The Hidden Side of Health You're Missing: Dr. Sean Gibbons on the Gut Microbiome

Speakers: Matt (Interviewer), Sean (Guest) **Total Duration**: 83:18

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Matt: Today I'm excited to be joined by Dr. Sean Gibbons. Sean is an associate professor at the Institute for Systems Biology and one of the leading experts in the human microbiome. We'll be diving into the cutting edge of microbiome science, and I'll be asking him a bunch of questions. We've all heard about good bacteria and bad bacteria; is that a real thing? Antibiotics can have a very negative impact on your gut health, right? Where do you stand on probiotics? Are there general recommendations for probiotics? My two cents on that is I think you're going to find this conversation both enlightening and actionable. Let's jump right into it. Well, thank you for coming out to Optispan HQ today, Sean. It's really a pleasure to have you here. I thought maybe we could start kind of big picture and just talk about first of all what got you interested in the microbiome as an area of research, and then maybe even just to lay the foundation, let's talk about how do we even or how do you define microbiome.

Sean: Thanks, Matt, for having me. That's a big question. I'll go back in time a little bit. I'm from Montana originally; that's where I grew up, so I grew up around a lot of nature and ecology. I was always interested in ecology, and in undergrad, I started to think about microbes and microbiology and kind of putting the two together. My early work was actually tramping around Glacier National Park. We were digging up sediments from these streams that were flowing out from the park and characterizing the microbes living in those sediments and kind of cultivating new organisms that hadn't been seen before. I started playing in microbial ecology at that point. Then I moved on in my master's degree to do synthetic biology and genetic engineering of microbes for energy production and then moved back into microbial ecology in my PhD, where I again kind of kept focusing on the environment. I worked on what was called the Earth Microbiome Project at the time. My PhD adviser, Jack Gilbert, ran that project—everything from the bottom of the Mariana Trench to the top of the Himalayas. We were sampling from all over the world, but as a subset of that project, we did sample the human body, so we had some human microbiome in the Earth Microbiome Project. I kind of got drawn into that work, and then my postdoc with Eric Alm at MIT was focused exclusively on microbes in the human gut. It kind of became a model system that I got attached to and what I designed my trajectory towards in my faculty career.

Matt: When we talk about the microbiome, again, maybe you can just sort of define for people what do we mean by that.

Sean: Micro is small; biome is essentially a word for kind of an ecosystem, right? You have the tropical biome or the rainforest or the desert. So, microbiome is just a microbial ecosystem. In the human body, we actually have different terms. The microbiome usually refers to the genetic material within the microbial system, whereas the microbiota actually refer to the individual organisms themselves. It can be bacteria, it can be fungi, archaea, viruses, protists—any kind of microscopic single-cellular organism that we can't see with our naked eye.

Matt: Just to make sure I got it right, microbiome from a technical perspective, you're really talking about the DNA itself, the genomes there. Microbiota is the species diversity. I guess those terms now get used almost interchangeably in a lot of contexts, at least outside of scientific literature, but is that kind of what you were describing?

Sean: It's getting into the arcana of terminology in science, but there's omics, right? There's a lot of these omes—the proteome and so on. So, the microbiome is usually referred to as the nucleic acid sequence, as the way you're detecting those organisms. The microbiota is more when you're talking about, oh, I actually grew a cell from someone's poop and I have it in a dish, or you have a fecal transplant, and that material is the microbiota, right? The living, breathing cells.

[05:00]
Matt: I wasn't actually planning to go here, but your comment about Glacier National Park, looking for undiscovered microbes—one of my favorite science memories is, so my wife, for her PhD, was studying uncultivable microbes at that time. She was working at the seawater-sediment interface and trying to culture microbes there. When we were living in Boston, when we drove back to Seattle for our wedding, we got permits to go to Yellowstone and collect microbes from Yellowstone National Park. The idea that there are these extremophiles that are present there was super fun. It makes me wonder because we were married in 2000, right? So, this is our 25th anniversary. It makes me wonder, 25 years ago, the feeling was the vast majority of microbial diversity, we had no idea what was out there. Is that kind of still the current thinking, or are we really starting to understand what's there, both in the worldwide global environment and even within our bodies? Related, I guess, how much better are we at being able to culture these things in a controlled setting?

Sean: It's a great question. If you go back a couple hundred years, that's the beginnings of when we first started learning how to isolate organisms from the environment and grow them. You could only really see things that you could grow back then, right? Louis Pasteur and Badgereni and a few of these people who were doing this kind of work, they could characterize these organisms, grow them in different media, and kind of learn stuff about these microbes. In the 20th century, we realized that about 10 to one to 10% of the cells that we could see under the microscope ended up being cultivable; we were able to grow them. So, there's something like 90 to 99% of all those cells we couldn't grow; we didn't know how to do that. We didn't talk about microbiomes before 25 years ago because we didn't have high-throughput sequencing. It was only with that advent of high-throughput sequencing that we were able to peer into this dark

matter of the microbiome and see all these things that before were invisible to us because we just couldn't grow them. Now we're better; in certain ecosystems where we've spent a lot of time working on them, like the human gut, we're closer to maybe being able to grow half to 60 to 70% maybe of the different things. But then, other environments like the deep ocean or soils, we're still far away from being able to grow stuff. So, a lot of undiscovered microbes out there; at least maybe we've seen their DNA, but we've never actually seen a cell.

Matt: It's just super fascinating to think about, but I think if I hadn't gone into longevity, maybe I would have gone into microbes that we can't culture yet. I think there's probably a lot of interesting biology, but then probably molecules that these bugs produce that could have therapeutic value or other interesting properties.

Sean: That's a gold mine. Think of all the new drugs and new therapies that exist in that dark matter that haven't yet been discovered.

Matt: Cool. Okay, so here's an easy question for you, maybe. We've all heard about good bacteria and bad bacteria; is that a real thing?

[10:00]
Sean: Well, there are pathogens, right? There's the unequivocal bad guys that are out there. But even the pathogens, like take Vibrio cholerae, for example, it's unequivocally a pathogen to humans, but it lives in the environment; it has another life cycle where it's sitting in water, essentially swimming around. Many of these pathogens that are really bad, we've labeled those as the bad guys. So, for sure, there are a set of bad guys. Then there's a set that are on the fence; they're opportunistic pathogens. They only maybe cause problems when you're immunocompromised or when there's some issue. Things like Clostridium difficile fall into that camp. A lot of infections you get when you take antibiotics or have some sort of bone marrow transplant, those kinds of organisms are on the fence, in between good and bad. They can be one or the other depending on context. Then there's a set of commensals, which the word commensal just means that they're getting a benefit from us, and they're not costing us anything. Most of our microbes you can put into that bin; they don't really harm us, but they're not really helping us. Then there's a small subset of those that are necessary for our health, kind of mutualistically entangled with us. It's even then not that clear-cut because you can take some commensals or even some mutualists, but on certain dietary backgrounds or in other weird contexts, they can turn against you in some ways and cause problems. So, it is really kind of how you have to integrate things at a system scale to understand what the functional consequences are of a given microbe at a given point in time and place.

Matt: I think that's a really important concept, right? That it is really even a system of systems interacting within our bodies. We haven't talked yet, but there are even different microbial communities within our body, right? We talk a lot about the gut microbiome, but there's a skin microbiome, an oral microbiome, a vaginal microbiome, probably three or four others, right? That are all interacting with the physiology of our bodies, and the way they interact is also

dependent on the environment that we're in, and diet's part of that. So, how do you think about a systems biology approach to studying these microbial systems? Maybe that's too broad of a question, but I'm not really sure how to ask it in a more precise way.

Sean: No, it's a good question. It's framed well. I think you can break it down into the old way of thinking and the new way of thinking. Biology was so complex that a hundred years ago, there was no way you could integrate things at a system scale; you couldn't put all the pieces back together again because we didn't even know what the pieces were. The molecular biologists of that century focused on diving down into a single protein or a single gene and dissecting its function and coming up with this huge parts list, so knowing what are all the individual constituent parts of the system, take them all out one at a time and look at them. But we know that if you're taking a car apart or you have some big complicated machine, any individual piece is not going to behave as it does outside of the system as it does in the system. Towards the end of the 20th century or the beginning of this century, we began to realize we picked the low-hanging fruit that we could pick, and we realized that a lot of what we understand about biology is limited by the fact that we haven't put it all back together. This is at this time people like Lee Hood, who's the founder of our institute, was thinking about how do we stitch it all together again and get this more complete and more emergent picture of the functionality of biological systems. What enabled that is data. We were able to collect omics, like I mentioned before. You can measure the small molecule metabolites with metabolomics or the proteins with proteomics or the DNA with shotgun sequencing, and with all these methods that you can measure from the same system together at once, you can suddenly assess all the different layers of the system at the same time. Then the trick is putting Humpty Dumpty back together again, and that's the kind of computational and analytical toolsets that have been built over the last 20 or 30 years to start to enable that process. Our lab focuses a lot at this interface: how do we take this parts list and stitch it together into an emergent picture of what is the functionality of the system.

[15:00]
Matt: When you try to do these types of studies, are you, one of the places you're looking, is it the fecal microbiome, right? What are the things that you measure in that case? So, you can measure DNA, right? Which bacterial, fungal species are there and in what prevalence? You can measure metabolites, metabolomics. You could potentially measure RNA. So, what are the kinds of things, when you think about trying to construct this system, what's all included in the different things that you can measure there?

Sean: That's a five-hour conversation if you want it to be, right? I think you really have to constrain what your question is and what your system is. I'll stick to the human body, and with a fecal microbiota, if we wonder how is it influencing our physiology or the rest of our body, you definitely want a snapshot of who's there. So, you take stool, you can sequence the DNA there and get a snapshot of all the genomes of all the organisms that are living in that stool sample. It turns out, for any individual human, that's very stable over time, so your microbiome today is

going to look like it does tomorrow and maybe a year from now. It changes a little bit with diet and lifestyle, but it's frustratingly hard actually to shift.

MattIs it the case that antibiotics can be something that has a major shift, so independent of if you take a course of antibiotics, relatively stable? I would assume that's if your lifestyle stays relatively stable, is that correct?

Sean: Yes. If you take antibiotics, you definitely tank the biomass of your microbes in the gut, so you see this big perturbation; their biomass goes way down. But as soon as you stop taking those antibiotics, they bounce back within a couple of weeks. The question is, does it come back the same, or does it come back different? Because there's this perception at least that antibiotics can have a very negative impact on your gut health because of changes to the microbiome, microbiota.

Sean: Yes, and there's a lot of reasons there. So, when you perturb the system, when you smack down the commensals, a lot of the metabolic resources that are normally not available become available, so opportunistic pathogens have a heyday. If you happen to be exposed to a C. diff spore during one of those periods, it's going to get in and start to stick and grow, and then even after you recover, it's still in there at some low level. Then, if you got another round of antibiotics later, it can cause disease. A lot of opportunistic pathogens can get a toehold in these transient periods of disruption, but by and large, most of the organisms that were there before come back; you bounce back usually. The more healthy your diet is, the more dietary fiber you're eating, the faster and better you tend to bounce back. There are subpopulations of microbes in the gut that are kind of smaller; a good example are Bifidobacterium species. This is a group of organisms that specializes on dairy, so babies tend to have a lot of this in their guts; they help us digest breast milk. Many of us keep this bug throughout our life. In fact, most humans lose their ability to digest lactose as they become adults; there's a couple of small subpopulations in Scandinavia and Africa who have that gene turned on throughout their whole life, but most of us don't, so almost all of us are lactose intolerant, but functionally, most of us can still eat lactose, and it's because we maintain a population of bifidos in our gut. But it's not a big population, and when you smack it, sometimes it goes extinct and can't come back. People have frequent experiences of when they go on vacation and get food poisoning or they take a round of antibiotics, suddenly they can't tolerate drinking milk anymore. That kind of sudden intolerance is driven by extinction events that can occur, and those extinctions can compound over time as you take more and more rounds of antibiotics, so you can erode and degrade your microbiome. So, if you suddenly become lactose intolerant after a round of antibiotics, that's why.

Sean: Okay, and the way to come back from that, so a lot of people think you can take a probiotic because you can buy bifido at the store. But if you're not also eating dairy, you can't keep it, right? Because there's no food for them to eat; they're just going to wash right out. So, I think most people think, well, they'll first take the probiotic, and then they'll stop, and then they'll

take the dairy. No, try to take a little teeny bit of dairy and like titrate it in and work your way back up to levels that you want.

Matt: Okay, cool.

[20:00]
Matt: One of the things that I think is interesting, and there's been several papers on the role of the microbiome in certainly health generally, longevity sort of broadly speaking, but let's start with, like, what do we know about how the microbial communities in our body, and we could focus on the gut if you want to, but if you want to talk about other microbial communities as well, I think that'd be interesting, but how they change with age and why they change with age? What do we know about that?

Sean: Thinking about these other ecosystems on the body just quickly, you can think of the human body as a donut; it's a torus, and so there's a hole going through the middle of us, and all of that outer surface is covered in bacteria, whether that's our skin, our mouth, our gut. But the colon has about 99% of all the cells of microbes in the body, so it is by mass the vastly dominant over the others. There are important things happening in the other places, but I'll stick to the gut for now. So, how does a gut change throughout the lifespan? It's very dynamic early in life; you're actually born sterile, you have no microbes in your mother's womb. As you come out, mom is your sourdough starter culture, if you will; she gives you your first dose. But then we see a lot of strain sharing between the father and the mother and the siblings within a family, anyone who's socially interacting with that child. It's very plastic and formable in those first few months. You see a lot of these bifidos that are specializing on milk, but as you transition to solid food, the microbiome starts to kind of solidify and crystallize into an ecological composition that is very unique to each individual; even identical twins actually show very unique compositions. Once that kind of solidifies into an adult-looking microbiome with solid food intake, it stays pretty darn stable for multi-year periods. There can be perturbations that push you off a cliff, and occasionally people have big turnovers, but most people stay very stable on many-year timescales. Then over decades, you do see a bit of a drift, so there's a little bit of change happening every year; a few strains come in, a few strains go out, but most of our adulthood is characterized by stability. Then around 40 or 50, we start to see signatures of later aging changes in the gut. Our lab has actually shown that there are different patterns in healthy people; you actually see that there's a lot of dynamics and change as you age in the later decades of life. In less healthy people, you actually see that they hold on to a microbiome that looks more like a younger person's, counterintuitive even, huh?

Matt: Do we have a feel for why that is, or do you have a hypothesis? You must have a hypothesis.

Sean: We do, so we can kind of tell what's going on. In that study, we calculated a value called uniqueness, and it's sort of how similar are you to everyone else in the population, and that number gets bigger and bigger and bigger as you get older. You're drifting away from everyone

else, right? What's driving it is there are a few core abundant organisms that are dominant in youth, and they tend to be gram-negative anaerobes like Bacteroides or Prevotella. These organisms are declining in their dominance through time in these older folks, and the other taxa that they harbor in their guts, they tend to be kind of clostridia, gram-positive anaerobes. We tend to have different sets of these; we all have our unique set, and these are rising in dominance in that group. So, the decline in the core taxa and the rise of these non-core taxa seems to be driving this uniqueness signature that people are drifting apart.

[25:00]
Matt: I guess the question in my mind is, why is it that the people who seem to be in a poorer health status are maintaining the youthful microbiome, for lack of a better way of saying it, right? Is it because there's something adaptive that's happening in the healthier population that's then allowing or causing this change in microbial diversity, or I don't know? Again, it's super interesting, but not what you would predict, I think, in the absence of actually doing the experiment, right? In fact, most of the field is focused on rejuvenating the microbiome and maybe doing fecal transplants from young people to old people, which is also super interesting.

Sean: It is interesting, but if you look at the mouse studies and the human studies, I would actually suggest against that; it might do more harm than good. Here's a few things we think are going on. The human body is changing as we age; one thing that changes is the crypts with the stem cells that generate our enterocytes, right? We're getting fewer and fewer stem cells with aging, and so you get less production of goblet cells, which are the mucus-producing cells in the lining of the gut, so our mucus layer production is going down. A lot of these gram-negative anaerobes that are dominant in youth, they are facultative mucus degraders; they can eat dietary polysaccharides, but they can also switch to eating you. So, if your dietary intake is going down or if your fiber intake is going down, they will actually start to erode that mucus layer, and if your production of mucus is going down and you're maintaining these organisms that are capable of degrading that mucus, that thinning of the mucus layer would drive inflammation, which is definitely a positive feedback on aging. That's one piece that's going on. The other piece that's going on, and I should also say with aging, there's also a bit of a decline in appetite, so the bulk amount of dietary substrates going in is kind of going down a little bit. The clostridial species, the gram-positive firmicutes, these organisms produce certain kinds of organic acids like butyrate, which is very healthful and anti-inflammatory. We find, if we have some modeling that can simulate the microbiome and predict this butyrate production, we see that people with more unique microbiomes who are older, their butyrate production capacity is increasing as well. So, it could be that the production of this healthful organic acid is also kind of helping to counteract the inflammaging that's happening with getting older.

Matt: You made a comment about how you might predict that fecal transplants from young into old could be counterproductive because that would, in principle, cause the healthy older microbial composition to look more like the composition that in unhealthy older people is the situation, right? But there is a little bit of data, and again, it's not many studies, I think, in mice on these sorts of transplant experiments and potentially showing some benefits.

Sean: I'm actually very familiar with this work. That's out of John Cryan's lab; there's a paper on fecal transplants from younger mice into older mice. They didn't do the control where they did the inverse, right? From older mice into younger mice. There are papers that have done that as well. So, what I'll say about that Cryan paper is, yes, they did a huge battery of tests, essentially, and measured all kinds of cytokines, and they were able to tell a story of some sort of benefit from this younger transplant. I would also point out in that paper, there's also signatures that maybe it wasn't so great. IL-10, which is this kind of anti-inflammatory cytokine, it was actually, it went down with this transplant. There are a few other studies showing that older mice transplanted into younger has a physiological benefit. There's a recent one on menopause, looking at post-menopausal mice transplanting their feces into younger mice actually increased their fertility and reproductive capacity, whereas from a matched young mouse, it didn't. So, it's a little equivocal in the mouse literature, and no one's really done it in the human side. I would also suspect that for some people, your microbiome might just be so disrupted through drugs or what have you that they would benefit from any fecal transplant from a healthy person, right? But maybe, on average, a younger, a 20-year-old microbiome maybe shouldn't be in a healthy 80-year-old's gut.

[30:00]
Matt: That sort of leads me to one of the things I was wondering about when you said that in most people, the composition of the fecal microbiome is fairly steady throughout adulthood. How dependent is that on people maintaining a similar environment, you know, diet, exercise regimen, like, if people make a big change in their diet, let's say, do you then, in that case, often see significant changes in the microbiome, or is it still, is it still sort of set, and then it's pretty much set for you more or less independent of things that you may change about your lifestyle factors?

Sean: I think there's some that we don't quite know how to answer that question totally yet. What we can observe is that even with radical dietary shifts over the short term, it seems to be hard to budge the microbiome; that's at a global level. I assume there are specific microbial species where you do see wiggling.

Matt: If you make dietary changes, you can wiggle it around on the margins. Is that even with, like, a big shift in fiber intake, even switching from, like, a Western diet to a high-fiber diet?

Sean: You can, it's kind of hard to actually drive a global ecological change. A lot of that is driven by the fact that there's strong priority effects, so if populations are established, it's really hard to dislodge them. There's also kind of nonlinear things going on where sets of taxa sort of stabilize each other's existence in the system, and you might need to knock out a few to get rid of that set to replace them with something else. So, part of it is understanding what the niche space is and where the holes are in it, and if there's holes to fill, you can just fill them, but if you have to maybe subtract certain things in order to put in another set of things that live in the same niche. So, this is getting kind of in the weeds, but longer-term interventions do push your

microbiome around. There's great work from Dan Knights' group where he looked at Thai immigrants, I believe, so people sequenced who are living in Thailand and people who've been in the US for a few months, a few years, first generation, second generation, and third generation immigrants, and the longer you are here in the US, the more American your microbiome looks. So, that does happen, but it happens over months to years.

Matt: I do wonder too whether it's a matter of, let's make the assumption, and tell me if this is completely wrong, but I'm going to make the assumption that the microbiome is important for some aspects of health. I assume you would agree with that. Maybe it's just a matter of, at the global level, the changes are relatively small, but if you know which specific changes to look for, those could be biomarkers of, or maybe even mechanistically involved in, changes to health in response to, say, going from a low-fiber, low-quality diet to a high-fiber, high-quality diet.

Sean: Totally. The Bifidobacterium is a good example there because it's not going to make up that many cells in the population; it's going to be a small component of the ecosystem, but its presence or absence will have a big phenotypic effect on your ability to eat dairy, right?

[35:00]
Matt: So, related to that then, I guess a question would be these microbiome tests that are out there; let's just talk first about the direct-to-consumer tests, right? How useful do you think those are at detecting the meaningful changes, and how do they even work? Like, what is typically done in these microbiome tests that will tell you your good microbiome and your bad microbiome and then give you recommendations? Like, are those useful?

Sean: There's different camps of these companies, and some are more science-based than others. For many of them, I actually can't tell you much about what they're doing because they're not transparent about it; it's hard to say, and there's no published under the hood. You don't know what they're actually, how they're doing it, what they're measuring. I can kind of infer a little bit; it seems like a lot of them are just kind of looking out at papers that were published and saying, well, this organism was correlated with this thing in this population, and so that means you should eat more broccoli. It's kind of hard to draw the line between how they're making those connections; there's no underlying model per se. Other, more, I would say, gold-standard companies have some kind of science that is the foundation of their prediction. So, one coming out of Israel, Aron Segal's group, had a study, I don't know, 10 years ago at this point, where they had a bunch of Israelis eating various foods over a period of time, they had their microbiomes sequenced and clinical chemistries taken and a few other measurements, and they threw all that into a gradient-boosted decision tree and a machine learning model and were able to predict post-meal glucose response to a given dietary input given their microbiome composition. It worked pretty good, like, what was it, 40% of variance explained or something like that, and that became a company, a company called DayTwo, which sold the service of sequencing your microbiome, running it through their AI model, and then predicting for a given food that you would eat what would be your sort of glucose spiking that would occur. It, they demonstrated that it worked compared against, say, the standard Mediterranean diet, and they

beat the Mediterranean diet. For most people, they did better on the precision diet. Sadly, that company's gone out of business.

Matt: So, when you say they beat the Mediterranean diet, what do you mean? Is this in terms of, like, being able to, for an individual, say, we can recommend a diet that will reduce glucose spikes and then just as compared to across the board, everybody should eat a Mediterranean diet?

Sean: Exactly, yes. They had some people treated with a Mediterranean diet, some with the, no, it was a crossover trial, so the same people got both diets, the precision and the Mediterranean, and they did that area under the curve for glucose, and they found that the precision, for nine times out of 10, the precision beat the Mediterranean for reducing that glucose spiking.

Matt: Interesting.

Sean:So, but yes, that, and then there's a company called Zoe that's still in existence, and that's in the UK, and they have a similar machine learning approach to predicting kind of lipid profiles in the blood, and they do a fairly good job. But the other companies that I know of, they're all, it's entertainment; it's fun to kind of see what's in your gut, but what, the advice they're giving you, I have really little confidence in what they're saying.

Matt: That's what I was going to ask, I guess, is, outside of maybe these rare cases where you might be able to predict a dietary pattern that is going to be better than the Mediterranean diet for glucose response or lipids, is there, in your view, at least actionable information that you can get from a generic microbiome test? I understand there are certain people with certain gut issues where you can go look for a certain signature that can be predictive of a specific condition, but outside of those cases, do you feel like from a sort of global microbiome profile that you can get to personalized, like, you should take this supplement, or you should not eat this food, or do you feel like we're there yet, I guess, is the question, recognizing it's a moving target and all that stuff?

Sean: It's just opinions, right? Well, no, there is no FDA-approved microbiome diagnostic, right? So, in terms of, like, healthcare-reimbursable stuff, that doesn't exist yet because the evidence base doesn't exist for such things. In the more wellness space, which is less regulated, just a little, there are things that kind of work. I talked about a couple of these companies that have reasonable products. We are thinking about ways of doing this ourselves, and that boils down to building metabolic models of microbial systems, kind of making a digital twin of someone's gut in silico that you can feed different dietary inputs or probiotics and then ask how is that affecting the output of high-value metabolites like butyrate that you might be interested in turning up. That's all been partially validated at this point, but what now is needed is doing prospective human trials to actually prove that it works prospectively. Then I would also suggest, if you really wanted to do it to the best quality, you would also want to be certain that for this person, turning up butyrate actually is beneficial, right? So, there's some assumptions that go along with that.

But I certainly also recognize we can't wait for all of these things to be done at the highest level because if you do that, you're never going to get there. So, it's a balancing act.

[40:00]
Matt: Do you take microbiome tests to guide your own life? In your own lab, either your own or other people's, you don't have to say which ones, but...

Sean: Well, we're in the process of doing this modeling stuff, right? I have sequenced my microbiome, and we've run it through these models, and there's things I can learn from that. Maybe I'll change what prebiotic I'm taking.

Matt: Do you want to share what prebiotic you're taking?

Sean: I've sort of floated around. Generally speaking, I have a pretty healthy diet; I eat mostly a plant-based diet, although I do eat some meat. I'm an omnivore, but mostly a lot of legumes, beans, and nuts, leafy greens; I try to have a diversity of plant species that I'm eating, and that's a good rule of thumb. Generally speaking, just eat a diversity of whole foods and plants especially, and you're probably going to be doing pretty good. But I think resistant starch looks really good based on our modeling and also a little bit of self-experimentation. Resistant maize or potato starch seems to do a pretty good job in terms of at least turning up butyrate.

Matt: You think that's a pretty reasonable biomarker to go after?

Sean: That's the one we focused on because, in my opinion, it's pretty unequivocally good. There's not that many metabolites that are like that, right? Like you say, sometimes having higher of something isn't good for some people, but it is for others. Butyrate, I think you can turn it up across the board, and it's going to be good.

Matt: Just as an aside, I mean, again, it's not 100% this is the mechanism, but in my lab at the University of Washington, we worked in a mouse model of mitochondrial disease where we found originally that rapamycin rescued that model, but then later on that acarbose rescued that model, which is an inhibitor of...

Sean: Yeah, exactly.

Matt: And that, in those mice, in the acarbose-treated mice, there was an increase in short-chain fatty acids, including butyrate, and that's at least part of the mechanism of the rescue of the mitochondrial dysfunction. So, I'm in agreement with you that there are lots of lines pointing to butyrate and perhaps other short-chain fatty acids as having benefits in certain contexts. But there is some controversy, right? So, we could quickly touch on that. There are some papers showing that butyrate has negative associations with certain diseases.

Sean: There's some cancers, and so when you say negative associations, you mean people who have these diseases have higher levels of circulating butyrate, is that typically?

Matt: Yes, in the blood, and that's what's been measured, and that's what's been postulated. My two cents on that is the gut is a pretty good barrier, and there's a lot of control about what metabolites actually cross into the bloodstream. Generally speaking, butyrate has a very peripheral effect in the gut; it doesn't get into the bloodstream very often. It's, for the most part, signaling to receptors on the intestinal epithelium, and when it is absorbed into the enterocytes, it's consumed; it's the predominant carbon and energy source for enterocytes. They actually eat it preferable to blood glucose, so it's turned into CO2, essentially, and whatever little bit is in the bloodstream is kind of stochastic noise almost. However, if you were to poke a hole in the gut and suddenly a bunch of metabolites were flooded into the blood, you would see a boost, a jump of these short-chain fatty acids, and it would maybe look like they're associated with maybe a tumor causing a disruption to the epithelial wall. But that's sort of a fluke, right? It's not saying that butyrate is causing the tumor; it's that it's like a side effect of that damage, having leaky gut phenomenon. That's what I suspect is happening.

[45:00]
Matt: So, I guess we've talked a bit about how the sort of global microbiome changes with age, and there's a lot of interest in aging clocks right now, a variety of different aging clocks, and people have talked about microbiome aging clocks. Given what you've described, how could you construct a microbiome aging clock, like what would that be based on? Obviously, changes in the genome sequences and prevalence, but if it's the case that it seems to be health status is most closely associated with diversity or lack of diversity in the case of unhealthy people, is that really what people are measuring in these microbiome aging clocks, or is it more sophisticated than that?

[45:04]
Sean: The ones I've seen have kind of been more machine learning approaches where they're training some sort of algorithm to predict age from the microbiome and then applying that model to out-of-sample sets. They do kind of work, right? So, you look at them; there's, they're not as good as, like, the methylation clocks, but, yeah, if you squint, there's some prediction there. My concern with them, though, is that we are clearly seeing variable patterns happening in people who are maybe less healthy and people who are healthier, and so if you're averaging across all of those folks, whatever your training set is will kind of skew what the model is telling you, and the interpretation for many of these clocks is that older is worse, but our interpretation would actually be the opposite, that having an older microbiome is actually appropriate for an older person, and you don't necessarily want a younger microbiome, at least at the global level.

Matt: I would assume the way these clocks are being developed, they're agnostic to what the signal is, right? So, you can measure, I don't know how many, you tell me, how many species do you get, or how many different microbes do you get on a typical microbiome sequencing at the species level?

Sean: At an appreciable abundance, probably a couple of hundred.

Matt: Okay, so it's not a huge number, right? So, like, these methylation tests, you've got hundreds of thousands of locations in the genome to choose from; you can pick the hundred that are most correlated with whatever your metric is, chronological age or health outcome. In this case, if you only got a few hundred, that seems like a pretty small set to start from. I guess I'd be wondering what are these machine learning algorithms picking up on that are driving those correlations?

Sean: That's even more complicated than that because, of those few hundred, the two of us maybe share about 20% of that set, and you take three people, and maybe it's less than 10% sharing across those three. So, there's tens of thousands of species across the human population; we only, just particular, we have a couple hundred of those. The sparseness of the matrix is very high, unlike a genome where everybody pretty much has a similar set of sites in their genomes. So, it is noisy; it's messy; it's really skewed by the training set that you're putting in. Like, if you're predicting a population in the US who may have a certain set of taxa on average, it's not going to maybe work so well for a Chinese population who has a very different set of species in their guts.

Matt: Let me say what I think you said, which is that the variability from person to person might be much more of a driver in the microbiome space than it is in genetics or genomics, even though, of course, variability in genomics is important, right? That's what leads to a lot of our phenotypic variability. But in terms of both diagnostics and I would then assume interventions, the person-to-person variation in the microbiome is so big that it is likely to make it very complicated to, first of all, say there's a one-size-fits-all, and then secondly, to identify personalized solutions based on the microbiome as we understand it now.

Sean: Hopefully, we'll get there someday, right? I mean, I think that that is the challenge: there's a huge universe of microbes; each one of us pulls down a set, and for most of us, that is a functional set, and there is redundancy in those functions. So, maybe the metabolites that are being produced by our microbiomes are actually pretty similar, even though we have completely different species. So, I think part of the solution to precision or personalized medicine in this space is translating from that ecological composition to a more relevant functional output microbiome, and that tells you more about the kind of clinical outcomes.

[50:00]
Matt: That makes perfect sense. So, we talked about prebiotics and then kind of skipped right over probiotics, but I want to touch on that. Where do you stand on probiotics, and especially given what we were just saying, which is that it's so individual, like, are there general recommendations for probiotics, and kind of where do you land on that topic?

Sean: I don't know if I have a strong opinion to land anywhere, but if you look at the data, so there's a paper from Jens Walter's lab from a few years back where they gave a bunch of people Bifidobacterium longum, which is a common species of probiotic. 30% of them, after they stopped taking it, that bug stayed in their system, but for everyone else, 70% of the population, as soon as they stop taking the probiotic, it's undetectable from their system; it just bounces right off. I think that's true for almost any probiotic that we're taking. Only a very small fraction of us have it actually growing in our guts; for the rest of us, it's just washing right out of the system, and growth isn't always correlated with the functional effect of the probiotic. There are examples of, for Akkermansia muciniphila, it's been shown that pasteurized Akkermansia muciniphila has an anti-inflammatory effect in human trials, so even dead microbes can have a drug-like effect. But for much of the effect of a probiotic, it is actually, it's kind of growth and activity. So, each one of us has maybe a different set of probiotics that would be useful to us, but there's no one probiotic that we should all take.

Matt: That makes sense to me, although there's going to be the effect of the probiotic while you're taking it, which could be different from the effect after you stop if it's lost. Are there any probiotics where you feel like there's sufficient evidence out there that either everybody should be taking it all the time or that there is a test you can take that says, for you, this probiotic is the right probiotic for you?

Sean: That's a good question. There are a ton of clinical trials in this space that have been run, and even for the, I would put probiotics into two different camps. One is the classical set that we all know, which are like lactobacilli and bifidobacteria, and the reason we have this set is because they're sort of grandfathered in by regulators; they're considered food because they're already present in yogurt and cheese and sauerkraut, and so we're eating them anyway; why not put them in a pill? The other things that are actually dominant constituents of our microbiomes, our adult microbiomes, are not that set; that set's, like, maybe common in babies, but they're very low prevalence in the adult gut. The bugs that I'd like to give people, like Faecalibacterium prausnitzii, which is a butyrate producer, or Akkermansia muciniphila, these organisms, you have to run phase one trials.

Matt: but you can buy Akkermansia supplements, can't you?

Sean: Because they've gone through phase one trials. So, some companies have put in the effort of actually pushing these bugs through these trials, so you only have to go through phase one for probiotics in order to be able to market it.

Matt: Exactly, you just need safety, huh? But still, that costs a million bucks, right? To run a phase one trial. So, all of these next-generation probiotics that would maybe be more useful are still making their way through this pipeline of getting the phase ones to get to the approvals of being able to give them to people.

Matt: Let me ask you, I don't mean to interrupt, but I want to ask this question before I forget about it, because I've heard a lot about Akkermansia; I've listened to some podcasts; it seems really interesting, promising, and part of me thinks, even though maybe it's gone through phase one trials, we really don't know, sort of, long-term what the consequences of a probiotic could be. Is there a risk that you will permanently change your microbiome from these kinds of probiotics in a way that, in the long run, I mean, it's sort of an open-ended question, of course, there's some risk, but is it, I guess, am I defaulting to the fear side of things, which is natural, I think, in many ways, or is that something we should really be thinking about? Because my feeling is, even these phase one trials are usually pretty short-term, so is that something that we even need to think about with things like Akkermansia or other probiotics that could have a long-term impact on your microbiome?

Sean: No, I mean, it's always something we should worry about in the back of our minds. Nothing's unequivocally good, right? Everything's got a risk-reward to some extent. The question was, I sort of, when I hear about these things, I wonder how well established is it that Akkermansia is good, question number one. Question number two is, is there any reason to think that by taking a probiotic, you are permanently changing your microbiome in a way that is going to be detrimental in the long run?

Sean: So, one of my philosophies is, if you build it, they will come. So, you can think about your microbiome in that sense; if you're eating substrates in your diet that a particular group of microbes can grow on, they're probably going to make their way in there. We're constantly touching our mouth and taking up microbes from the environment; if there's a hole in the niche space in our guts, something's going to fill it pretty quick. I just want to make sure I understood something you said before, which is that, for you, it might be microbe species X that has that function that fills that gap; for me, it might be microbe species Y, but it still has that same function. So, if we were to sequence, we would have different compositions, right? But functionally, they would be doing the same thing; it would be hard for us to swap because, once Y has established in you, it's hard for X to hop in, got it?

Matt: Right, yeah. That's one thing; it's like, I think you can be rest assured that your microbiome is going to be pretty stable no matter what you do to it. So, you can eat a lot of different things, you can take a lot of different probiotics, and I think that's fairly safe. But Akkermansia is a good one to pick on because it is a mucus degrader, so it specializes on eating your mucus, and it lives on the mucus layer. It's a keystone species in a way; it actually, when breaking down those glycans in the mucus layer, it provides downstream products for a lot of other organisms to cross-feed on. Some people associate it with a thinning of the mucus layer, so it's been associated with constipation; it's been associated with total sort of feeding through a tube in your arm. So, when you stop eating food, the only food left for the microbes is mucus, and so the organisms that can leverage that resource become more abundant. But I think, for the most part, Akkermansia is, looks like a criminal by accident there because it's not having necessarily a detrimental effect; it's just enriched in those disease conditions because mucus is the only game in town for what's left to eat in that system. Akkermansia also can

stimulate mucus production, and so it's helpful in that sense. There's another piece to Akkermansia that gets to, like, indigenous cultures versus developed world, where you don't really see Akkermansia as much in these indigenous societies that have really high-fiber diets. My thinking there, some people say it's one of these microbes that we've gained in the developed world; I actually think that it's low enough abundance that, when you're eating a 100 grams of dietary fiber in, like, an indigenous society, and we normally eat less than 10 in the US, there's so much biomass of the non-mucus degraders in that sample that, when you sequence that sample, the Akkermansia is just falling beneath the detection threshold. So, I think it's still there; it's just, I don't think it's associated with poor diets; I don't think it's associated with disease. I think the evidence seems to point towards it being beneficial, but then again, we should be cautious about all this.

[55:00]
Matt: I think what you just said is an important point that's maybe worth emphasizing for people who don't think about the way these technologies work, but when you're sequencing the microbiome, right, what you detect is going to depend both on how many cells there are of a given species, like Akkermansia, but also the total number of cells, because what you're really trying to detect is that signal from all of the noise. So, if there's just a lot higher abundance of total microbes or total genomes in the fecal sample, your likelihood of detecting something that hasn't changed in abundance compared to when there's a lower total amount of DNA goes down. Is that exactly what you're getting at?

Sean: The really hard part about the microbiome in terms of detecting rare things is, it's an exponential distribution, and there's a few things that are very, very abundant, and then there's an enormously long tail of really, really rare taxa. So, I said we each maybe have a couple hundred species; if you sequence deep enough, you might be able to find a couple thousand, right? Or at least strains. But, like, at appreciable abundance, yeah.

Matt: Do you think that that will change as the, presumably, the sequencing technologies will continue to get better; sequencing will come down in cost; that we'll start to be able to sequence deeper and deeper and deeper?

Sean: We will. What, how relevant it is, is another thing. We could be picking up something that was on a piece of lettuce that I just ate, and it's just passing through, but I'm not actually, it's not doing anything, right?

Matt: Right, but having said that, I mean, this is very similar, I think, to the problem we had with the serum or blood proteome, but, of course, a lot of the stuff that's in circulation, even at very low concentrations, is really important. So, I suspect it'll be a combination of things that are irrelevant at low concentration in the microbiome and then things that are really important, and that's the hard part is teasing that out.

Sean: If you know what you're targeting, you can target it with, like, qPCR, for example, so you can target things in that rare tail.

Matt: Okay, one more question on probiotics. Do you take a probiotic currently?

Sean: I am not, but I was a little while back; I was taking this glucose control probiotic from Pendulum, and they're the ones who make the Akkermansia one. I don't know if other people do as well, so they're one of the few that do these next-gen probiotics, which I find cool. There's a few of those companies out there doing that.

Matt: Okay, great. So, let's switch gears because I want to talk about the work that your group has done on identifying what foods people eat from the feces, right? So, maybe you can take us through how that works and what you did and what you see the potential applications of that.

Sean: Totally, yeah. One of the major factors to understanding the microbiome in general is knowing what people are eating because that's the major force shaping the composition. If you build it, they will come; if you put the substrates in, they'll grow. But it's really hard to get that information; questionnaires are onerous, they take time, people lie; they'll say, like, I ate a salad; I didn't eat the candy bar. So, having unbiased ways to extract that information directly from data would be really useful. We had this crazy idea that we eat things with DNA in them, like apples and asparagus, and some amount of that DNA is going to survive passage through the gut into poop. How much is there? It's hard to say; we don't know, but if, when you're sequencing DNA from stool, well, can you pick up enough of that residual DNA from your diet to actually reconstruct what you're eating? This is actually a technique that was pioneered in wildlife, so people looking at working with whales or grizzly bears have used these methods to actually try to dissect their diets. There's another group at Duke, Lawrence David, who's also using this in a slightly different way with amplicon sequencing, where he's actually amplifying out genes from chloroplasts and mitochondria from food and then sequencing those amplicons. We took a shotgun approach, so we just took all the DNA, chopped it up into little pieces, fed it through an algorithm, and saw if we could predict these food items. That's the method; it's called metagenomic estimation of dietary intake, or MEDI, and it was just published a couple of months ago in Nature Metabolism.

[60:00]
Matt: What's the take-home from this? So, first of all, you can do it, right? You can tell something about people's diets from the DNA that's in their poop from the food that they ate. It's accurate, I assume, ish, right? Nothing's perfect.

Sean: We had some validation data; we could validate in silico, so we could create synthetic metagenomes and then feed them in and see that we could actually predict what we put in. But the harder prediction is, like, real human feeding trials. So, we had a couple of feeding trials; one was a very simple one from Hannah Holscher's group at UIUC; she had an avocado study where they had two groups of people; they're eating the same exact diet, but one group gets

one large Hass avocado for lunch, and it was funded by the avocado industry, a big avocado. I'm an avocado guy; I love avocados. So, when we asked, what is differential between these two groups, what food is detected in these two settings, there was only one thing that popped up as significantly different; it was avocado. So, that was a good sanity check. Another study we had, more quantitative information on the amount of intake, and we did see, slightly noisy, but if people had a higher frequency of eating a particular type of food, we predicted a higher detection of DNA from that food, and vice versa; lower frequency, lower detection. For some food types, we weren't doing a good job, so shellfish was really hard; we weren't getting a lot of quantitative agreement there. We suspect it's because, when you eat shellfish, it tends not to be a huge amount of biomass, and shellfish biomass is very fragile and degrades pretty easily. Plants degrade less easily, so we kind of over-detect plants, and we under-detect meat or animals. So, we know there are some biases; we also know that our model gets tricked by processed foods.

Matt: I was going to ask about the processed foods.

Sean: Yeah, so we can predict total energy intake, total protein intake, total carbohydrate intake, and a few other things really quantitatively, but for other things, our model chokes. One of them is dietary fiber, and the other is total fat, and we think the reason for this is a lot of food processing actually removes the bran from grains and reduces the fiber content. So, when we detect a wheat DNA piece of DNA in our sample, we assume we're translating that wheat into its whole food intake, right? So, we're assuming the amount of fiber you get from a whole kernel of wheat, but that's not the case, probably, in a lot of these people's diets. So, we know that's breaking the fiber prediction. The fat prediction, we actually get a significant association, but it's a negative association. That was a head-scratcher for a little while, but what we came up with is oils, which are in foods, processed fats, do not contain a lot of DNA in them. People who have higher fat intake tend to eat more of these oils, whereas people who get more of their fat from whole foods, like nuts or avocados, tend to have less total fat in their diet. So, our model, or our algorithm, is actually predicting more fat in the low-fat intake group because they're eating more of their fats in their whole foods, and the people eating more fat have more of the oils in their diet.

Matt: It's a very cool story. Is there any sort of practical applications you see from that technology or that approach?

Sean: Yes, so one practical application is, we just don't have good data that pairs dietary intake in a stool sample and the microbiome profile at scale. So, having, like, hundreds of thousands of samples where we can have those two profiles together and really get a really, really good answer to the question of, if I eat an onion, which species are enriched in the human gut? That's ongoing; we have a collaboration with Nicola Segata, where we're going to try to look at this in a few hundred thousand metagenomes, and because you're sequencing DNA, it's, I mean, the technology is maybe a little bit different in the way you do the sequencing, but you're sequencing DNA.

Matt: Two different types of DNA from the same sample, right?

Sean: Essentially, right, exactly. So, it's enabling this new kind of lens into understanding diet-microbiome interactions. I'll throw out another weird one. We actually had a preprint from a group in Germany come out right when our paper was published that used our method, and they had an interesting phenotype; they were looking at people who got the mRNA vaccine for SARS-CoV-2, and some people had a higher fever than other people. This is from Ruth Ley's lab, and they wanted to understand, like, what was distinguishing these high and low fever producers. They looked in the diet questionnaire data, and they found that people who were omnivores had higher fevers than people who were vegetarians. Interesting, didn't know how to interpret that. They looked at the gut microbiome, and a certain gene function was enriched in the high fever producers, which was flagellin; this is the little tail that bacteria make to move around; it's also piquant to the immune system, right? It activates innate immune receptors. So, there's a good reason to think why people would have a higher fever if you have more of this produced, and the organism that was producing it was also sort of enriched in these people's guts. So, they ran our method on these samples, and they found that there was an enrichment in hibiscus in their guts, and this was something we discussed in our original paper; hibiscus was associated with processed food intake; we didn't quite understand why, but one of the limitations of our model is, if there's a plant species that's kind of related to another one, sometimes you get mistaken cross-contaminations. Cotton is really related to hibiscus, and cotton happens to be a really common additive to processed foods; carboxymethyl cellulose is a derivative of cotton; it's an emulsifier and a kind of non-caloric bulking agent. This was really enriched, and it was kind of quantitatively associated with this fever temperature. They took the bug that was this flagellin producer out of the gut and grew it in culture along with about a dozen other human gut commensals and found that none of them could grow on this carboxymethyl cellulose except for that one bug, and when they grew it in the presence of carboxymethyl cellulose, it had, like, 10 times more flagella being produced. So, they found, what I'll say to end this off is, I think another outcome of this method is, it's going to be pointed at clinical trials where there are responders and non-responders or variability in response that you don't understand, but maybe if there's a dietary component to it, you can extract that information and look at it.

[65:00]
Matt: Let's dive into that topic just a little bit deeper, this idea that the microbiome can influence the way different people respond to different interventions, drugs, and then using our understanding, our growing understanding of the microbiome, or in this case, the dietary components that lead to changes in the microbiome, to stratify people for clinical trials. Can you just talk about that for a few minutes?

Sean: Totally. The most experience we have with this is with statins. So, we have a paper from a couple of years ago where we looked at statin use and variability in response to statins, both in terms of the on-target effect, which is lowering LDL cholesterol, and in terms of side effects. So,

one side effect of statins is insulin resistance; you get a, people who take statins are a little more likely to transition into pre-diabetes or diabetes, and it's still a little unclear exactly why, but it is an observed phenomenon. We had this Arivale population of people, kind of a wellness cohort.

Matt: Maybe we can just quickly, you can quickly describe Arivale, because probably not everybody listening or watching is familiar with Arivale.

Sean: Right, so Lee Hood and Nathan Price, who are my colleagues at the Institute for Systems Biology, started a company called Arivale; it was a sort of precision health wellness company where people would sign up and pay a fee, and they would have their bodies quantified, soup to nuts; their genomes would be sequenced, they had their blood proteomes, they wore Fitbit trackers and had all these questionnaire questions, and microbiomes and lots of other data collected. This company existed for about five or six years and got up to around five or six thousand people, and 95% of those folks signed a waiver saying they wanted their data to be used for science, and so those data came back to ISB to be used by us researchers. We published a lot on this cohort, and this drug application was just one of the examples of something we could look at because we had collected so much data from this population of people. So, within that Arivale cohort, there are about 1,200 people with clinical records on their drug use, where we could either definitively say they were or they were not taking a statin, got it? About 300 or so, maybe, were taking a statin out of that 1,200, and for about half of those, we had dosage information, so whether it was a high, medium, or low-intensity statin dose. So, then we could kind of look at their microbiome composition and stratify people and ask, cross-sectionally, those people who are taking statins, do you see variation in the effect of the statin and the side effect of the statin? We did. We used a proxy in the blood called HMG to measure the on-target effect, so statin inhibits an enzyme called HMG-CoA reductase, and if you inhibit that enzyme, you get a buildup of HMG in the blood as a kind of instantaneous marker of efficacy. Sadly, we didn't have longitudinal data, so we couldn't do the traditional change in LDL as the efficacy marker, so we used this HMG as a proxy. When we looked at the microbiome, we found that people with higher-diversity microbiomes tended to have less efficacy in their statins; their LDL lowering wasn't as much, and they also tended to have, so people with lower diversity had better efficacy but more insulin resistance, so they had a bigger perturbation to their A1C, essentially. So, it was all sort of cross-sectional, a little bit correlational; we didn't have that longitudinal data to look at, but we published this and showed that we could kind of predict who would or wouldn't respond most to a statin. A follow-up study out of China then looked a little more deeply at this and had an actual intervention trial, putting first-time users on statins and then following them, and they saw the exact same patterns that we saw, but they saw them longitudinally. One of the observations was that taking the statins depleted certain clostridial taxa in the gut; these organisms are producing certain bile acids, and in particular, ursodeoxycholic acid was something that was produced by these bugs, and it was depleted in people taking them statins. If they supplemented a human cohort with ursodeoxycholic acid, which is, it happens to be a drug people give to patients as cholesterol-lowering medication, those people were protected from the A1C change, so their metabolic disruption was abated.

Matt: Did they still get the benefits in terms of lipid-lowering?

Sean: Their LDL lowering was the same.

Matt: Yeah, so it kind of opens up the idea that you could use the microbiome to stratify patients and maybe target them with co-interventions to augment that effect of the drug while protecting from side effects, right? Very cool. I'm guessing that, for most drugs, there's not a ton of data on microbiome changes correlated with individual metrics of drug efficacy or bioavailability or pharmacokinetics, or whatever we're looking for. Is that a place where the Arivale dataset you could potentially do this for other, like, at least common drugs, right?

Sean: That's the thing with the Arivale, you're just sort of capturing people in the wild, so it's a sort of a random sampling from the population, right? So, you're going to get the most prevalent drug.

Matt: So, I wonder about metformin; that's the other one that jumps to mind.

Sean: That's the other most common drug in that population; it's still much less than the statins, so, whatever power we had to see what we saw with statins, we would be much less for metformin, so, we haven't seen any really, really strong signals, so we haven't pursued it, but I'm always looking out for these large, deeply phenotyped cohorts where you have paired drug and outcomes data.

Matt: Yeah, I mean, metformin is interesting because, I mean, we still don't really know exactly what all of the different biochemical targets are, but one thing it does is that it's a complex one inhibitor, and given bacteria are sort of pre-living mitochondria, I mean, obviously, that's a massive oversimplification, but you might predict metformin would have effects on a lot of bacterial cells. I'm sure there's literature on this; I don't know the literature, but it would be shocking to me, I think, if metformin doesn't impact the microbiome.

Sean: In fact, actually, David Gems had some work in C. elegans where they attributed the effects on metformin on lifespan due to effects on the microbiome; I think it had to do with folate metabolism. So, yeah, I've actually seen papers that kind of oscillate back and forth; he's like, “Here's this is how it works, and no, this is how it works.” There's a guy at Yale, Andy Goodman, who does these drug microbiome interaction studies, big screens where he grows out microbes with these drugs, and I think metformin was in that screen, so I, but I actually forget, like, are there microbes eating it or being killed by it, but that's been done, probably, on that.

[[70:00]
Matt: I think this is a really rich area to mine in terms of these drug interactions and effects on the microbiome. Cool, so I guess I'll finish up with a very open-ended question, which is, when you kind of look at the landscape of microbiome research or microbiome products, wherever

you want to go, like, what's the thing that most excites you? What's the, where, what are the areas where you see the largest sort of potential over the next three to five years in the space?

Sean: It's a good question. There's waves of things that are coming that I think are really exciting. The first wave of clinical translation was fecal transplants for the treatment of recurrent C. diff infections, and now we have FDA-approved fecal transplant alternatives, or not alternatives; they're sort of fecal transplant light. There's a few companies that have created cocktails of bacteria, like a few strains that you can just grow in vitro, put together in a pill, and give to patients. A company called Vedanta just had one of these cocktails pass a phase two trial and go into phase three to essentially replace fecal transplants altogether with a defined cocktail. I would label that as bugs as drugs. What are the conditions, the short list, where you see the most likelihood that we'll have bugs as drugs in the near future?

Matt: This VE303 drug that Vedanta is pushing into phase three trials, it's likely that that will have successful outcomes and become approved in the next year or two. So, we should see our first bugs-as-drugs intervention available to patients in the next year, and that's for what condition?

Sean: That's for recurrent C. diff, right, which has been the easiest, it's the lowest-hanging fruit; it's been the easiest thing to treat with a microbiome intervention because you just need to recover the ecology of the system to exclude the pathogen. There are a ton of trials, though, for different diseases that are taking this bugs-as-drugs approach, and it's sort of old-school pharma; you do these massive screens, often in mouse models, until you find a cocktail that seems to work, and then you do phase one trials, and you keep pushing things through that pipeline. That's great, and that's all happening, and we'll see our first drugs available soon. Next, there are the more algorithmic or precision interventions that are on the horizon, and much of this is happening in precision nutrition. The NIH actually had a big push in the microbiome and precision nutrition in the last couple of years, so they've dumped tens of millions of dollars into development of these technologies. But the basic idea there is being able to leverage the composition of your microbiome to give targeted advice on what types of diets you should be eating. We know this works to some extent; like I said, there's this DayTwo company and this Zoe company that kind of do something like that.

Matt: Let me ask there, how precise can you get? Because you sort of said, like, they can predict something that, for glucose, for example, is going to be better than the Mediterranean diet. How precise does that get? Is this, like, daily meal plan precise, or is this generally you should eat less carbs and more protein level sort of precise?

Sean: I believe, so, it really depends on the training set that goes into their machine learning. I think for their training sets, they were specific food items, like whole wheat bread or broccoli, so it was, like, specific food items. Are there actually people where whole wheat bread is better than broccoli? Probably. I mean, in that paper, they had these kind of extreme outlier cases

where, like, for some people, eating a bowl of ice cream is better than an apple, for their glucose spiking, yeah.

Matt: I'm sure that's the case, right? I'm sure, for a subset of people, yes, it is interesting. I don't know if you've ever done CGM, but it definitely is interesting how different people, different foods will absolutely have totally very different glucose profiles.

Sean: Absolutely, yeah, spiking is only one thing you could measure, and there's a bunch of other effects of diet on the body that you're ignoring there.

Matt: I don't want to go down this rabbit hole too much, but obviously, even the shape of the spike is important because there's both the uptake, right, and then there's the insulin response and how fast it... So, I'm sure it's massively complicated, but super interesting. So, anyways, I'm sort of fascinated by this idea that you can predict dietary interventions, the efficacy of different dietary interventions based on the microbiome. I guess my skepticism comes as to how precise can that actually be, but I don't know because I'm not an expert in the space. Sean: I would say my skepticism has been a bit dissipated because it's already been done. I mean, I think DayTwo is telling you, you should eat this specific food item, and their prediction, if you look at their R value for their predicted versus measured, it's about 0.68, so it's not perfect, but it's pretty good.

Matt: I guess I would push back on that a little bit, though, is, and you just said this, right, if glucose is the only thing you're looking at, like, that's one piece of a very large health puzzle. I guess the other questions would be, over the long term, what other effects might there be from that substituting that specific food item?

Sean: Yeah, and so our lab is actually trying to get into this space with our metabolic modeling, and there, in the models, we can actually break down a banana into its molecular constituents, like this much inulin, this much glucose, and that can be fed into this metabolic network of someone's microbiota and then predict the good guy metabolites, like maybe butyrate, but there's also bad guy metabolites, like imidazole propionate, which promotes some insulin resistance. So, ideally, at the end of the day, with the microbes, we want to kind of turn up the good guys and turn down the bad guys, and what's good and bad might vary for context, like certain people might benefit from different sets of these things. I'm really interested in kind of hacking that, trying to get precision outputs for precision inputs and matching that to what's needed for the host.

Matt: There's a ton of research that needs to happen before something like that moves into the clinic, right?

Sean: Yeah, and I guess the question would be, maybe it's not even really a question, but my first thought is, probably what you're going to see is you're going to see people move that into the direct-to-consumer space because there's no regulation there; they can get away with it, right? So, I guess, and I mean, you see this already in the aging community or longevity with the

biological clocks, certainly with the microbiome tests. So, hopefully, that won't slow down the clinical development of these things. There's some challenges with the regulatory landscape there, right?

Matt: I mean, with pharma, you're banking on the fact that you have a billion-dollar payout on the other side with a small molecule. If you're telling people to eat bananas and avocados, what's the payout, right? Like, can you prescribe your algorithm at 30,000 bucks a pop to, like, recoup that investment?

Sean: That's right, that's why it probably will start out in the wellness space because the sort of economic bar is lowered, but you could, I have seen a few companies doing pretty well and developing some interesting things that have some scientific validation behind them, but, of course, you're also competing with companies that are pushing all their money into the marketing budget. It's a landscape that's very, very difficult to do high-quality science.

Matt: Totally. I mean, DayTwo went out of business, right? Because I think they were trying to compete in this market that they just couldn't protect their...

Sean: Yeah, that was hard. So, anyways, that's a whole longer conversation about the incentive structures around consumer-facing products.

[75:00]
Matt: In the microbiome space, it sounds like you're pretty optimistic that, in the coming years, the research, as it progresses, will get to the point where we may be able to take a test that will, with some level of precision, give recommendations on food, optimal food selection for, presumably, things like glucose, lipids, other metabolic markers. What about disease detection? Because that's the other obvious place where you could see, are there specific microbial signatures that could pick up, or even pre-disease, pick up on things that are coming early on so that you can then take steps to get that?

Sean: Yes, for sure, there's work in that space. One clear one is colorectal cancer. There's a strong signature where people who have these tumors or these polyps will show an uptick in organisms that are normally orally associated, like Fusobacterium; they're suddenly detectable in stool. That tends not to happen in people who don't have these things. I don't know if it's as good as the current host-based DNA testing that's out there on the market, but I suspect that you could leverage the microbiome data to get some amount of diagnostic information on colorectal cancer or risk of colorectal cancer. Beyond that, though, it's tricky because it is so heterogeneous and functionally redundant and varies so much across diets and cultures and continents. Coming up with a diagnostic for a specific disease is really, really hard. But there are, like, more general patterns that we should pay more attention to, possibly. So, we recently published on bowel movement frequency, and we can see in the bloodstream what your pooping frequency is; I can see in your blood whether you're constipated or whether you have diarrhea. If you look at the molecules that pop up, people who are constipated see an uptick in

these molecules associated with protein fermentation by the microbiota. Usually, our microbes like to ferment dietary fibers into these short-chain fatty acids, which tend to be healthful. But if you eat up all that fiber, the next thing you switch to fermenting are the proteins, and you get these protein putrefaction byproducts, like p-cresol or indole, and those build up in the blood, and they cause damage to the kidneys, the liver, the heart. Over the long run, I suspect that this might be somewhat causal in chronic disease. You see, in people with neurodegeneration, they've often suffered for years or even decades from chronic constipation before the dementia sets in. So, I think we should maybe more actively manage bowel movement frequency as a long-term health risk.

Matt: Because that's not something that is typically thought about or talked about.

[80:00]
Matt: Okay, we'll finish up with kind of a fun question, maybe. Which is, as this technology moves forward, there's going to be, I think, a drive to get more and more data, right? Do you envision, I mean, right now, it's honestly, it's kind of a little bit yucky; you got to collect your poop. Are there tools, approaches that can be taken to sort of make that process easier, and maybe even, as the sequencing gets further and further along, like, it's an all-in-one DNA sequencer toilet, call it the Super Bowl, right? Where you can actually get microbiome data on people in a much easier way?

Sean: It's not crazy because people are doing it. There are a few approaches people are taking. My colleague, Nathan Price, who's now at UIUC, they built a kit that's based on a wipe; you just have a toilet paper wipe, and you throw it in a tube and cap it; no scooping from a diaper or something. So, that makes it a little easier. The smart toilet, that is in development; there are a few companies working on it. My PhD adviser, Jack Gilbert, who's now at UC San Diego, he has a company; I think it's called BiomeSense, and they're targeted more towards clinical trials, because these are expensive toilets. These aren't, like, something for home use yet. Although, I've seen some crazy toilets in my travels—50 buttons, and I'm like, what does that do?

Matt: But, yeah, it's a way to automate the sampling, right? And let the human not have to deal with it.

Sean: Exactly. No, it's a what you said. For now, it's just sample preservation and processing; there's no DNA sequencer built into it yet because that's too expensive. There's a cartridge you have to pull out and then it goes to the lab.

Matt: Well, that's neat. I'm interested to see my first microbiome collection toilet; that'll be fun.

Sean: It's got awesome potential.

Matt: All right, well, thanks so much for coming by; this has been great. Is there anything we didn't touch on that you want to touch on?

Sean: I guess one final shout-out to your microbes. We have them; it's not bad; it's good, and you should feed them. We don't eat enough whole food or dietary fiber in the developed world, and, generally speaking, everyone should eat more plants, more diversity of plants. So, feed your microbes.

Matt: I think that's a great way to finish. Thank you so much for coming on.

Sean: Thanks.