ILSI 2021 Annual Symposium Session 2: The Microbiome beyond the Gut

Transcript of the presentation, The Oral Microbiome, Oral Health Resilience and Systemic Health, Egija Zaura, PhD, Academic Centre for Dentistry Amsterdam, University of Amsterdam, The Netherlands

Thank you. First of all, I'd like to thank the organizers for inviting me. And I see that the time is running, so without today time, I will continue with my talk. First of all, let's start with disclosure or conflict of interest. Research which we perform at [ACTA 00:00:20] has been sponsored by several companies, as you see there, they're mentioned here.

An outline of the talk today will be about focusing on what do we mean when we talk about healthy oral microbiome? We'll also show that it's quite heterogeneous, even when it's in healthy young individuals. And what happens when we expose oral microbiome to environmental stress. And I will also touch upon oral-systemic health link.

What is a healthy oral microbiome? There is no one single definition, just like in previous talks about the soil, it depends on which stage of your life you are in. If you're just infants, at the beginning, you don't have any teeth and then your first teeth erupt, and those are deciduous teeth, and then you get mixed dentition, and then you get permanent teeth, and then you get maybe braces. And then if you get older and you are unlucky, you might even lose your teeth and you have dentures. Each phase of life will bring also a different ecology in your oral cavity.

But in general, we always will have bacteria, and we will have fungi. We will also have viruses. Majority of those are bacterial viruses or bacteria phages. We also have protozoa. Depending on how often a person cleans teeth, and depending on a water source, you can have quite a lot of protozoa because they feed on bacteria in your mouth.

And besides those, we also can have archaea, which are usually ... Well, the only methane gas producers. They're not only in the gut, but they're also in oral cavity.

Every day, we actually harm a lot our oral microbes. We do everything to remove them, to kill them, to perturb them by cold and heat foods, and foods containing antimicrobial substances, toothpaste mouth washes containing antimicrobial substances, but also our own saliva having immune components and antimicrobial components. All these attack continuously our microbes.

You might think that every day we wake up with a completely new oral ecosystem, that it changes due to all these perturbations. Well human microbiome project, which had been mentioned already before, collected microbes from different niches in the human body. And in part of the individuals, they collected these microbes at two occasions with few months in between. And then they looked at

similarity or correlation between two related samples. What you see here is the Spearman correlation coefficient. When it's high, the samples are very similar.

And here are the different niches they took, and the number of individuals these niches were collected on. And as you see here, the least reproducible niches are places on all over our skin, but also vaginal tract, also the nose. And then you see here in red, these are all sites collected, samples collected in oral cavity. And the green here in between is the gut. And the champion actually, the most stable microbiome in human body appears to be tongue. No, we are not waking up every day with a different oral microbiome. There is this very specific stability of the oral microbiome.

We also tested what happens to human microbes in the gut and in the mouth, when we expose healthy individuals to different antibiotics. And we followed these individuals for a year, and we looked at both feces and saliva right after antibiotic exposure, after one month, two months, four months, and one year.

And here you see part of the data, where we compare placebo, ciprofloxacin or clindamycin groups, so it was randomized clinical trial. And here, similarly to the previous graph, the higher the value, the more similar the samples are. And here we compare the baseline samples with right after antibiotics. Baseline plus one month, two months, four months and 12 months since antibiotic exposure.

The green triangles are the placebo samples, fecal samples in this case, and you see that the similarity drops from 0.8 to 0.6 in time, even if you don't expose to any antibiotics. And we were really shocked by seeing the results. What happens when you expose healthy individual to clindamycin, because the similarity of the baseline and the consecutive samples actually dropped and stayed low during the whole year. And it was not significantly different from placebo after one year, but not all individuals had reached the complexity of microbiome they had before this exposure.

And imagine if these were sick individuals, or these were individuals receiving antibiotics more than once. But today we were not supposed to talk about gut. And the reason I'm showing this graph is actually that we also looked at saliva in the same individuals at the same time point. And what you see here, what's also striking, that almost nothing happened. Also, the green triangles which are placebo saliva samples, they were stable. They didn't drift like the gut. And when you see the clindamycin, which was so dramatic in the gut, actually only had some effect right after the antibiotic exposure. And after one month, it differed slightly from the placebo, but further on nothing happened. Again, this confirms the high resilience and stability of oral microbiome.

But then I said, I would like to actually address the question, what is then a healthy oral ecosystem? We have these healthy microbiomes, but what is the whole ecosystem? What makes it healthy?

And usually, my background is dentistry. And as a dentist, if I look into somebody's mouth, and if these squares are individuals, I can say that these are really clinically healthy. I can say that these do have caries or tooth decay, and these two have periodontal disease. But then sometimes I'm in doubt, and I cannot really tell if these people are at risk of some disease. And that's what I would like to say.

If we set up a study with the aim to describe actually the boundaries, and the heterogeneity of healthy oral ecosystem, and we wanted to assess the relations between the bacterial composition in saliva, the metabolites in saliva, and the related biochemical salivary parameters, and the diet of individuals. And

we looked at almost 300 healthy, young individuals who actually were collected, we collected all types of samples, and part of this data was published in [Eastman 00:07:07] Journal.

I will just show you the summary of the study. If you want, you could read the study yourselves because it's open access. What appeared? Here in a corner, you see the heat map of the microbiome. And as you see here, these different squares, we found several groups or ecological types of microbes.

And what also appeared, that they correlated to certain metabolites and certain biochemical parameters of saliva. And what was most striking, that what a certain dichotomy in our groups. Some ecosystems for more [sacrolitic 00:07:43] for breaking down sugars, and some were more proteolytic, breaking down proteins. And it was related to different bacteria and different metabolites.

And then also we found that some individuals were most specialized, and some were more adaptive. We ended up with these five different types. And these specialized, here on the left side, you see they were really specialized in breaking down sugars. And these on the right side were specialized in breaking down proteins. And these upper parts were actually doing everything right. They had microbes which were adaptive. They could do both.

And what we actually think, and this was a cross-sectional study, so we cannot confirm at this stage, but we think that these two groups here at the bottom might be at the early stage of dysbiosis. Really losing the balance because they're too specialized. But remember, when we looked into the mouths of these individuals, they were still healthy.

We call these ecological types, or oral eco-types. And what we actually think, that if this is true, that even if we are clinically healthy and we are all different, one size does not fit all. We all will need a different oral care, according to the ecological type we carry. And we also think that these most specialized ecological types could be more susceptible towards disease or could indicate a disturbed ecosystem at the preclinical level.

One size of prevention does not fit all also. If we are healthy, but we will all use the same toothpaste or the same preventive tools, it might not be the best for our oral ecosystem. The challenge for us, for oral research professionals, is to identify which are the right individuals for the right preventive strategy.

Let's look what happens when ecosystem is exposed to these daily perturbations. Like I said, we are having this complex ecosystem, imagine this sphere being oral cavity, and it goes through the trajectory of somebody's life. And then it's exposed to different, there are different factors, and these factors are exposed to different stresses. I've illustrated the factors as springs, and it shows the resilience of ecosystem. You can press the sphere, and it will resist the stress and it will inflate back.

Basically, this is what happens every day when we chew, when we expose our oral microbes to different treatments, so they can handle this, because all these issues are collaborating in this stability. But we also wanted to see if we are all equally resilient, and what happens if we expose ecosystem to quite a severe stress. It was all ethically approved. We performed an experimental gingivitis study, which is very an accepted protocol for clinical studies. You ask volunteers actually to abstain from toothbrushing or from any other oral hygiene measures.

These individuals, we assess them clinically at the baseline. We actually duplicated the baseline so that we have replicated measurements and assessed it two weeks after abstaining from toothbrushing. And

then also we gave them time to recover, and we assessed them again. And in this time, we also took samples for microbiome analysis, and we also took images, which we call QLF, quantitative light, light fluorescence. I'll explain you in a minute.

This visualization of dental plaque, it's quite a specific measurement for dentistry. You can color dental plaque like you see on the right picture, so it's a colored with a staining solution, which stains very young plaque pink, and older plaque dark blue. We see this dark blue line here, and this is corresponding, white light image without staining, and you can see that there is a dental plaque here because this person did not brush for two weeks, but it's hard to see.

What you also can do, you can take a picture under specific light wave. We call it fluorescent image, and you can see here without any staining, I hope your screen resolution allows seeing it, but you can see here this red layer, which corresponds with this blue layer. Basically, what we see here that old mature dental plaque fluoresces red under specific light. We used this property in this study, so we also took these fluorescent light images throughout the experimental gingivitis study.

What you see here are selected individuals, A, B and C, at the first day, before they abstained from brushing, so they were supposedly clean. And you see here, I hope here at the bottom already, some red, red fluorescence appearing. And also, here, you see red fluorescence in individual-B. When these individuals continued with the study, you could see that individual-C actually started fluorescing quite a lot. And if you continue, it even became like glow in the dark individual, if you would see this person somewhere under fluorescing light.

Well, nothing, hardly anything actually happened in the individual A, even though this individual also didn't brush teeth. And we checked it, because you could see in the white light, there is dental plaque, but this dental plaque didn't become mature and didn't become fluorescing red.

When we analyzed, we could quantify these fluorescence images. You could see the different ... We could split our individuals in three response groups. This group with very low fluorescence change, this intermediate, and this very high. And actually, what was also interesting, the response was high in this group, but they were also having very high red florescence values at the beginning, at the baseline. They differed already, even before we started this intervention.

What we actually also found, that when you continue such a study, the whole idea of experimental gingivitis is, that after two weeks you create an ecosystem which is diseased. These individuals should have bleeding gums, but not everybody became inflamed, so not every ecosystem collapsed. And here, when we correlated the fluorescent plaque after two days of not brushing, so at the beginning of the study, it already correlated with bleeding at the end, after two weeks. Basically, what we see here, that by measuring this fluorescence, we could already predict who would really be harmed by not brushing.

And when I put here this picture of dentist appointment, I mean there that before going to dentist, most people actually do their best. They brush, they floss, they rinse, but they should not do so, because by coming without brushing, it gives us an opportunity to predict the risk for inflammation, at least in this case.

With this example, I wanted to show that we expose these individuals to the same perturbation, but they're differed extremely in their responses. And I only showed one part, this red fluorescing plaque, but they also differed microbiologically, they differed in their bleeding, so they differed in many aspects.

And you could see that yeah, the [anchor 00:15:21] system is not equally resilient at each ecological type. Maybe this also is related to the host eco-types we discovered in the previous study. But that's, again, something we are investigating currently.

Well, in the last part of my talk, I will discuss the benefits to the host and interaction with the host, of the oral microbes. This is example from Sweden, where they had a study on small infants and they asked parents, what did they do when the infant uses pacifier, and the pacifier falls down on the ground? There was part of the parents who always meticulously cleaned the pacifier and cooked it. And there were parents who were licking it like Britney Spears here. And they followed these families in time. And here you see microbial communities. It is PCA plot. Each triangle is one saliva sample. And here you see cluster of saliva from pacifier-boiling families from the infants, and here from pacifier sucking.

This was not a surprise, that by inoculating your child with your own microbes, your child creates a different microbiome. But what was more interesting, that when the children reached two-and-a-half years of age, the children who were exposed to salivary microbes of their parents by sucking the pacifier, had lower risk for eczema, asthma and sensitization. Basically, their immune system was trained, and more mature, and developed healthier than infants who were not inoculated at a timely manner.

Another example is for instance, the oral microbes, when they're there, they protect us from intruders. They keep the niche occupied so no other microbes can sit there. But they also, when they are killed, the niche is available. An example of that is outgrowth of opportunistic pathogens like candida albicans. It is a very painful and debilitating condition. There's this white part on your tongue, when the microbes or bacteria are killed by antibiotics, then these opportunistic pathogens, fungi, can proceed and grow out of proportions.

Well, the third example on benefits of oral microbes will be about nitric oxide. As you might know, this is a very active, physiologically active molecule. And I will focus on its role in vasodilatation. If endothelial cells release nitric oxide, the blood vessels dilate, and your blood pressure goes down. But how do we get nitric oxide in our blood? Well, mainly it is from nitrate-rich foods. These are leafy vegetables.

And these foods, so they contain nitrate, it has to be reduced in [nitrite 00:18:14]. And then in your stomach, it is reduced to nitric oxide. There's one problem. That's this red arrow because our own cells don't produce enzymes for this reduction. What we need, we need oral bacteria for this step.

And these oral bacteria do it, but they cannot do it at the moment while the food is still in mouth, because that should be very quick job then. Basically, they get chance to do it slower because nitrate goes through stomach, intestines, and is absorbed into blood and is reabsorbed and concentrated into salivary glands. And while we are doing something else, two or three hours after the meal, this nitrate is released in the oral cavity, and oral bacteria can reduce it to nitrite.

And does it work? Yes, there are several studies, I'm showing you only the oldest studies, the pioneering studies, which tested the nitrate supplements, and actually showed that supplementing a diet with nitrate protects your cardiovascular system and reduces your blood pressure. You can also inhibit this effect by killing oral microbes using antiseptic mouthwash. And so again, it was indirect proof that oral bacteria are involved in this process.

When we look at the oral ecosystem, I said it is different at each stage, but when it's healthy, it's definitely in balanced with the host. They perform these beneficial functions. I just showed some examples. However, things go wrong if things are losing the balance. And for instance, here, if somebody snacks too often, sugar-containing snacks, then you can result in very low diversity, aciduric microbiota, and you can lose your teeth. Or on the other hand, if there is an overruling inflammation, and these pro-inflammatory bacteria grow along the gums here, and that person is susceptible to these pro-inflammatory molecules, then you can lose the bone, which keeps your teeth in mouth, and your teeth can become loose.

If that would be all the problem, it wouldn't be a big issue because you could also extract the teeth and have a denture. But the problem is that all these inflammatory products actually end up in our bloodstream. They end up in bloodstream and end up in different parts of our body where they don't belong. And not only these pro-inflammatory products, but also bacteria themselves can enter the bloodstream via the gums here. They get easily into the bloodstream.

And here I will show you some examples of this so-called mobile oral microbiome. Oral microbes have been associated with very many conditions, debilitating conditions, such as cancers, but also with brain tissue and Alzheimer's disease, but also with pregnancy complications and [trauma 00:21:16]. These are just a few examples, and I don't have time to go into details. There are several reviews on each of these, where they explain how this could happen, that these microbes actually have such an impact.

The causality is not proven, but there is certainly association. If you have colon cancer, examples for instance, they do find oral bacteria, such as Fusobacteria in these kinds of tissue, but they don't know so far if these bacteria initiated it or they actually went there when the situation was already out of hand.

Of course, we don't want to have these disease-producing microbes. We do want to modulate them and keep our ecosystem healthy. There are different ways how to modulate it, like using pre and probiotics or exchanging your microbes maybe or transplanting them from the healthy individual. But can we actually modulate it? My final slide is actually about the challenges. Of course, many of you have heard about fecal transplants, which are quite successful in certain cases like Clostridium difficile infections. This whoever would not work that successfully in the oral cavity.

And just few reasons why these are big challenges to modulate, because as I showed you at the beginning, this extremely high stability of the oral ecosystem, it will actually work against any changes we would like to introduce. And so, you would first have to remove all existing microbes, that's also this colonization resistance. If the niche, if the site is already occupied, there's no place for a newcomer. So how to remove all existing microbes, and how to prevent those opportunistic ones will start growing out?

And lastly, like I showed you, even if we're all healthy, we are all different. And our own intrinsic properties, like salivary pH, different enzyme activities, will affect which microbes prefer our oral ecosystem and which won't. It's not like you can have a probiotic strain and implant into somebody's oral cavity and it will grow there. Indeed, there are a lot of challenges.

And take-home messages for you from this talk. Healthy oral microbiome is very stable, and it is resilient to stress. While maybe not everyone, like I showed you, some people suffered more from stress than others. Healthy oral ecosystem is highly heterogeneous, so one type of prevention will not fit us all. And a healthy mouth is a key to a healthy body, so please take care of your oral microbes.

And with this, I would like to thank the collaborators for the large study we performed on healthy individuals and would like to thank my group at Department of Preventive Dentistry. And thank you all for your attention.