut microbes and enteroendocrine cells, but also between other cell types. My broader aim is to continue to develop techniques that enable us to decipher this complex molecular language and learn about how these communications shape us. ■

A PIONEERING MICROBIOME MODEL FOR THE SMALL INTESTINE

With his Global Grant for Gut Health, Purna Kashyap plans to create the first humanized mouse model of the small intestine to determine its role in modulating intestinal physiology and influencing gastrointestinal disease symptoms.

Purna Kashyap is a consultant physician at the Mayo Clinic College of Medicine in Rochester in Minnesota, USA. His research into functional gastrointestinal disorders is driven by direct experience of working with his patients, with the overarching goal of improving understanding of these complex, debilitating health conditions. He studies gut bacteria, dietary carbohydrates, and associated metabolites, and explores their influence on host physiology, with the aim of developing new biomarkers and targeted therapies for gastrointestinal disorders.

What drew you into gut microbiome research?

I work with people who suffer from functional gastrointestinal disorders and have a myriad of symptoms, many which change over time. These diseases are diagnosed based on symptoms rather than standardized tests, because we still don't have a good understanding of what is driving symptoms in different groups of patients. Each symptom can stem from the disruption of individual or multiple processes inside the gut. Recent studies have found these processes to be inextricably linked with the microbiome — the collection of all organisms (bacteria, fungi, viruses and parasites) and their genes in a given site. Understanding how the microbiome affects these mechanisms will transform how we help patients with these debilitating conditions. Our intestines are densely packed with bacteria that could kill us and yet live harmoniously within us. The intestines are very vascular; they have their own immune system and regulation mechanisms. But

Experiments with a Ussing chamber help to assess the effect of microbes and their metabolites on gastrointestinal physiology.

the ways in which changes in the microbiome result in changes in the functioning of the intestinal cells are still unclear.

Why study the small-intestine microbiome?

The microbiome of the small intestine is distinct from the colon, and likely plays important roles beyond digestion. An overgrowth of bacteria in this region, most commonly a result of slow movement often seen after certain surgeries, has been associated with weight loss and gastrointestinal symptoms similar to functional gastrointestinal disorders. Just like when you leave a cup of water sitting for days and it gets covered in microbes, the small intestine can experience microbial overgrowth, disrupting the delicate balance of that environment. We recently conducted a pilot study where we looked at small-intestine bacterial composition in patients where bacterial overgrowth was suspected based on their symptoms. We found that patients with symptoms like diarrhea, abdominal pain and bloating had very different small-intestine microbial compositions than those of healthy asymptomatic individuals.

Disentangling the causes and effects of these differences and what it means for functional gastrointestinal disorders is incredibly difficult; the complex nature of humans means that we need a simpler model. The advent of DNA sequencing technologies has allowed us to gain considerable understanding of the gut microbiome, but the focus has largely been on the colon, while the small intestine has been left behind. I hope to help change that by generating an effective mouse model that will allow us to investigate the biological effects of the small-intestinal microbiome.

How will you create the mouse model?

We will use germ-free mice — mice bred with no bacteria in their guts — to trial three methods of recreating the human small-intestinal microbiome in mice. Previous mouse models have had human stool samples implanted to recreate the microbiome, but we don't know if this accurately recreates the entire gastrointestinal tract, including the small intestine, or if it only represents the colon. Do we need stool plus small-intestine bacterial samples? Or just one or the other? We will try all three options to determine the best model for recreating the small intestine (ideally, the small intestine and the colon). Of course, there are challenges ahead in achieving this — samples are difficult to collect and the small intestine harbours fewer overall bacteria and fewer kinds of bacteria than the colon and these may not always survive the process.

What will the model allow you to observe?

We will look at how different small-intestine microbes affect gastrointestinal function, and how these mechanisms are influenced by dietary choices. The microbes depend on us to provide them with nutrition — they eat what we eat. We will measure how small-intestinal microbes respond to high- and low-fibre diets, and examine changes in the ability of the small intestine to release fluids into the lumen, in the time it takes for food to transit the small intestine, and in the small-intestinal permeability. I don't like the term 'leaky gut' — our guts are meant to leak to a degree, otherwise we couldn't absorb all the nutrients we need to survive. The problem lies in the gut becoming more permeable and allowing unwanted molecules to pass through. Both host cells and resident microbes have a vested interest in the gut staying healthy: the microbes don't want the host cells to attack them, and the host doesn't want the immune system to be constantly active. However, different diets provide different nutrients, which in turn can change the way these microbes behave. We plan to identify what metabolites the bacteria produce in response to diet and how they might contribute to gastrointestinal symptoms.

What do scientists know about how diet affects the small intestine?
Very little. We know that the small intestine and diet are intimately linked because the small intestine absorbs nutrients from our food. The bacteria in the small intestine have adapted to live in a fast paced, dynamic environment that differs greatly from the colon. Dietary fibre can change bacteria in our colon which has been blamed for symptoms like bloating but the effect of fibre on the small-intestinal microbiome and resulting effects on gastrointestinal function has never been studied. This is unexplored territory in terms of how small-intestinal microbial behaviour might be involved in generating symptoms. Our mouse model will allow us to examine the effect of small-intestinal microbes on different parameters of gastrointestinal function in response to diet. This is easier in mouse models because humans are so complex and you can't rule out the influence of factors like medication use or comorbidities. While several factors affect the small-intestinal microbiome, we will start with diet, because we expect it has the largest effect; but we will eventually have to tackle additional factors.

How might your results inform future treatments?
When we treat small-intestine bacterial overgrowth, we don't know which bacteria are the offending agents, so we use standard antibiotics to suppress their numbers. However, bacteria are smarter than us, and they can quickly develop resistance. Non-specific treatments can have many off-target effects and long-term ramifications in terms of promoting growth of antibiotic-resistant bacteria. If we can pinpoint specific microbes or microbial products that are driving symptoms, it would entirely change the current treatment paradigm. We have ways of delivering products to the small intestine separately from the colon; we can use strategies like phages, which home in on specific bacteria. We can also use narrow-spectrum drugs that are not as broad as antibiotics, or we can use healthy bacteria that are more efficient at surviving in the small intestine to try and outcompete the unhealthy ones in an effort to change the microbial landscape.

What are your hopes for your future research?
Patients with functional gastrointestinal disorders face increased morbidity, reduced quality of life, and even stigma associated with their disease with the suggestion that they are imagining symptoms. We want to show how specific mechanisms can drive symptoms in these disorders. Ultimately, this would validate the disorders themselves. The small intestine is uncharted territory, but it is also a place where I hope to find many answers. ■

EXAMINING THE INFLUENCE OF THE SMALL-INTESTINE MICROBIOTA ON GLUCOSE RESPONSES

Guido Hooiveld's proof-of-concept study, examining how the small-intestine microbiota influences different blood glucose responses when people eat the same foods, will be enabled by his Global Grant for Gut Health.

Guido Hooiveld is an assistant professor at the Division of Human Nutrition and Health at Wageningen University, The Netherlands. As a molecular nutritionist, he is interested in how the food we eat interacts on a molecular level with the microbiota, and how this in turn influences host responses and ultimately health. Previously, he focused on dietary lipids and regulation of their digestion and absorption in the body, before turning his attention to examining the differences in control of glucose responses in healthy and diseased states.

What inspired you to work in gut microbiome research?
Nutrition at the molecular level fascinates me. I trained in molecular biology and biochemistry, and initially focused on dietary lipids and regulation of their absorption by the body. Several years ago, DNA-sequencing technologies took us into a whole new realm of microbiome research, and I became intrigued by the differences in gut microbial communities between healthy people and people with metabolic diseases, and whether these could be linked to differences in processing of foods by the body. How nutrients are absorbed, how they are metabolized directly after eating — these processes differ greatly between healthy people and, for example, people with diabetes. It became clear that various microbial communities in the gut are also involved in different responses to diet — even to individual foods in a person's diet.

Why is glycemic control of interest?
Dysregulated blood glucose responses are associated with a higher risk of developing metabolic diseases such as diabetes. A seminal paper by Segal's group in Israel demonstrated that you could improve the prediction of a person's postprandial glucose response to different food types with considerable accuracy by including details of their faecal microbiome in a computer model. Previous predictions had only been based on a person's diet, BMI and so on, and were only about 30–35% accurate. This clearly suggested that the gut microbiome somehow influences glycemic responses at an individual level. The team also showed that if different people eat two foodstuffs containing the

same amount of carbohydrate — say bread and cake — some people will have high glucose levels in their blood after eating bread but not cake, or vice versa. No two individuals' responses were the same, even when they consumed the same food. This was something we also observed in our human studies. This all begs the question: why?

Why focus on the small-intestine microbiome?

The small intestine is an underexplored microbial organ, largely because it is very difficult to access in humans. Most microbiome research to date is based on faecal samples, but these are taken from just one end of the intestinal pipe and only tell part of the story. We know that people respond differently to different food products, and that glucose responses change in individuals depending on what they eat. Because the small intestine is the region of the gut where nutrients, including glucose, are absorbed, it is likely that the small-intestine microbiota is involved. This indeed is shown in preclinical studies. For our Gut Health project, we will focus on the role of the small-intestine microbiota in glycemic control in humans and how this differs between and within individuals in response to different dietary inputs.

What drives the rapid changes in the composition of the small-intestine microbiome?

It is multifactorial — from a bacterium's perspective, it has to grow. Bacterial species depend on the substrates, the food available in their environment — these are largely sourced from an individual's diet. The small intestine is a very dynamic environment; food passes through depending on the varying speed of a person's digestion, and the pipe expands and contracts. Other components, such as digestive enzymes or bile acids, may be harmful and impair the growth of some bacteria. All these processes determine which microbes are able to thrive and grow there over time, but we know very little about glycemic control and the small-intestine microbiota. A few studies have shown a correlation between glucose and the colon's microbiome, and animal studies have found that lipid absorption is manipulated by the small-intestine microbiota. But when it comes to carbohydrate absorption and associated glycemic control, this is new research.

What does your project propose to do?

We will screen for 70 participants that respond in very different ways to several standardized food products with the same amount of carbohydrates. We know from our previous work and published results which food products can produce such differential responses, and we hope to replicate our findings. The chosen foods could be bread, cake, cookies — it doesn't matter as long as the portions have the same carbohydrate load. What we need is each person to react differently to the two chosen products so we can pinpoint what drives individualized glucose responses.

In a subset of participants, we will use catheters to take multiple samples from their small intestines in the minutes before and hours after consuming each of the food products. We will measure the composition of each person's microbiome over time, the carbohydrates in the given food, and the person's glucose responses, and we will do this several times to check for reproducibility. We will see whether there are differences in each person's small-intestine microbiome composition in response to each food, and how this correlates with glucose levels in the individual's blood. This will provide fundamental insights into the role of the small-intestine microbiome. It is a small, proof-of-concept study, but if it works, we will expand on our findings in the future.

How might host responses be influenced by the small-intestine microbiome?

Differential glycemic responses could be driven by the fact that certain small-intestine bacteria can metabolize one carbohydrate source better than another. It could also be that different bacteria have differential impacts on host responses. To explore this, we will use human intestinal cell culture models that will be exposed to the small-intestine content obtained through the catheter, and see what effect these samples have on markers of glucose uptake and metabolism in the host cells.

What conclusions might be drawn from this project?

This is about gathering valuable data on an under-researched microbial ecosystem, and improving our understanding of what drives variable glucose responses. In time, we aim to verify the role of the small-intestine microbiota as an additional factor in gut-host responses, and provide concrete evidence that diet drives small-intestine microbiota composition and influences overall health. We hope to identify new microbial targets that could be used to improve glycemic control and give better, evidence-based dietary advice. There's so much misinformation out there in terms of nutrition; it's vital that advice is based on rigorous scientific evidence. ■

Samples of tomato juice being prepared. By comparing the responses of individuals to various foodstuffs, researchers can find out how the small-intestine microbiota influences glycemic responses at an individual level.

THE GLOBAL GRANTS FOR GUT HEALTH

Supporting innovative research into the human microbiome

A growing body of evidence has emerged in the last decade supporting the major role the human microbiota plays in health and disease.

To encourage the development of this promising field, in 2018 Yakult and Nature Research launched a multi-year competitive grant programme for research into the human microbiota.

2020 AWARDED GRANTS

Deciphering the molecular language of the small intestine

A pioneering microbiome model for the small intestine

Examining the influence of the small intestine on glucose responses

2019 AWARDED GRANTS

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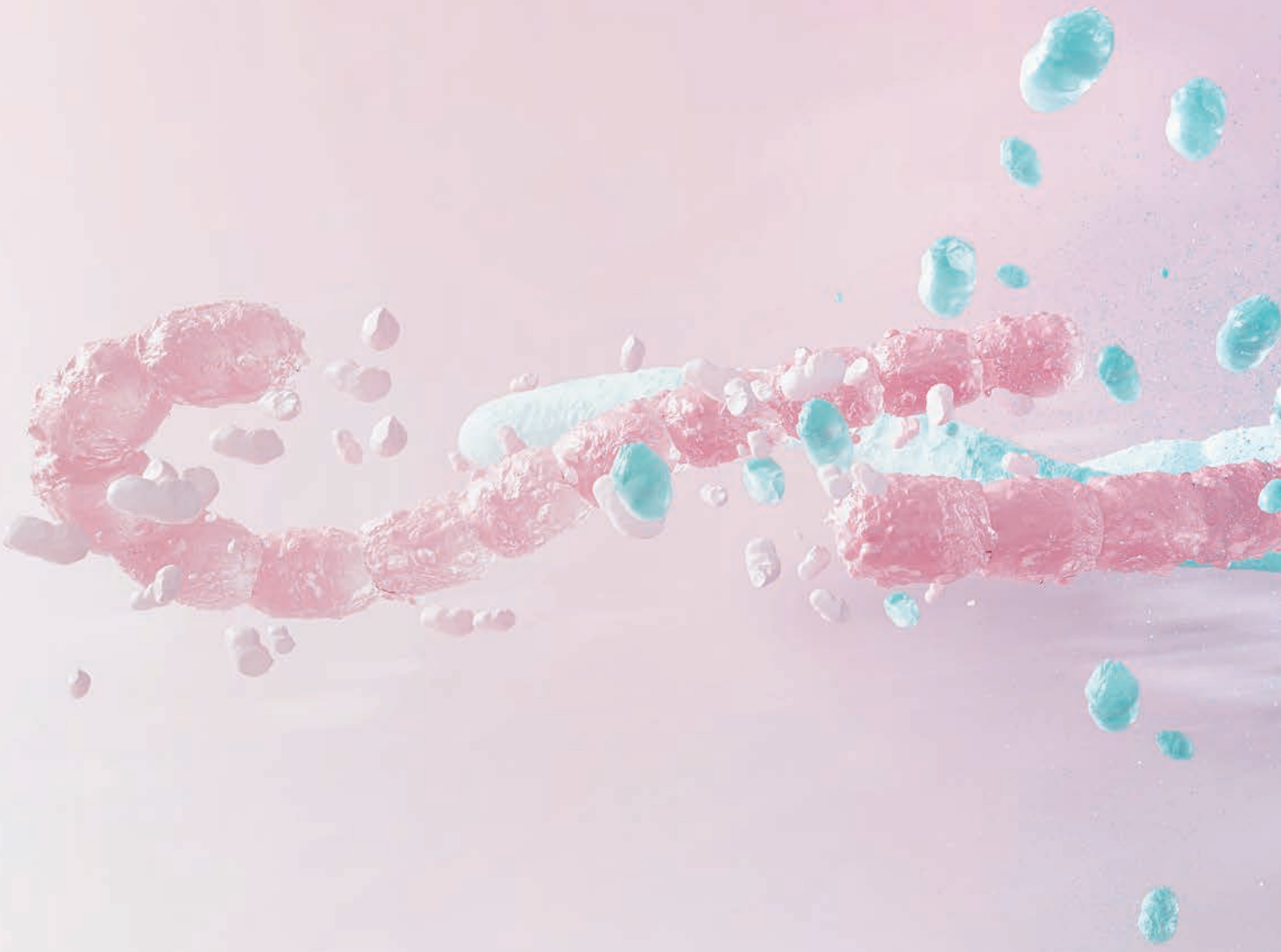

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