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ORAL MICROBIOTA: A PREDICTOR FOR ORAL AND SYSTEMIC DISEASES

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ABSTRACT

Introduction: The oral microbiota is a wide ecosystem formed by microorganisms including bacteria, virus, fungi present in the oral cavity (saliva, dental plaque, mouth mucosa, etc. …). Oral microbiota is composed by different species existent since child's birth including *Bacteroides*, *Firmicutes* and *Streptococci* family and fungi such as *Candida albicans*. New techniques for studying microbiota have been developed thanks to the DNA sequencing.

Objectives: The primary objective proposed in this thesis is to study the oral microbiota and the techniques used to sequence the DNA. The secondary objective is to study oral microbiota as an effective predictor of oral diseases (caries, periodontal diseases, aphthous and oral cancer) and systemic diseases (Diabetes and Lupus Erythematosus) and to characterize the most important microbiota biomarkers associated to these diseases.

Materials and methods: To study the relation between the oral microbiota and oral and systemic diseases, a comprehensive search, through Medline, Pubmed and Google Scholar, was conducted.

Results and discussion: In deep dental caries high level of *Streptococcus mutans, Streptococcus spp.* and *Lactobacillus* were found; *P.gingivalis, Treponema and Filifactor Alocis* are the most represented in early and severe periodontitis; *Synergisteae, Actinomyces, Firmicutes and Proteobacteria* in Recurrent Aphtous Stomatitis; *Streptococcus* and *Capnocytophaga* families in oral cancer.

In diabetic patients with periodontal diseases, *P. gingivalis*, *T. forsythensis* were highly represented and *Lactobacillus* and *Veillonella* in patients with advanced systemic lupus erythematosus with ulcerative lesions.

Conclusions: The evolution of techniques, such as 16S rRNA sequence analysis, has made possible to discover many bacterial species in the oral cavity. These findings help healthcare professionals to properly diagnose and treat oral and systemic diseases.

RESUMEN

Introducción: La microbiota bucal es un amplio ecosistema formado por microorganismos que incluyen bacterias, virus y fungos presentes en la cavidad bucal (saliva, placa dental, mucosa bucal, etc…). La microbiota oral está compuesta por diferentes especies existentes desde el nacimiento, incluyendo la familia *Bacteroides, Firmicutes y Estreptococo* y fungos como *Cándida albicas*. Se han desarrollado nuevas técnicas para el estudio de la microbiota gracias a la secuenciación del ADN.

Objetivos: El objetivo principal propuesto en esta tesis es el estudio de la microbiota oral y las técnicas utilizadas para secuenciar el ADN. El objetivo secundario es el estudio de la microbiota oral como predictor eficaz de enfermedades bucales (caries, enfermedades periodontales, aftas y cáncer bucal) y enfermedades sistémicas (Diabetes y Lupus Eritematoso) y caracterizar los biomarcadores de microbiota más importantes asociados a dichas enfermedades.

Materiales y métodos: Para estudiar la relación entre la microbiota y las enfermedades orales y sistémicas, se realizó una búsqueda integral, a través de Medline, Pubmed y Google Scholar.

Resultados y discusión: En lesiones cariosas profundas las bacterias más frecuentes son *Estreptococos mutan, Estreptococos spp*. y *Lactobacilos*; *P.gingivalis*, *Treponema* y *Filifactor Alocas* son los las bacterias más representadas en la periodontitis temprana y severa; *Prevotella*, *Actinomices*, *Firmecitas* y *Proteobacteria* en la estomatitis aftosa recurrente; Las familias de *Estreptococos* y *Capnocytophaga* se hallaron en el cáncer oral. En pacientes diabéticos con enfermedades periodontales se encontraron muy representados *P. gingivalis* y *T.forsythensis* y *Lactobacilos* y *Veillonella* en pacientes con lupus eritematoso sistémico avanzado con lesiones ulcerativas.

Conclusión: La evolución de técnicas, como el análisis de secuencia del ARNr 16S ha permitido descubrir muchas especies bacteriana en la cavidad oral. Estos hallazgos ayudan a los profesionales de la salud a diagnosticar y tratar adecuadamente enfermedades orales y sistémica

ABBREVIATIONS

CO2 : Carbon dioxide

DNA : Deoxyribonucleic acid

EBV : Epstein-Bar Virus

IDD : Insulin-Dependent Diabetes

HCMV : Human Cytomegalovirus

HHV : Human Herpes Virus

HMP : Human Microbiome Project

HSV : Herpes simplex virus

HIV : Human immunodeficiency virus

NIH : National Institute of Health

NGS : Next generation sequencing

OTU : Operational Taxonomic Units

OSCC : Oral squamous cell carcinoma

RNA : Ribonucleic acid

rRNA : Ribosomal ribonucleic acid

SLE : Systemic Lupus Erythematosus

SCFA : short-chain fatty acid

T1DM : Type 1 Diabetes Miellitus

T2DM : Type 2 Diabetes Miellitus

WMS : Metagenome shotgun sequencing

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INTRODUCTION

1.1 The Human Microbiota

The human microbiota is defined as the most dynamic ecosystem in our body (1). Microbiota is composed of microorganisms living in the digestive track, the skin, the oral cavity, the respiratory tract, the vagina flora and many other organs playing a role in the body's physiology (1). It evolves throughout the host's life and is influenced by genetic and environmental factors, as the delivery type - natural delivery or caesarian, or even the diet of a new born in their early life (1).

It plays a key role in different processes, as metabolic, nutritional, physiological and immunological (2,3) (Figure 1).

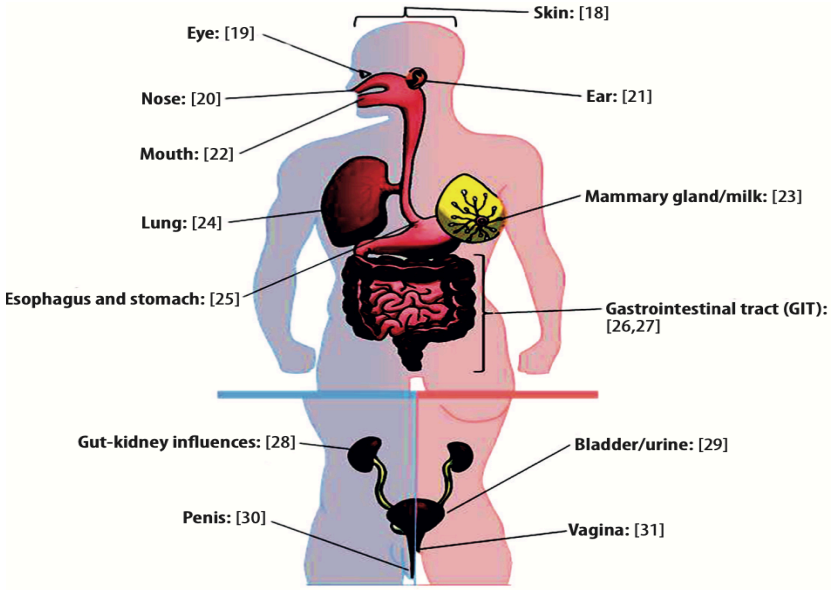


Figure 1: The human body and his different microbial organ hosts. (Illustration made by A. Conrad) (34).

Our initial knowledge of the human microbiota composition comes from microscopy with the observations of Antoni van Leeuwenhoek, who showed a collection of bacterial morphotypes in dental plaque. The characterization of bacterial components of the microbiota was carried out and allowed the identification of numerous microorganisms with many metabolic functions. After great

interest in the bacterial diversity of the microbiota, researches are currently focusing on its functional aspect. A bacterium is no longer studied individually, because the predominant interests of researchers is to study interactions between bacteria and the host (1).

The acquisition of the microbiota in a new born is made specially maternally. It has been shown, that the way of giving birth is important for the early development of the baby's microbiota (2). If the delivery mode is by caesarean, the composition of the mother's skin microbiota is found on the baby's skin; in contrary, a new born with the vaginal delivery will present bacterial communities coming from the mother's vaginal microbiota (2).

It is then established and starts a new microbial system with the early presence of *Bifidobacterium* - specially in breastfed babies - and stabilizes during the first years of life (3).

Once this human microbiota is acquired, it becomes essential in one's life, augmenting the diversity of species inside it, and reaching more or less 1.5 kilograms of bacterial weight in the body; 70% of it living in the gastrointestinal tract, making the human intestinal microbiota - gut microbiota - the richest part and the most studied (2).

Within these characteristics of the gut microbiota, the most dominant bacteria present in the intestine are the *Bacteroidetes* and *Firmicutes*, constituting 90% of the bacterial system. The ratio of these two may change during life and specially in various pathophysiological conditions. They depend on different factors, as aging, malnutrition or even bad oral hygiene (Figure 2). This ratio is considered as a marker to compare healthy and unhealthy subjects, which will be studied in this review (4).

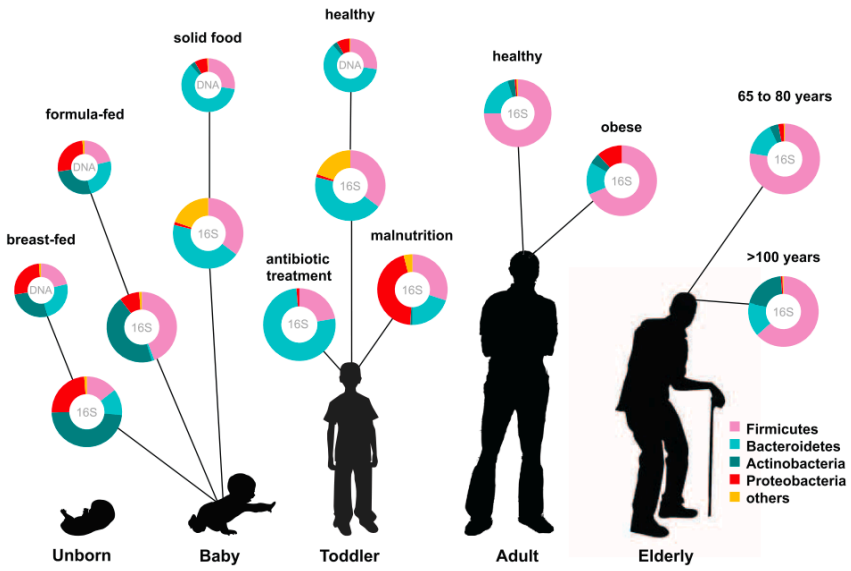


Figure 2: Onset and shaping through life stages and perturbations of the human microbiota (Illustration taken from N. Ottman) (3)

The human intestine microbiota presents functions on the metabolic, structural and protective scales (2). The gut microbiota is considered as a full part metabolic organ. Gut bacteria harbor enzymes to metabolize diverse carbohydrates, such as glycosyltransferases or polysaccharide lyases, maintaining the flora and providing energy for the host. Particularly, *Bacteroides* and *Firmicutes* get this energy from the fermentation of carbohydrates. What remains - undigested substrates - are transformed into fermentation components, such as short-chain fatty acid (SCFAs) and gases (CO2) (2).

These SCFAs can modulate glycemia by their enzymatic activities. They also stabilize the glucose homeostasis in the body and inhibit the repetitive production of cholesterol (2).

In addition, the intestinal microbiota contains other metabolic functions, as the synthesis of vitamins and enzymes cofactors, such as *Vitamins B1, B2, B6, B12, PP, K* and folic acid, and the absorption of calcium, magnesium and iron. It also plays a role in the transformation of the bile liver by the enzyme bile-salt hydrolase and lipid changes in cholesterol (2,4).

The energy production made by the gut microbiota alters the structural part of the digestive tract. It has an epithelium in a single layer composed of columnar cells, strongly bound together with tight junctions, adherens junctions and desmosomes. Some bacteria, as *E. Coli*, are able to decrease tight junctions' functions and influence on the permeability of the flora, thanks to the production of cytokines in the intestines (4).

In relation with the gut microbiota structural changes, the third most important function is the protective aspect. Some bacteria influence the immune system of the body by crossing the mucus layer and changing its permeability with the pH. Groups of mucin glycoproteins, secreted from goblet cells and united into a viscous gel-like layer in the intestinal epithelia, protect from the attachment of bacteria to this layer (4) (Figure 3).

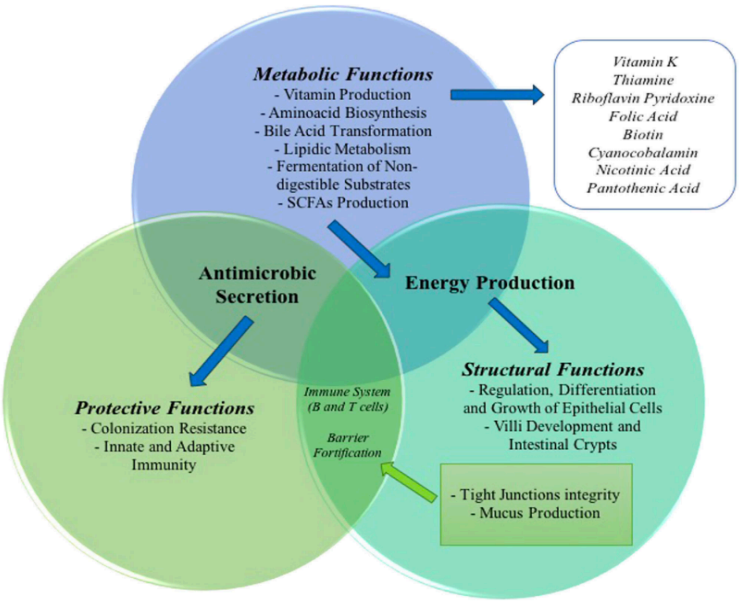


Figure 3: Gut microbiota functions (Concept map made by A. Pascale) (3).

It has been estimated that these functions are applied by at least 40,000 bacterial strains, which is an average of at least 9.9 million non-human genes added to the body ones (1).

The microbiome defines the collection of these bacterial genomes and non-human genes. New projects have been created with the goal of understanding these symbionts' key roles and their impacts

on our health. This symbiont term is used to refer to an organism living in a symbiosis, or in a relationship or interaction between two dissimilar organisms. It helped also to specify the definition of microbiome, but specially showed the differences and the complications in terminology of: "microbiota" and "microbiome" (1,5)(Figure 4).

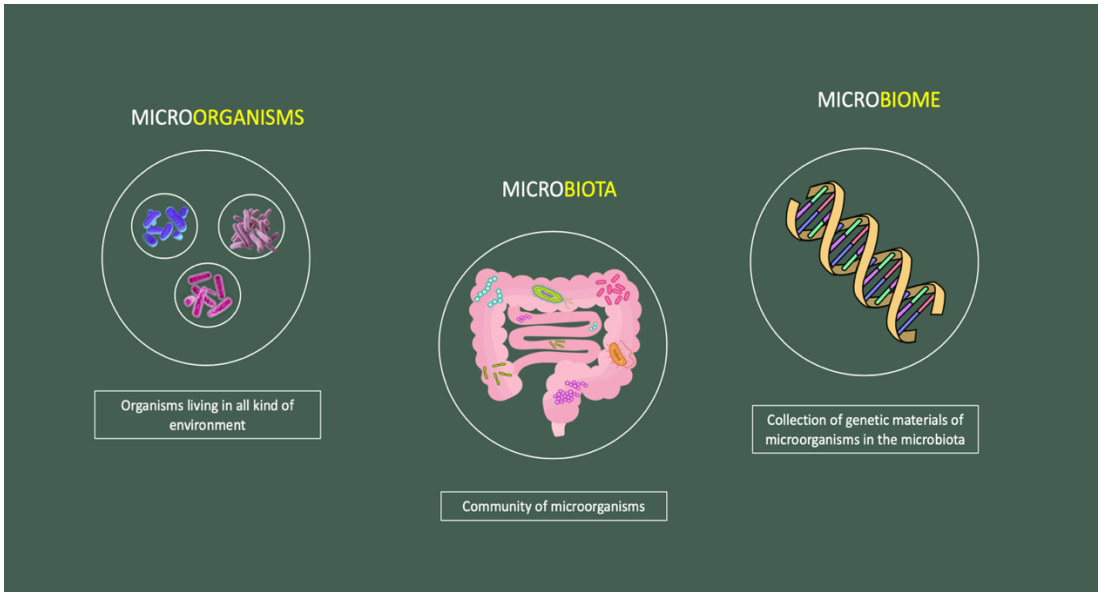


Figure 4: Differences between Microbiota and Microbiome (Illustration inspired by the European Food Information Council (EUFIC))

New studies are reaching us to understand these concepts, so the definition of the OTUs (Operational Taxonomic Units) was created to classify micro-organisms in groups that built the microbiota (5).

Our knowledge on the microbiota came from the evolution of DNA sequencing. One of the most important breakthroughs was the "characterization of the human microbiome and the analysis of its role in human health and disease" made by the NIH (National Institute of Health) (1).

Therefore, the human microbiome consists of all the micro-organisms, in a genetic scale, present through the body, the membranes and the gut. The microbiome links with the immune system to create a balance between the defensive mechanism and the symbiotic microbial factors (6).

This link helps to observe the changes of the bacteria composition and amount during changes occurring in the body, creating a possible dysbiosis (7).

Dysbiosis is defined by a structural and functional modification of the microbiome, which leads to the unbalance and the break of homeostasis in the body. It can link with diseases and inflammation through our system. It is an effective way for medicals and researchers to compare both modulations and links with oral and systemic diseases (8) (Figure 5).

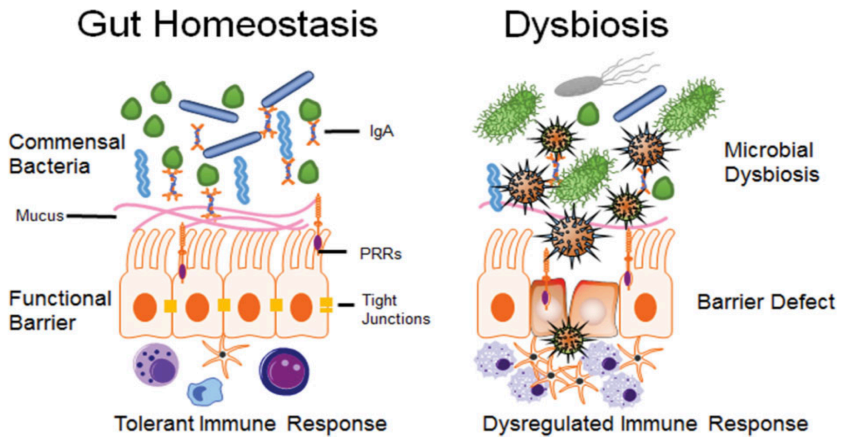


Figure 5: Illustration of a gut homeostasis and dysbiosis (53).

The transitions that induce dysbiosis is the subject of numerous studies. Marsh, in 1994, proposed the presence of a direct and dynamic relationship between the environment and the species present, in terms of diversity and abundance. For example, bacteria in dental plaque are frequently compared with environmental agents that will compromise the balance of the oral microbiota and induce dysbiosis (8).

1.2 Oral Microbiota

1.2.1 Oral cavity and description of oral microbiota

The oral microbiota contains, based on sources, an important segment of the human microbiota with hundreds to thousands of diverse species (1). The oral cavity is the first part of the buccopharyngeal - gastrointestinal tract, but has its own species, different than the rest of the gastro-enteric microbiota in the esophagus, stomach, intestines and colon (1).

In the neonatal period, the microbiota develops at the same time as the immune system. In the uterus, the oral cavity of the fetus is sterile, and the first colonization are made at birth by the mother's vaginal, intestinal and skin microbiota, then by the environment, as in the gut microbiota (9). We can find several ecosystems called “niches” colonized by different microorganisms. These niches are lived by a minimum of 700 species located into diverse areas of the oral cavity (2)(Figure 6).

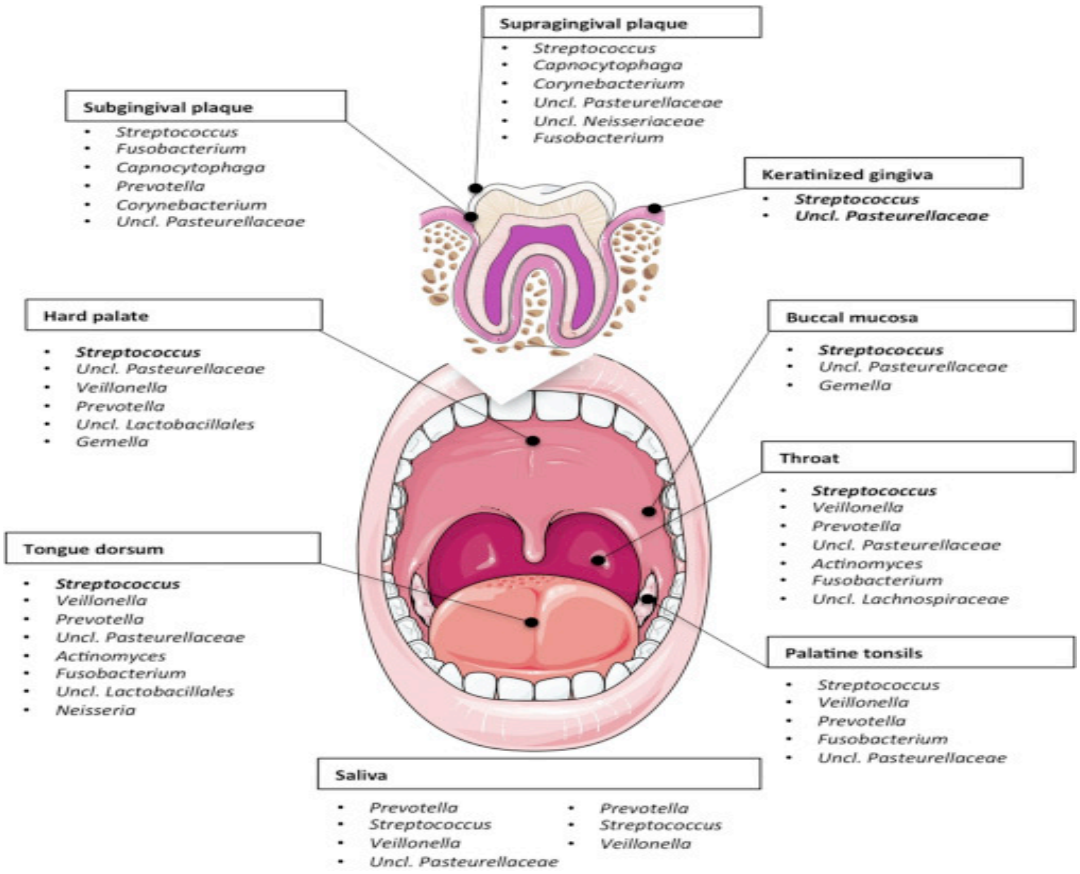


Figure 6: The oral bacteriome (Illustration made by B. Sampaio-Maia) (35).

The most important “niche” that occupies a great part in the oral bacteriome is the saliva, that can contain approximatively 10 times 8 the number of microbial cells (2).

Microorganisms are constantly swallowed, which corresponds to about 5 g of bacteria that disappear every day in the stomach and move to other floras. The bacteria present in the saliva do not multiply, unlike the dental plaque. We can therefore, consider that saliva does not have its own resident microbiota, and bacteria present in it, result from the desquamation of oral tissues, mainly the tongue (1). It is possible also to find bacteria in the soft tissues or the hard ones like dental biofilm - plaque -, or even hard metals like complete or partial removal dentures (1,6).

The oral microbiome can form structures called biofilms (10). Microbial biofilm is defined as an accumulation of microorganisms, different species, adhering to a surface generally in relation to an aqueous environment. It is formed by microorganisms contained in saliva and gum fluid. Microbial cells develop within a matrix made up of biopolymers and excrete polysaccharides by microorganisms during their cell cycle (Figure 7). The architecture of this biofilm is complex and the organization of different microorganisms does not happen randomly. This depends particularly on their nature and their varying affinities of co-adhesion. Also, it is important to note that the biofilm is in constant interaction with the environment and can therefore, vary depending on it but also, depending on the external physico-chemical conditions and the activity of microbial metabolisms (11).

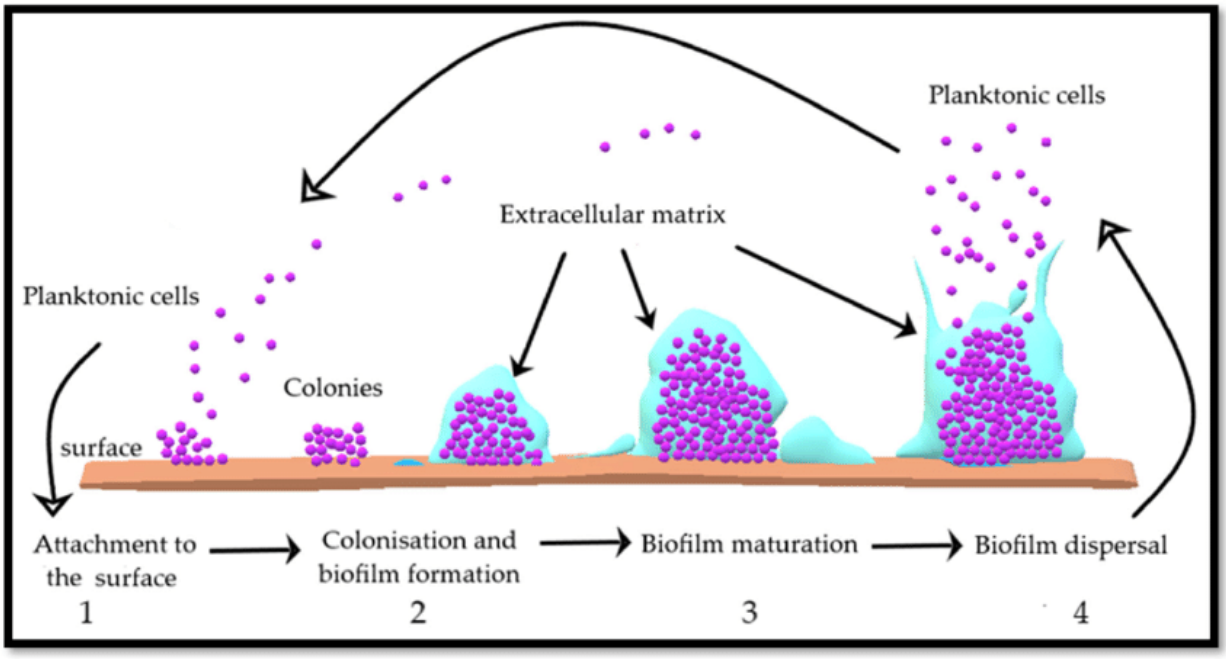


Figure 7: The different stages of microbial biofilm formation (Illustration provided by M. Idrees) (55).

1.2.2 Composition of the oral microbiota at glance

The physiology of the oral microbiota associates with the host in different proportions. The oral microbiome presents itself mostly in biofilm form. It is an important part of the oral homeostasis, conserving the buccal cavity, and avoiding disease development (12).

It helps specially for the metabolic, physiological and immunological roles, for example aliments' digestion (12). Microbiota assists also in the formation of energy and the development of the immune system. By the time, they create the possibility to protect and barrier tissues of the body (12). At the level of bacterial species, the composition of the oral microbiota is very variable from one individual to another, but studies showed a set of bacterial genes shared in the oral cavity of a majority of healthy individuals, called « core oral microbiome » (10).

A common point is not found at the microorganism's amount, but at the functions that bacteria carry. It is considered that the biological functions associated with the core microbiome are rather

redundant despite a high bacterial diversity (6). The “variable microbiome” is specific to an individual. The latter develops in response to a unique lifestyle, diet, environment, and as a function of phenotypic and genetic determinants (6).

The oral microbiota contains a set of diverse microorganisms. One part is common to the different microbiota of the body, for example the fungus *Candida albicans* present in the intestinal microbiota and the vaginal microbiota, the bacterium *Helicobacter pylori* which is also found in the stomach, or the anaerobic bacterium *Porphyromonas gingivalis* found in periodontal pockets and "migrate" into the body via the vascular system. But, another part of these bacteria is quite specific to the oral cavity. We will thus find many aerobic bacteria due to the air breathed, as well as anaerobic bacteria that hide in pockets that are difficult to access and are therefore more difficult to eliminate (2).

Saliva microbial composition is identical to soft tissues, even-though their colonization differs with dental plaque. Studies proved that, in the saliva, a considerable amount of bacterial taxa were found, and *Firmicutes* (*genus Streptococcus* and *Veillonella*) and *Bacteroidetes* (*genus Prevotella*) were the most represented ones (9). In the soft tissues’ surfaces, the oral mucosa is constantly colonized by bacteria. Cheeks’ surfaces and palate have different monolayers of bacteria emanating routinely. But in contrary, the tongue presents multilayers of them. It has been shown that a great density and diverse organisms occupy the tongue confronting mucosal surfaces. Bacterial dominant species on the dorsal part of the tongue were *Streptococcus salivarius*, *Rothia mucilaginosa*, and uncharacterized species of *Eubacterium* (strain FTB41) (9). Whereas, the microbial composition of supragingival plaque shows some differences with the subgingival plaque ones. *Firmicutes* and *Actinobacteria* (*genus Corynebacterium* and *Actinomyces*) dominate the supragingival plaque, while subgingival plaque has shown the presence of almost the same bacteria adding *Prevotella* and more *Streptococcus* (9).

Due to its anatomical situation and its physiological role, oral microbiota is the most complex system in the human body. It has a necessary role in the manifestation of oral diseases but it

communicates with other microbiota, in particular the digestive microbiota, but also with the respiratory microbiota due to the anatomical proximity (2).

Among these bacteria, some settle very early (*Streptococci* in particular: *S. oralis, S. mitis*, *S. gordonii*), others have a preponderant role (*F. nucleatum*) because they play the role of a bridge between the colonizing bacteria of the beginning of biofilm formation and those which adhere later (*A. actinomicetemcomitans*, *P. intermedi*, *P. gingivalis*, *Spirochetes*...) (13).

Bacteria counts for the head part of oral microbiota, and the comprehension of oral bacteria's composition are due to different cultures and metagenomic techniques that will be seen below (9).

Even if the perspective of oral microorganisms has gradually expanded and improved in these past years, a great number of bacteria that cannot be observed or cultivated are present. Many microorganisms have demands for survival, such as determined nutrients, precise temperature, pH levels, and interaction with other microorganisms within their colonies (9).

Fungi are colonizing mostly the oral cavity. They also take part of the healthy oral microbiota. It has been detailed and searched that fungal species are also present in healthy subjects (9). It was also observed that the *Candida* species were the most frequent, followed by *Cladosporium, Aureobasidium, Saccharomyces, Aspergillus, Fusarium,* and *Cryptococcus* (9).

Archaea composes an inconsequential part the oral microbiota and contains less breeds to introduce them in the ecosystem as the ones above. The found species are *Thermoplasmatales* (9).

Viruses present in the oral cavity are associated to conditions. *Herpes simplex virus* (HSV) can give primary herpetic gingivostomatitis, mucocutaneous orofacial disease and recurrent lesions on the face and lips for example. In addition, HIV infection can cause several oral manifestations in an indirect way, such as oral candidiasis, oral hairy leukoplakia, linear gingival erythema, necrotizing ulcerative periodontitis and Kaposi's sarcoma (9).

The classifications of these microorganisms are done by different multiple techniques, that improved during time, starting from the most basic and primitive ones to the new generations (‘’next gen’’).
It will be reviewed in this bibliographic review, the new methods of studying these bacteria and their colonies.

1.3 Methods of studying of the oral microbiota

In the 1880’s, Robert Koch studies helped a lot for the discoveries of the existence and the dominance of microorganisms, along with the evolution of new study techniques. Culture independent methods were applied to studies of the microbiome, so new microorganisms’ breeds were discovered with more details about microbiota’s changes (10).
It is possible to characterize the colony by:

- Its configuration: DNA and metagenome,
- Analyzing transcripts: metatranscriptome,
- Analyzing proteins: metaproteome,
- Analyzing terminal productions coming from the action of the microorganism’s colony (10).

In the 70’s, Frederick Sanger created the ‘DNA sequencing technology’. It was based on chain-termination method (also called the Sanger sequencing). After it, Walter Gilbert found another sequencing technology based on changes in the DNA by chemicals and successive cleavage at specific bases. High efficiencies allowed Sanger’s sequencing to be accepted as the main technology in the “first generation” of sequencing applications (14).
Traditional microbiology has focused for a hundred years on the study of species as isolated units in cultures. The genome of a bacterial strain grown in the laboratory was then analyzed (14).
Among 50 to 60% of oral bacteria are considered to be uncultivable. They cannot be cultured for analysis due to the demand of particular nutrients, sensitivity to oxygen and its dependency to

surrounding organisms. Scientists therefore encounter great difficulties in experimentally reproducing the complex microenvironment of the oral cavity (10).

1.3.1 16S rRNA amplicon sequencing and metagenome shotgun sequencing (WMS):

To come through these constraints, the 16S ribosomal RNA's sequence study was the most used technique. 16S rRNA is an RNA component of 30S small subunits in prokaryotic ribosome. Their sequence is widely used in phylogeny - study of the parent genetic lines - to reconstruct the evolutionary history of organisms. These genes are present in multiple copies within each organism and is universally present in all prokaryotic organisms, and with the use of universal primers it has been possible to characterize species in a provided sample, even if they are unidentified. Using 16S rRNA amplification, cloning and Sanger sequencing has shown that the oral microbiome is composed of around 700 species belonging to 13 different phyla (10).

Moreover, the 16rRNA sequencing has been generally an important tool in metagenomic studies, giving greater study models. The 16S rRNA gene is familiar to all prokaryote cells and archaea, and has greatly preserved locations, which concludes that it is an important gene's marker for the use of universal primer sequences to remove the 16rRNA for sequencing. In the metagenome shotgun sequencing (WMS), the fragmentation of the DNA occurs several times, allowing short sequences to a parallel reading, and reuniting into genomic sequences. It permits the discovery of species, counting also functional annotations of the microbiome (15).

1.3.2 Next generation sequencing, « Nextgen »

Next-generation sequencing (NGS) technologies gave researchers the tools that permit the profiling of the microbiomes and metagenomes at uncommon deepness, not reachable with any of the remaining techniques (12). The first NGS application (454 Life Sciences) was introduced by Roche

in 2005, based on pyrosequencing, which is the pyrophosphate release on nucleotide adding, instead of chain termination with dideoxynucleotides, seen in the Sanger sequencing (12).

We know that bacterial species and their entity were defined by the genomic DNA, which makes the DNA sequencing important to research structures and functions of cells, and the interpretation of life mysteries. This is why these techniques help biologists and health care workers in a great range of works as cloning, breeding and discovering pathogens (14).

However, Next-gen techniques should also be, in different factors, faster, more specific and specially at a lower cost (14).

But in the past years, next-generation sequencing (NGS) techniques have remodeled all studies of the microbial's diversification. This has let the completion of larges sequencing projects, in shorter durations - only in a few days or sometimes hours (14).

The most famous NGS technologies are:

- Roche 454 pyrosequencing
- Applied Biosystems
- Illumina NGS technique

To interpret them correctly, their analysis requires bio-informatics characteristics counting data like quality controls, aligning and mapping genomes, filtering the quality and standardization across samples and populations (10).

Bioinformatics are defined as the biological study and terms of molecules by applying informatic techniques, such as mathematics, computer and statistics. They helped to observe and organize these molecules information on a greater scale (16).

Its aim is to classify data to allow researchers to reach existing information and submit new entries produced. They are also used to improve the analysis of data, for example protein sequencing, by comparing the past characteristics (16).

OBJECTIVES

This thesis' main objectives are to describe and review the oral microbiota as a predictor of oral and systemic diseases. For this aim, the following objectives are proposed:

1. To study the oral microbiota, biomarkers and the techniques used to sequence the DNA as an effective predictor for oral and systemic diseases.
2. To study the relationship between oral microbiota with oral diseases.
3. To study how changes in the oral microbiota can be related with systemic diseases like Diabetes and Lupus Erythematosus.
4. To characterize the most important microbiota biomarkers associated to oral and systemic diseases.

MATERIAL AND METHODS

This bibliographic review was carried out through the review of numerous scientific articles found in impact journals. Electronic databases have been used such as the Crai Dulce Chacon Library of the European University of Madrid, ScienceDirect, PubMed, Google Scholar and Medline.

To define and explain the relation between the oral microbiota functions, characteristics, compositions and oral and systemic diseases, data was collected from the medical journal Advances in Experimental Medicine and Biology, as well as scientific articles, allowing us to understand the bases of the oral microbiota and the oral and systemic diseases. Languages included were English, French and Spanish.

Key words were used to perform the general research: oral microbiota - systemic diseases - oral infections - oral biomarkers – bacteria - viruses - fungus - protozoan - intestinal flora - buccal flora

- The **inclusion criteria** of our research were:

- Date from 2010 to nowadays
- Language: Spanish, English, French

- The **exclusion criteria** were:

- Small samples
- Children patients (under 13 years old)
- Before 2010

RESULTS & DISCUSSION

The oral microbial community is an important indicator of oral and general health. It became a key focus of the Human Microbiome Project (HMP) and the "National Individual Microbiome Testing Project" due to its importance in oral and general health (17).

Microbiota can be used as biomarkers that is defined as “a characteristic measured as an indicator of biological processes, pathologic processes or responses during a therapeutic intervention” (60). Biomarkers can be obtained from a tissue biopsy or a liquid biopsy (blood, urine, saliva, etc.) and subsequently molecules as DNA, RNA, proteins, metabolites, bacteria, etc. or can also be used to predict oral or systemic diseases (18).

In research and clinical therapies, they can be used to diagnose diseases or predict their risks. They help also to monitor healthy people for early signs of the disease and determine whether a treatment is effective or not. Moreover, biomarkers permit to researchers to have a global view of the events and changes that are constantly occurring within a cell, and in the pharmacological field to produce safer medications by predicting the potential for side effects earlier (18,19). Nowadays most diagnosis - especially *in vitro* ones -, are based on biological markers. The first step in developing a diagnosis is to identify one or more biomarkers associated with a normal or pathogenic biological process (18). Methods of studying on genomic, transcriptomic, proteomic or metabolomic biomarkers make it possible to develop a precise diagnosis. This is why their finding helped for the improvement of a field called “precision medicine”. It requires the identification and clinical validation of a large number of biomarkers to predict the course of the disease. In order to then, follow it and identify the different subpopulations of patients and predict the response of the disease (18).

Individualized diagnosis and treatment of related systemic diseases is an urgent requirement for effective prevention and treatment of oral infectious diseases and oral-related systemic diseases (20).

1.1 Microbiota in oral diseases:

1.1.1 Dental caries

Dental caries is known to be oral pain and tooth loss primary cause. They are the most common oral disease in the oral cavity. It is due to the acid production of bacteria and fermentation of carbohydrates that can produce the destruction of dental tissues. High intake of carbohydrates frequently leads to increase acid production, and decrease the buffering capacity of the saliva. Which leads to an environment with a low-pH, and so augmenting the colonization of bacteria on teeth (9). *Streptococcus mutans* and *Lactobacillus* have been studied as specific pathogens and markers in dental caries. Some other bacteria, produce ammonia from urea and increase the pH, which plays a role in the pH homeostasis and may modulate the advancement of dental caries (9).

Two studies were reviewed to compare the different composition of a normal microbiota and an altered one in dental caries of young adults - teenagers.

In the first one made by Becker *et al*., the presence of bacteria was compared by sequencing the 16S rDNA ribosomal subunit of bacteria (Nextgen) (21).

The association of 23 known bacterial species or species groups with caries was determined by a reverse capture checkerboard assay taking into account that *Streptococcus mutans* has already an epidemiologic link with caries. For this study, lesions were collected from 30 subjects with caries and 30 healthy controls. A hybridization blot – also called Southern blot which looks for fragment of DNA in an electrophoresis gel – was performed on the bacterial population distribution in these subjects. Samples with caries and healthy subjects were used for clonal analysis as described below and have been observed and compared (21)(Figure 8).

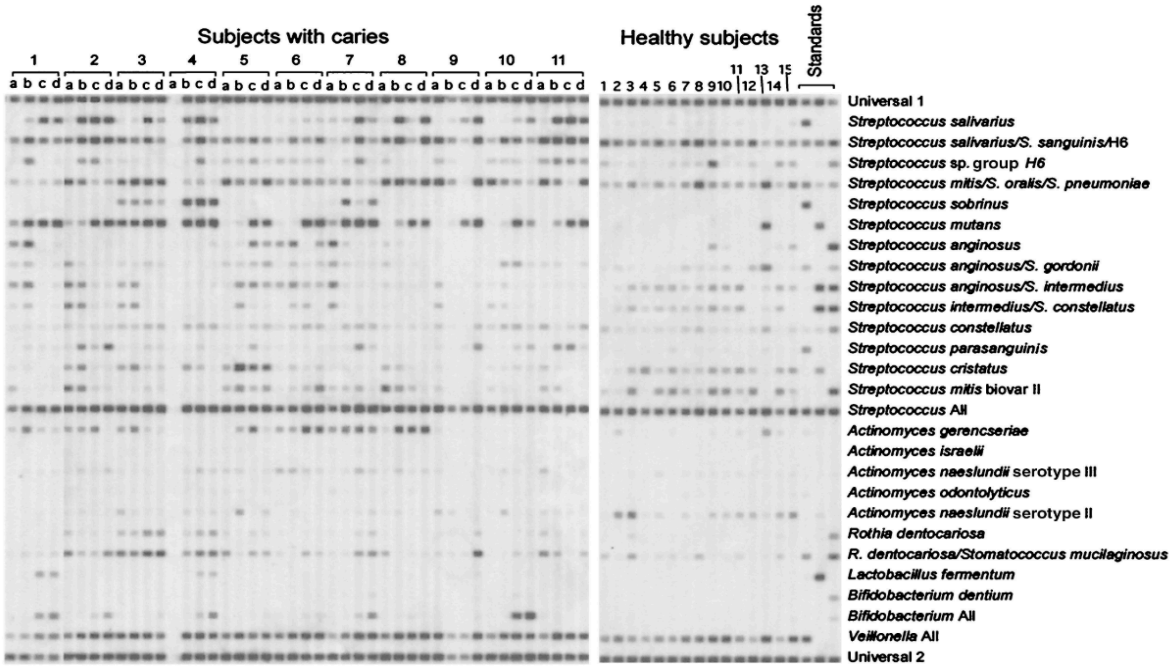


Figure 8: Capture checkboard hybridization blot for healthy teeth subjects and subjects with caries (Made by Becker) (21)

The authors observed in this study, the presence of a link between species already present in a healthy tooth, as *Streptococcus sanguinis* and *S.mitis,* and other like *Streptococcus mutans* present almost exclusively in dental caries (21)(Figure 9).

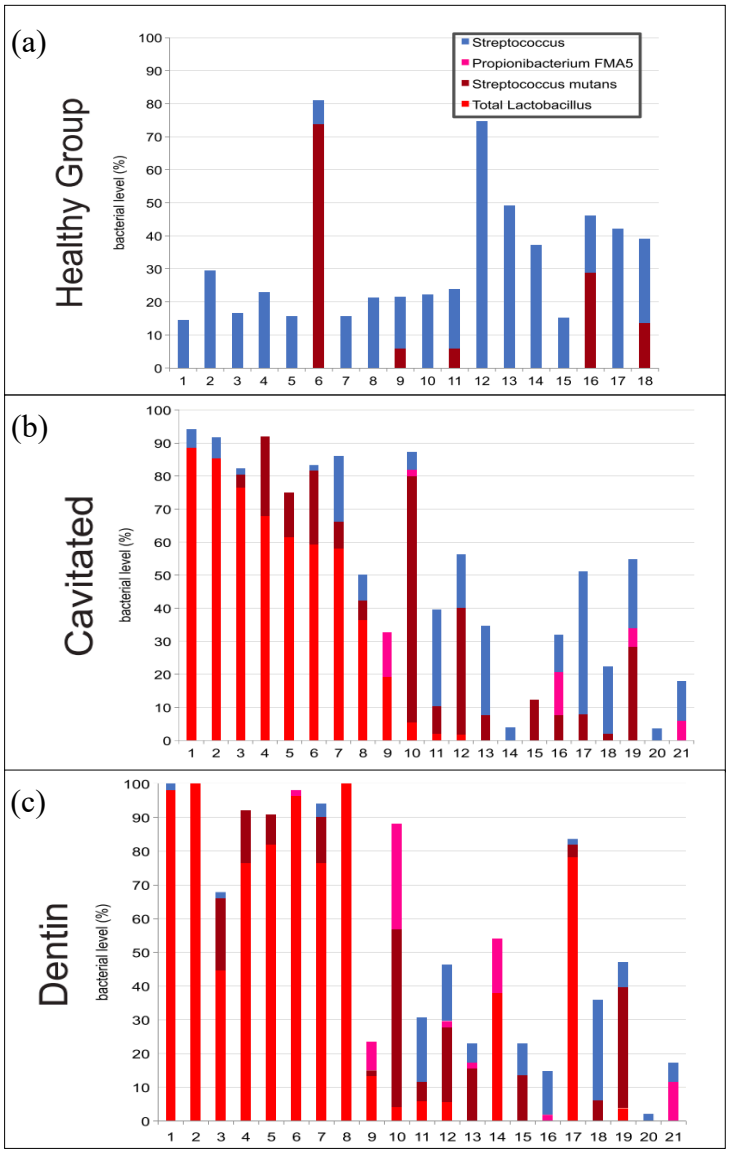
Species or phylotype	No. of times clones were recovered from the following subject and site:					
	Healthy	Caries				Total
		Intact enamel	White spot lesions	Cavitated lesions	Dentin	
Streptococcus mutans			11	30	19	60
Veillonella dispar or *V. parvula*			4	21	7	32
Streptococcus sanguinis	25	1	1			27
Bifidobacterium sp. clone CX010		2		4	11	17
Corynebacterium matruchotii		11				11
Abiotrophia defectiva	7	1				8
Leptotrichia buccalis		7	1			8
Actinomyces sp. clone AP064			3	2	2	7
Fusobacterium animalis			7			7
Streptococcus mitis or *S. oralis*	1	2		1	2	6
Lactobacillus fermentum				4	2	6
Neisseria mucosa	6					6
Streptococcus salivarius				4	1	5
Selenomonas sputigena			1	4		5
Streptococcus mitis biovar II	2	2				4
Actinomyces gerencseriae			3	1		4
Leptotrichia sp. clone DE081		3	1			4
Leptotrichia sp. strain A39FD		1	3			4
Fusobacterium nucleatum subsp. *polymorphum*		4				4
Streptococcus anginosus		1	2			3
Gemella haemolysans			3			3
Leptotrichia sp. clone BU064	3					3
Capnocytophaga granulosa	1	2				3
Abiotrophia adiacens			2			2
Gemella sp. strain 933-88			2			2
Additional species or phylotypes[a]	4	17	13	4	6	43
Total no. of species or phylotypes	11	29	27	13	13	68
No. of novel phylotypes	1	7	2	0	1	10
Total no. of clones	50	58	61	74	51	294

Figure 9: Microorganisms clones identified from a healthy subject and a subject with caries (21).

Authors concluded that some species might play an important role in the initiation of caries and others play a key role in the development of more pathogenic deep caries. In fact, the amount of *Streptococcus sanguinis* drops drastically in the evolution of a decay; *Actinomyces gerencseriae, Fusobacterium animalis* and *Corynebacterium matruchotii* colonization were found in initial decays – intact enamel or white spot lesions. Finally, *Bifidobacterium, S. salivarius, S. constellatus, S. parasanguinis, Lactobacillus fermentum* and *Veillonella dispar* colonize, in a smaller or greater amount than the present bacteria, the oral microbiota at a high infected level of decay – cavitated lesions and dentin exposure (21).

These changes bring to conclusion, even if further studies for bacterial quantitation are needed, the importance to analyze biomarkers in the oral microbiota, possibly contributing in the prevention and treatment of dental caries (21).

In another study made by Gross *et al.*, 21 subjects with caries and 18 healthy controls were chosen in an intervention study with young adolescent with young permanent teeth, comparing different subjects depending on the decay level in the dental tissues: healthy enamel, deep enamel cavitated lesions and carious dentin (22). The 16S rRNA genes were amplified from the DNA using PCR amplification technique and then observed by hybridization blot. Levels of *Streptococcus spp.*, *Streptococcus mutans, Propionibacterium FMA5* and *Lactobacillus* at each level of caries experience were correlated (22) (Figure 10).



Figures 10: (a), (b) and (c) Levels of Streptococcus spp., Propionibacterium FMA5, Streptococcus mutans, and Lactobacillus spp. at each level of caries (tables provided by E. Gross) (22).

These studies from Gross *et al.* have observed significantly higher levels of S. *mutans* in subjects with caries than in the healthy ones as Becker *et al*, and they confirmed its already known role as a biomarker in oral caries. In addition, they showed that in deep decays and deep infected dental tissues, the microbiota changed significantly (22). By the help of the amplification of 16S rRNA, profound enamel lesions and dentin cavities are correlated with bacterial taxa changes. The predominant presence of *Streptococcus spp.*, a great amount of *Lactobacillus*, almost displacing *S. Mutans'* locations, are noticed (22).

It confirms that there is a change of the microbiota between a healthy patient and one having caries. Time is also a factor observed in this study, because if species colonize a tissue for a long term, new bacteria invade the flora taking the place of the ones already present and creating new colonization with their own functions (22).

Limitation in the study of species were admitting that the only bacterium that served as a biomarker in the evolution of dental caries, was *S. mutans*. Authors of both reviews suggested that a closer look of the link between acid-base reactions and 16S-based bacteria with the amplification techniques, as *Lactobacillus, Actinomyces, Fusobacterium and Bifidobacterium* could be helpful in the prevention of dental caries' evolution and their treatment (21,22).

1.1.2 Periodontal diseases

Periodontitis is an inflammation of the periodontium - ligaments surrounding the tooth - due to bacteria that infiltrate the roots of teeth and the pockets or spaces in surrounding gums. They can produce bleeding, loss of bone and tissues supporting the teeth. The microbial community shows variations that can cause destructive inflammation and loss of bone. The inflammation can occur when bacteria meet leukocytes in the epithelium or connective tissues, dropping the patient's immunity; and can originate from dental plaque (9). Dental plaque is defined as a diverse microbial community found on the tooth surface embedded in a heavy matrix of polymers of bacterial and salivary origin. Plaque that becomes calcified is termed calculus or tartar. It can attach to the dental surface in two different areas creating the supra gingival plaque - biofilm placed in tissue above the gingiva - and the sub gingival plaque (23).

Periodontal diseases are characterized by a massive cluster of bacteria. Analysis made by the pyrosequencing techniques proved the presence of bacteria like *Porphyromonas gingivalis* (*P. gingivalis*), *Treponema denticola*, *Prevotella* and *Fusobacterium*, but introducing also, the presence

in the oral cavity of *Streptococcus* and *Actinomyces* operating together normally during dental caries and showing the easy “attraction” of microorganisms in this disease (24).

It is one of the most infectious disease in the human body, having a link - by its great number of bacteria in its ‘’healthy’’ microbiota - with other diseases and other parts of the human microbiota (9).

Also, in subgingival colonization, the presence of yeast and specially *C. albicans* was observed to be linked with the periodontitis’ severity. *Herpes viruses*, including *Human cytomegalovirus* (HCMV), *Herpes simplex virus* (HSV), *Epstein–Barr virus* (EBV), and *Human herpes virus* (HHV), have been as well discovered in subgingival bacteria (9).

Greffin *et al.* studies have collected two set of samples: healthy controls compared with patients suffering from periodontitis. Pyrosequencing of the 16S rRNA has been performed to compare in a broader scale both microbiota (25) (Figure 11).

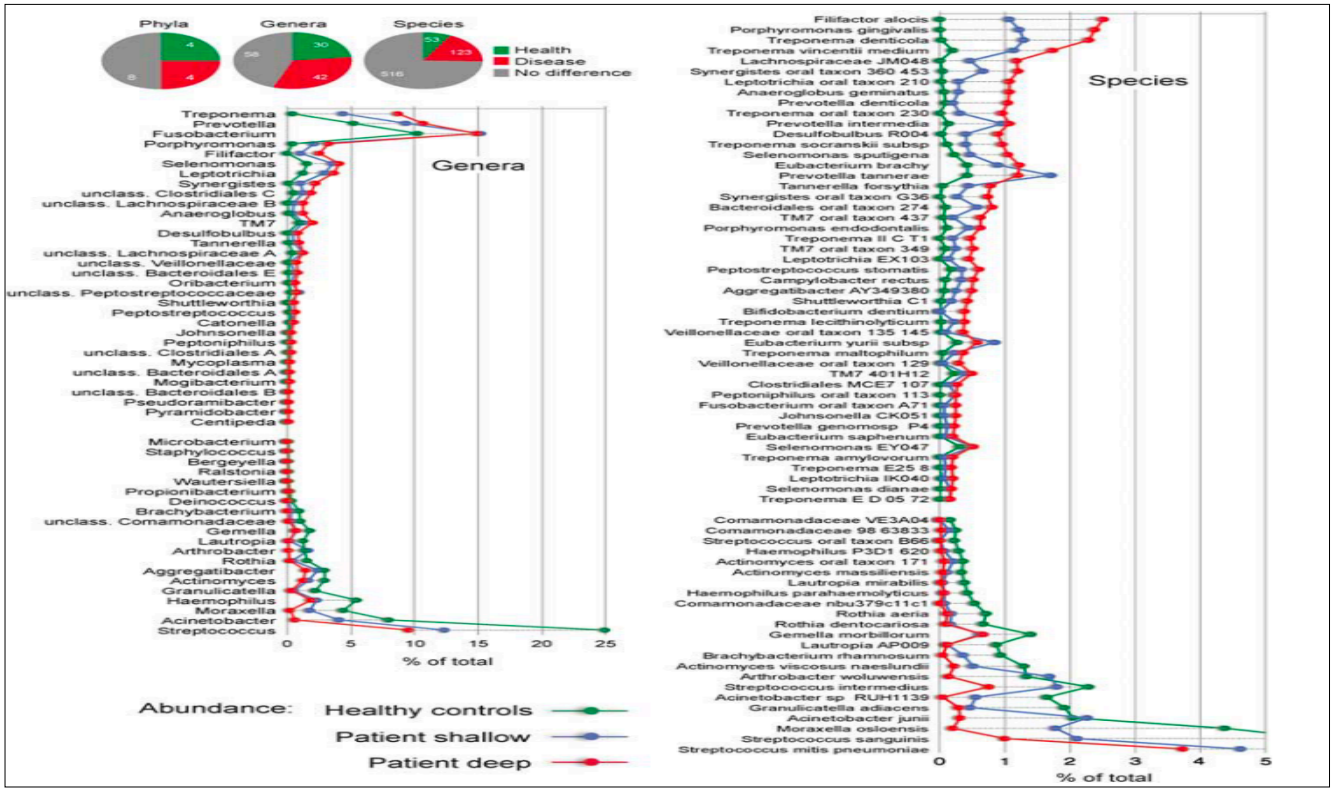


Figure 11: Bacterial taxa in healthy periodontal patients and periodontitis cases (25).

The authors highlight that a healthy periodontium normally present mostly a Gram-positive community with, or not, a small inflammation response. It has been shown through this study, that severe periodontitis brings a great modification of the oral microbiota (25).

The main changes in a periodontal disease are the modification in the amount of Gram positive and Gram negative. The small increases of gram negatives are due to the decreases of two main species, *Streptococcus mitis* and *S. sanguinis*, present mostly in healthy patients, letting place for new colonizing bacteria to colonize as *Filifactor Alocis*. This bacterium appeared to be prevalent and strongly diseased, associated with the periodontitis, making it a biomarker for periodontal diseases. The research demonstrated as well, that *Porphyromonas* and *Treponemas* increased (25).

All the species might be used, in the future, as potential markers to prevent this disease and provide a good prognosis for future periodontitis (25).

1.1.3 Recurrent aphthous stomatitis

Recurrent aphthous stomatitis (RAS) is the most common disease present in the oral mucosa. The disease is presented by constant and painful round or ovoid inflammatory oral ulcers with yellow or grey floors. Host genetic, nutritional deficiencies or even systemic conditions in the body are the most common factors in RAS, even though it can be associated with the presence of microorganisms in the mucosal and salivary microbiota, whom will modulate the inflammatory responses (9,26).

Hijazi *et al.* compared three samples of: healthy control patients, healthy sites from individuals having RAS and others having RAS but extracting ulcerated spots. The extraction of DNA was done followed by bioinformatics analysis (27) (Figure 12).

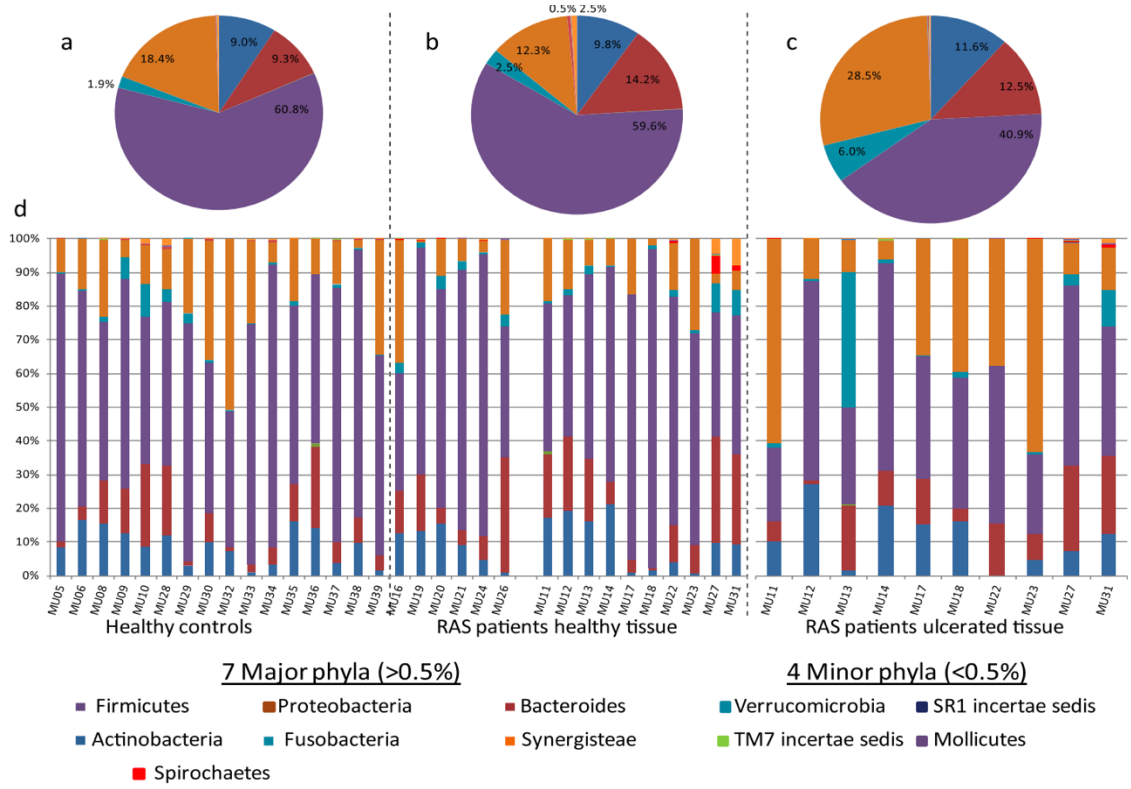


Figure 12: Samples of Hijazi *et al.:* (a) healthy controls (b) healthy tissue in recurrent aphthous stomatitis (RAS) patients, (c) ulcerated tissue in RAS patients. (d) Individual patient sample diversity at phylum level as stacked bars (Tables made by K. Hijazi) (27).

Authors' comparison concluded that *Prevotella* and *Actinomyces* were present only in the mucosal microbiota of RAS patients but not in healthy person. They revealed the decrease of *Firmicutes* and the increase of *Proteobacteria* in RAS in ulcerated sites. Higher abundance of total *Bacteroidales* in RAS patients with healthy sites over healthy controls were found: *Porphyromonadaceae* was associated with their periodontal health and *Veillonellaceae* predominated in ulcerated sites over the healthy ones. In addition, lower levels of *Streptococcaceae* were found in ulcerated sites of patients with RAS (27).

This study demonstrated that these changes suggest a key role of the oral microbiota in the beginning of RAS, but showed its limitations to provide data on causality (27).

1.1.4 Oral tumor

Oral squamous cell carcinoma (OSCC) is a malignancy pathology that induces from the epidermis of the oral cavity. It counts as 90% of oral cancers. Oral carcinoma surfaces harbor a high number of aerobes and anaerobes bacteria. Through times and from previous researches, saliva has been used for its functions and composition as a potential marker to prevent cancer and because some species coming from the digestive tract and colonizing the oral cavity are found inside (9).

Mager *et al.* provided a study based on the culture-independent 16S rRNA in patient cancer-free (OSCC-free) and patients with OSCC positive, comparing within the saliva of each participant. A total of 29 cancer free patients were chosen in one sample and 45 diagnosed OSCC patients placed in another sample by biopsy (28).

They have shown an apparent difference in the distribution of the microbiota between tumorous and non-tumorous mucosa. *Streptococcus sp. oral taxon 058*, *Peptostreptococcus stomatis*, *Streptococcus salivarius*, *Streptococcus gordoni*, *Gemella haemolysans*, *Gemella morbillorum*, *Johnsonella ignava* and *Streptococcus parasanguinis* were associated with tumor sites. Authors also showed also an increase of *Capnocytophaga gingivalis*, *Prevotella melaninogenica* and *Streptococcus mitis* in the saliva of subjects with OSCC (28) (Figure 13).

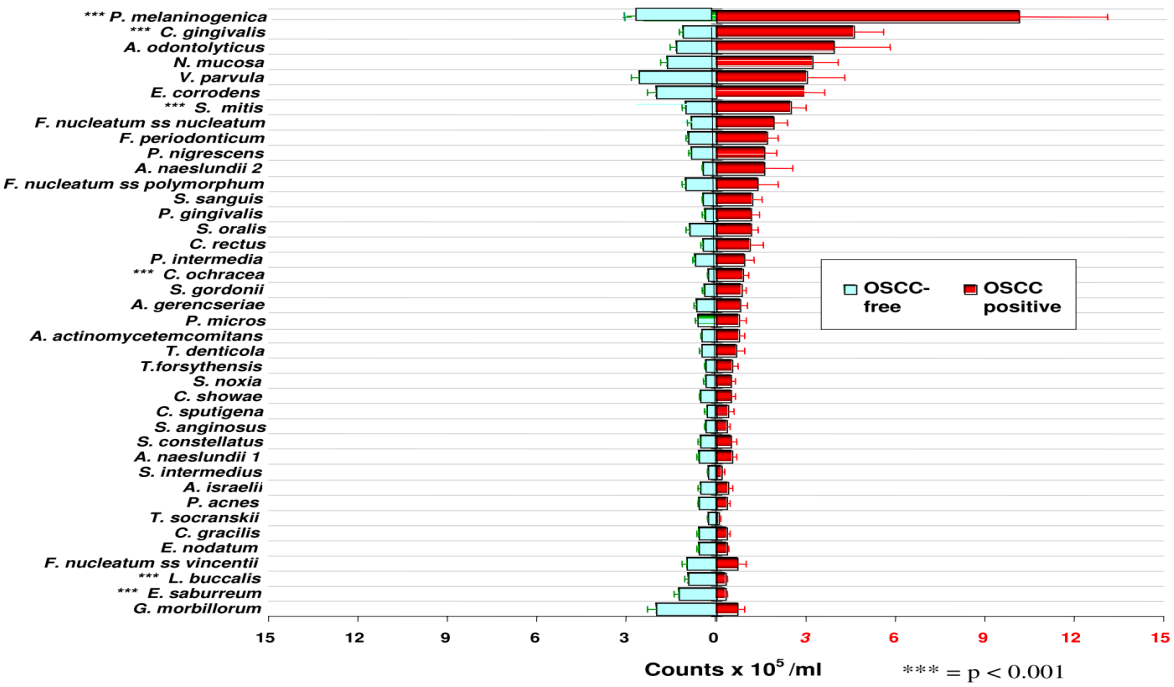


Figure 13: Salivary count of 40 test species in OSCC-free patients and OSCC-positive patients (figure provided by Mager) (28).

These differences between samples with OSCC and healthy patients indicate the role of microbiota as a biomarker for monitoring oral cancer advancement and frequency. But due to lack of studies and the complexities of carcinomas, the functions of oral microbiota in oral cancers are still not characterized and further studies are needed (9).

It is also known that bacteria are able to provoke some chronic inflammation. These mediators produced cause proliferation, mutagenesis or angiogenesis of cells and could permit a potential prevention of oral tumors within further studies and improved techniques in a near future (9,28).

1.2 Microbiota in systemic diseases:

1.2.1 Diabetes

Diabetes is a disorder in the absorption, use and storage of sugars from food. This results in a high level of glucose in the blood - also called hyperglycemia. The blood sugar level rises after food intake. The pancreas senses the increase of glucose in the blood sugar and secretes insulin. Insulin allows

glucose to enter the body's cells: in muscles, in fatty tissue and in the liver where it can be processed and stored, producing a decrease in the blood (9). Two types of diabetes are found:

- Type 1 diabetes: which affects about 6% of diabetics and formerly called insulin-dependent diabetes (IDD), is usually found in young people: children, adolescents or young adults.
- Type 2 diabetes which affects 92%, usually appears in people over the age of 40. Overweight and obesity are the main causes of type 2 diabetes in genetically predisposed people. Two irregularities can cause for hyperglycemia: either the pancreas is still making insulin but not enough, called insulinopenia; either this insulin acts badly, we speak of insulin resistance (9).

It has been shown that, in the oral cavity, periodontitis can frequently be one of diabetes' chronic complications due to the immunosuppressive status they acquired. Uncontrolled or poorly controlled diabetes was associated with increased susceptibility to oral infections, including periodontitis. Systemic diseases have an important influence on periodontitis, with diabetes mellitus having one of the more potent relations. Type 1 and type 2 diabetes mellitus (T1DM and T2DM) affect the periodontium in children and adults, with an increase in periodontal inflammation similar to the increased inflammation in other tissues affected by diabetes (29).

Campus *et al*. studied 212 individuals divided in two different group, patients with uncontrolled diabetes Type 2 and control ones, and compared them with different factors linked with their periodontitis. The authors analyzed the presence and stage of periodontitis, periodontal plaque, bleeding and amount of calculus in both groups. In order to see if any bacterial modifications were present, they extracted samples of *P.intermedia, T.forsynthensis and P. gingivalis* - most common bacteria found in periodontal pockets in periodontitis - and carried out the amplification of their 16S rRNA by PCR amplification, from the subgingival plaque microbiota; in order to describe and compare the different taxa and their prevalence with the analyzed criteria (30)(Figure 14).

	D N (%)	C N (%)
Periodontitis		
No	10 (14.1)	16 (11.3)
Low	40 (56.3)	96 (68.1)
Medium	16 (22.6)	22 (15.6)
Severe	5 (7.0)	7 (5.0)
$\chi^2 = 1.53$, $P > 0.05$		
Plaque		
No	8 (11.3)	33 (23.4)
Yes	63 (88.7)	108 (76.6)
$\chi^2 = 4.46$, $P < 0.05$, $OR = 2.40$, 95% CI = 1.04-5.53		
Bleeding		
No	17 (23.9)	52 (36.9)
Yes	54 (76.1)	89 (63.1)
$\chi^2 = 3.60$, $P < 0.05$, $OR = 1.85$, 95% CI = 0.98-3.53		
Calculus		
No	24 (33.8)	61 (43.3)
Yes	47 (66.2)	80 (65.7)
$\chi^2 = 1.76$, $P > 0.05$, $OR = 1.49$, 95% CI = 0.82-2.70		
T. forsythensis		
No	57 (80.3)	95 (67.4)
Yes	14 (19.7)	46 (32.6)
$\chi^2 = 3.87$, $P < 0.05$, $OR = 0.50$, 95% CI = 0.25-0.993		
P. intermedia		
No	28 (39.4)	50 (35.5)
Yes	43 (60.6)	91 (64.5)
$\chi^2 = 0.32$, $P > 0.05$, $OR = 0.84$, 95% CI = 0.47-1.51		
P. gingivalis		
No	49 (69.0)	112 (79.4)
Yes	22 (31.0)	29 (20.6)
$\chi^2 = 2.80$, $P < 0.05$, $OR = 1.73$, 95% CI = 0.90-3.31		

Figure 14: Two ways comparison between Type 2 diabetes patients and their periodontal pockets and healthy patients (D = Diabetic patients, C= Control patients, N = Number of patients in the sample, % = percentage of N in the sample) (Table provided by Campus) (30).

Authors observed no significant differences between T2DM and controls regarding the presence of periodontal disease and calculus, while a significant association was detected regarding plaque presence and bleeding on probing. Concerning bacteria prevalence, a positive association was noticed in two of the three main bacteria present. *P.gingivalis* and *T-forsythensis* showed a significant association ; *P.gingivalis* increasing from 20% in the control group up to 32% in the diabetic group, and *T-forsythensis* decreasing from 32% in the control group to 20% in the diabetic one (30).

There was an important difference observed in the subgingival bacterial composition between subjects having periodontal diseases and diabetes, and others having periodontal diseases and no other systemic disease. In this study, even though, the amplification of only 3 bacterial species groups was

carried out and showed a modification of the oral microbiota between the diabetic and control groups; thanks to the bacteria *T.forsythensis*, and *P.gingivalis* whom can be considered as promising biomarkers (30).

Another study, from Ganesan *et al.,* analyzed 2.7 million 16S sequences from 175 non-smoking normoglycemic individuals (controls), smokers, diabetics and diabetic smokers, all with periodontitis (31).

While comparing only the core microbiomes of periodontally healthy normoglycemics, periodontally healthy hyperglycemics and hyperglycemics with periodontitis; the core microbiome (*Campylobacter gracilis, Corynebacterium matruchotii, Fusobacterium nucleatum, Gemella sanguinis, Granulicatella adiacens, Capnocytophaga, Streptococcus oralis and Streptococcus sanguinis*) was shared by all the three groups (31) (Figure 15).

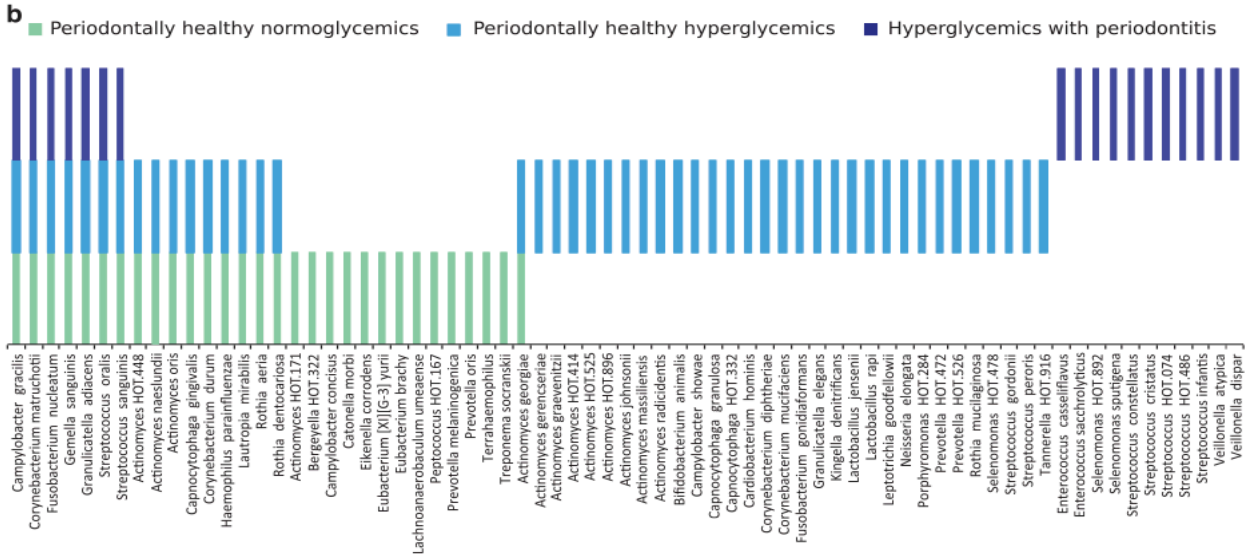


Figure 15: core microbiomes of periodontally healthy normoglycemics, periodontally healthy hyperglycemics and hyperglycemics with periodontitis (31).

It also appears that disease-associated phyla are established in diabetics with healthy periodontium; showing a decrease of health-compatible species (*Capnocytophaga* – family of *T.forsythensis*) , and

an increase of species belonging to the genera *Porphyromonas, Prevotella, Campylobacter and Fusobacterium*, confirming the results obtained by Campus *et al*. (31).

The main purpose of these studies was to use periodontal diseases as a "type" of marker to prevent diabetes and to diagnose it earlier. As said above, the bacterial composition of the microbiota in periodontitis can play a role in diabetic patients in order to control the disease, treat it and specially prevent it. In the past, for instance, the occultation of the retina or the urine analysis were ones of the main tools for diabetes diagnosis, beside complementary tests and blood tests. Nowadays with the improvement of pyrosequencing, the microbiota (especially *T.Forsythensis* and *P.gingivalis*) is putting itself as an undisputed tool (30,31).

1.2.2 Systemic Lupus Erythematosus (SLE)

Lupus erythematosus is a chronic autoimmune disease in which autoantibodies bind to components of the cell surface, cytoplasm, and nucleus, including nucleic acids and nucleoprotein particles.

It had a greater prevalence in women than in men, and is considered as a serious illness because it affects different organs which include kidney, heart, skin and mouth damage. Oral manifestations are frequent - 5-40% of cases - and can take on a variety of clinical features, including lichenoid lesions, lupus cheilitis, and nonspecific ulcerations with painful symptoms (32).

They include lesions on the palate, oral mucosa and gum tissue. In addition, lesions can sometimes occur on the vermilion border of the lips. These lesions of the oral mucosa manifest as white streaks and erythematous, atrophic and hyperkeratotic areas, as well as erosions and ulcers. Sometimes these signs can be misdiagnosed as other oral diseases such as lichen planus (32).

Li *et al.,* collected and analysed oral microbiota by 16S rRNA sequence amplification of 20 patients suffering from SLE and 19 other healthy controls. The bacterial diversity and distribution of their oral microbiota were compared with the help of the PCR amplification technique (33) (Figure 16).

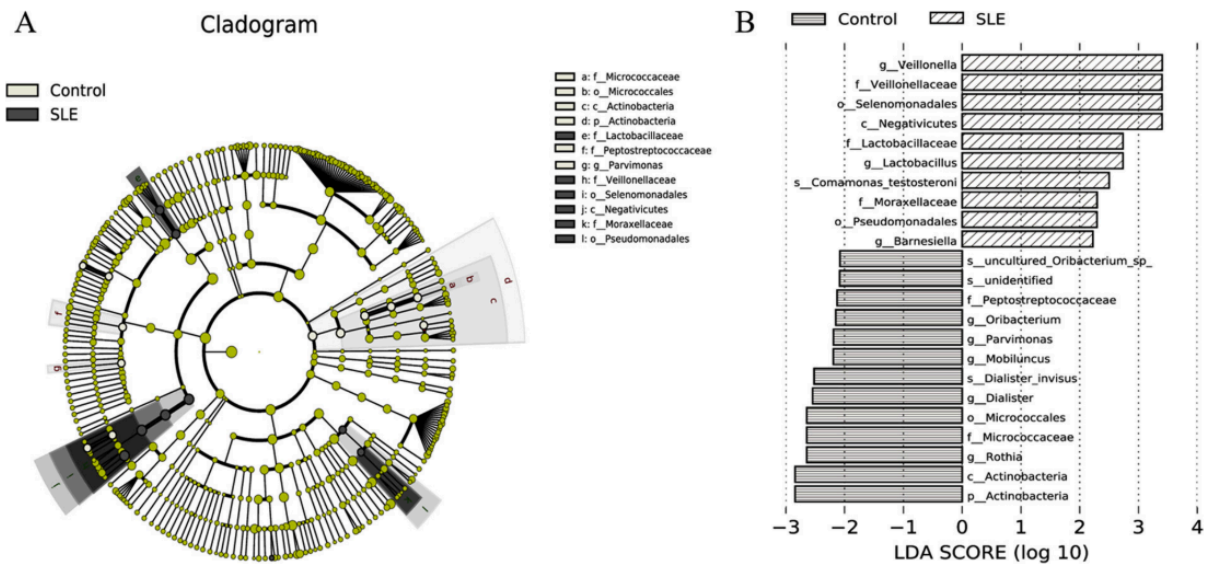


Figure 16: Analysis and identification of the bacterial taxa in healthy controls and SLE patients (Figures provided by B.Z. Li) (33).

At the genus level, the supply of *Barnesiella, Blautia, Lactobacillus, Pyramidobacter* and *Veillonella* were increased in patients with SLE. In addition, the oral microbiota may influence metabolic pathways, such as amino acid-related enzymes, and lysine biosynthesis. Finally, five genera (*Barnesiella, Blautia, Lactobacillus, Pyramidobacter and Veillonella*) and two phyla (*Actinobacteria and Tenericutes*) may be useful evidences for identifying patients with SLE and might be used as biomarkers (33).

In this study, authors found a reduced diversity and an alteration of the oral microbiota in patients with SLE (33). Oral microbiota dysbiosis and altered metabolic pathways were observed in patients with SLE. Although, a statistical difference between SLE patients and healthy controls has been seen in some microbial taxa, further large samples studies are still needed in the future. Besides, researches should focus on the elemental mechanisms related to amino acid metabolite and immunity on SLE, which may work as potential targets for SLE diagnosis and treatment (33).

CONCLUSIONS

- The evolution of techniques, as the sequence analysis of the 16S rRNA, allows the discovery of more species living in the oral cavity. Sequencing of DNA allowed researchers to understand the relation between oral and systemic diseases by pointing out a modification of the oral microbiota and presenting new biomarkers. Therefore, the use of the oral microbiota is considered as a tool to prevent pathologies.

- In oral diseases, the oral microbiota alterations were observed in dental caries, periodontitis, RAS and oral tumors. An increase of *Veillonella, Lactobacillus, Fusobacterium or Actinomyces* in dental caries was detected. *Porphynomas, Folifactor* or *Treponemas* increased in periodontal diseases and replaced species as *Streptococcus mitis* and *S.sanguinis* present in the "core microbiome" of healthy patients.
- In oral cancer, an increase of *Capnocytophaga gingivalis, Prevotella* and *S. mitis* was observed in saliva of OSCC patients.
- In subjects having recurrent aphthous stomatitis (RAS), the decrease of *Firmicutes* and the increase of *Actinomyces* was observed exclusively in RAS patients with ulcerated sites and *Bacteroidales* were detected in RAS patients with healthy sites.
- The oral microbiota can help health professionals towards the diagnosis of systemic diseases, thanks to specific bacterial biomarkers. This diagnosis tool can correlate diabetes with periodontitis: *P. gingivalis* increased, while *T. Forsythensis* decreased in patients with both diabetes and periodontal diseases.
- In Systemic Lupus Eryhematosus (SLE) with ulcerative lesions subjects, it was observed an increase of *Veillonella* and *Lactobacillus*, and a decrease of *Actinobacteria* and *Tenericutes* was detected in new SLE patients without ulcerative lesions.

RESPONSABILITY

This study aims to broaden the reader's knowledge of the design and understanding of the human and oral microbiota. It often happens that people know or have these oral and systemic diseases without really knowing the changes and the effects that are happening in their body. Knowledge can often stop at what we know and not look for the real etiology and the physiological alteration in our body.

The study of the microbiota brings a new medical category of diagnosis but especially prevention in connection with the advance of the Nextgen techniques. This method allows a faster and less expensive study of the bacteria of the microbiota, and therefore replaces all the biological studies which slowed down the diagnosis of diseases in the laboratory and brings another perspective of the prevention of diseases, which are sometimes difficult to treat.

The knowledge of what the microbiota is made of, also makes it possible to define these biomarkers, as said later, allowing a direct vision on the mark to be looked at by seeking it and going directly on it. That is why, the study of the microbiota is an advancement for the treatment of these systemic diseases, giving the hope of using the biomarkers present to prevent and maybe cure cancer over time, if these techniques of sequencing improve more and the isolation of DNA and RNA bring to a potential treatment.

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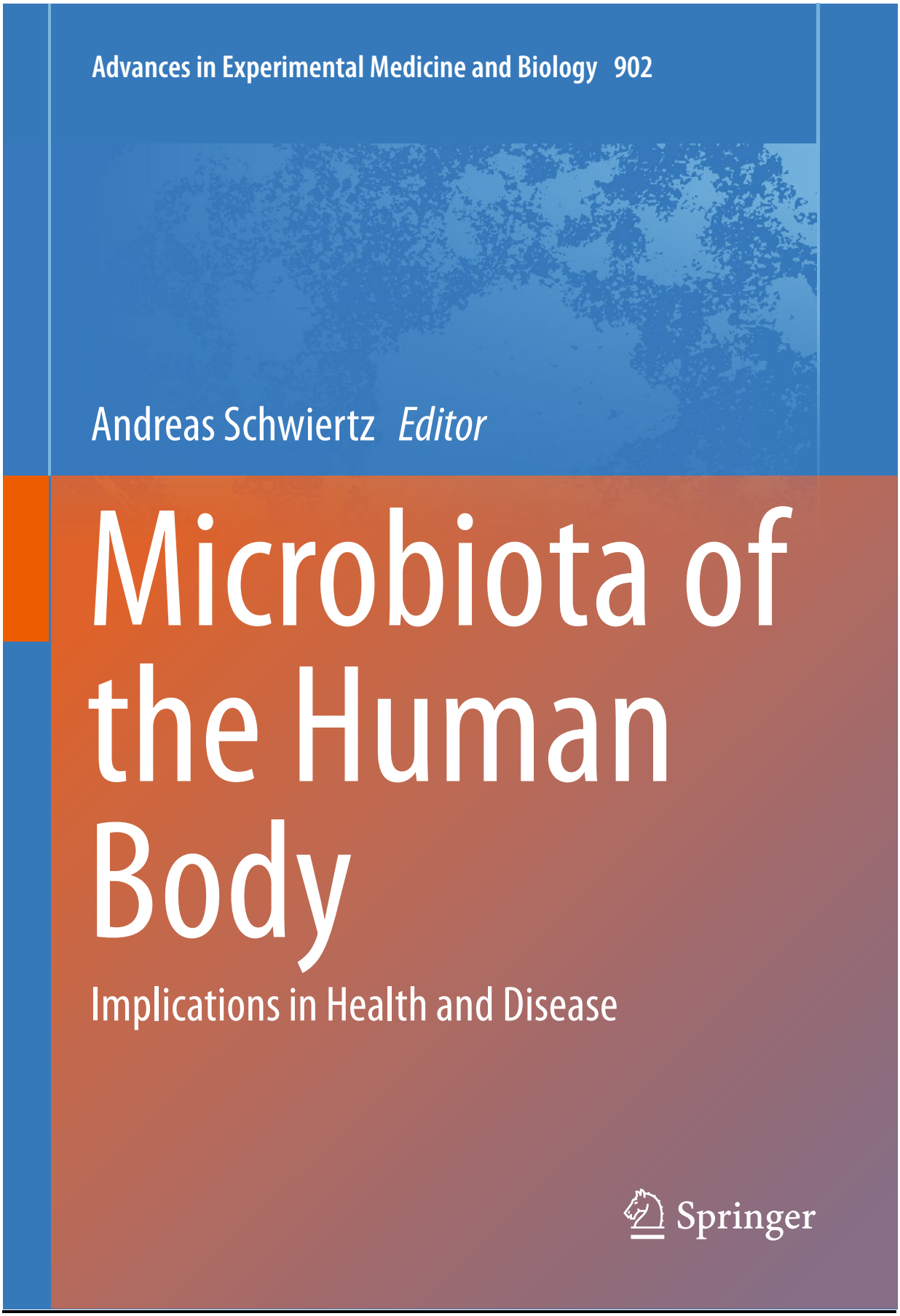
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REVIEW

Microbiota and metabolic diseases

Alessia Pascale[1] · Nicoletta Marchesi[1] · Cristina Marelli[1] · Adriana Coppola[2] · Livio Luzi[3,4] · Stefano Govoni[1] · Andrea Giustina[5] · Carmine Gazzaruso[2]

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Abstract
The microbiota is a complex ecosystem of microorganisms consisting of bacteria, viruses, protozoa, and fungi, living in different districts of the human body, such as the gastro-enteric tube, skin, mouth, respiratory system, and the vagina. Over 70% of the microbiota lives in the gastrointestinal tract in a mutually beneficial relationship with its host. The microbiota plays a major role in many metabolic functions, including modulation of glucose and lipid homeostasis, regulation of satiety, production of energy and vitamins. It exerts a role in the regulation of several biochemical and physiological mechanisms through the production of metabolites and substances. In addition, the microbiota has important anti-carcinogenetic and anti-inflammatory actions. There is growing evidence that any modification in the microbiota composition can lead to several diseases, including metabolic diseases, such as obesity and diabetes, and cardiovascular diseases. This is because alterations in the microbiota composition can cause insulin resistance, inflammation, vascular, and metabolic disorders. The causes of the microbiota alterations and the mechanisms by which microbiota modifications can act on the development of metabolic and cardiovascular diseases have been reported. Current and future preventive and therapeutic strategies to prevent these diseases by an adequate modulation of the microbiota have been also discussed.

Key words Microbiota · Microbiome · Diabetes · Obesity · Cardiovascular disease · Metabolic syndrome

Abbreviations

AMPK	AMP-Activated protein kinase
CVD	Cardiovascular diseases
FIAF	Fasting-induced adipose factor
FMI	Fecal microbiota transplant
GLP-1	Glucagon-like peptide 1
GLP-2	Glucagon-like peptide-2
HDL	High-density lipoprotein
HFD	High-fat diet
IR	Insulin resistance
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
MS	Metabolic syndrome
PYY	Peptide YY
RYGB	Roux-en-Y bypass
SCFAs	Short-chain fatty acids
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TLR4	Toll-like receptor 4
TMAO	Trimethylamine-N-oxide
VLDL	Very low-density lipoprotein

These authors contributed equally: Alessia Pascale, Nicoletta Marchesi.

✉ Carmine Gazzaruso
c.gazzaruso@gmail.com

[1] Department of Drug Sciences, Pharmacology section, University of Pavia, 27100 Pavia, Italy

[2] Diabetes and endocrine and metabolic diseases Unit and the Centre for Applied Clinical Research (Ce.R.C.A.) Clinical Institute "Beato Matteo" (Hospital Group San Donato), 27029 Vigevano, Italy

[3] Department of Biomedical Sciences for Health, University of Milan, 20100 Milan, Italy

[4] Metabolism Research Center, IRCCS Policlinico San Donato, 20097 San Donato Milanese, Italy

[5] Chair of Endocrinology San Raffaele Vita-Salute University, Milan, Italy

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The function of our microbiota: who is out there and what do they do?

Noora Ottman[1], Hauke Smidt[1], Willem M. de Vos[1,2] and Clara Belzer[1]*

[1] *Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands*
[2] *Department of Basic Veterinary Medicine and Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland*

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***Correspondence:**
Clara Belzer, Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB, Wageningen, Netherlands.
e-mail: clara.belzer@wur.nl

Current meta-omics developments provide a portal into the functional potential and activity of the intestinal microbiota. The comparative and functional meta-omics approaches have made it possible to get a molecular snap shot of microbial function at a certain time and place. To this end, metagenomics is a DNA-based approach, metatranscriptomics studies the total transcribed RNA, metaproteomics focuses on protein levels and metabolomics describes metabolic profiles. Notably, the metagenomic toolbox is rapidly expanding and has been instrumental in the generation of draft genome sequences of over 1000 human associated microorganisms as well as an astonishing 3.3 million unique microbial genes derived from the intestinal tract of over 100 European adults. Remarkably, it appeared that there are at least 3 clusters of co-occurring microbial species, termed enterotypes, that characterize the intestinal microbiota throughout various continents. The human intestinal microbial metagenome further revealed unique functions carried out in the intestinal environment and provided the basis for newly discovered mechanisms for signaling, vitamin production and glycan, amino-acid and xenobiotic metabolism. The activity and composition of the microbiota is affected by genetic background, age, diet, and health status of the host. In its turn the microbiota composition and activity influence host metabolism and disease development. Exemplified by the differences in microbiota composition and activity between breast- as compared to formula-fed babies, healthy and malnourished infants, elderly and centenarians as compared to youngsters, humans that are either lean or obese and healthy or suffering of inflammatory bowel diseases (IBD). In this review we will focus on our current understanding of the functionality of the human intestinal microbiota based on all available metagenome, metatranscriptome, and metaproteome results.

Keywords: human intestinal microbiota, functional metagenomics, metatranscriptomics, metaproteomics

INTRODUCTION

The human intestinal microbiota is known to play a key role in several metabolic, nutritional, physiological, and immunological processes, and recent years have seen a rapid development in the techniques for studying this previously overlooked organ (O'Hara and Shanahan, 2006). The human microbiota is established after birth and starts out as a dynamic ecosystem, dominated by bifidobacteria, that stabilizes during the first 2–3 years (Koenig et al., 2011; Scholtens et al., 2012). During life the microbial composition increases in both diversity and richness (Scholtens et al., 2012) (**Figure 1**) and reaches highest complexity in the human adult, with several hundred species-level phylotypes dominated by the phyla *Bacteroidetes* and *Firmicutes* (Rajilic-Stojanovic et al., 2009). Each human individual reaches a homeostatic climax composition, which likely remains relatively stable during most of a healthy adult's life. Although the individual microbial composition has an "individual core" that varies at the bacterial phylotype level and depends on the depth of the analysis (Zoetendal et al., 2008; Jalanka-Tuovinen et al., 2011), the overall phylogenetic profile can be categorized into a limited number of well-balanced host-microbial symbiotic states, the so-called enterotypes (Arumugam et al., 2011). At the late stages of life the microbiota composition becomes again less diverse and more dynamic, characterized by a higher *Bacteroides* to *Firmicutes* ratio, increase in *Proteobacteria* and decrease in *Bifidobacterium* (Biagi et al., 2010) (**Figure 1**).

The establishment of the bacterial ecosystem in early life is suggested to play a role in the microbial composition and disease susceptibility throughout life (Scholtens et al., 2012). A different microbiota composition is associated with chronic intestinal disorders and the severity of perturbation during disease and after antibiotic use (Sekirov et al., 2010). Diet is another important factor in microbiota composition development. Early in life there is already an impact of the diet on the microbiome: the microbiota of breast-fed and formula-fed infants was found to differ significantly in both composition and diversity. Breast-fed babies contain a microbiota that is more heterogeneous than that of formula-fed babies and contain a higher taxonomic diversity (Schwartz et al., 2012) (**Figure 1**). In addition, food habits can influence microbiota composition, and malnutrition results in lower abundance of *Bacteroidetes* that are shown to be specialized in breaking down the carbohydrates in energy rich western diet

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REVIEW

An insight into gut microbiota and its functionalities

Atanu Adak[1] · **Mojibur R. Khan**[1]

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Abstract
Gut microbiota has evolved along with their hosts and is an integral part of the human body. Microbiota acquired at birth develops in parallel as the host develops and maintains its temporal stability and diversity through adulthood until death. Recent developments in genome sequencing technologies, bioinformatics and culturomics have enabled researchers to explore the microbiota and in particular their functions at more detailed level than before. The accumulated evidences suggest that though a part of the microbiota is conserved, the dynamic members vary along the gastrointestinal tract, from infants to elderly, primitive tribes to modern societies and in different health conditions. Though the gut microbiota is dynamic, it performs some basic functions in the immunological, metabolic, structural and neurological landscapes of the human body. Gut microbiota also exerts significant influence on both physical and mental health of an individual. An in-depth understanding of the functioning of gut microbiota has led to some very exciting developments in therapeutics, such as prebiotics, probiotics, drugs and faecal transplantation leading to improved health.

Keywords Gut microbiota · Functions · Health · Therapeutics

Introduction

The life forms on this earth can be clustered into three broad domains: namely Archaea, Bacteria and Eukaryota [1]. All life has evolved from a simple unicellular common ancestor over billion years of evolution giving rise to a complexity of cells within an organism. The human is a superorganism that functions in harmony with trillions of symbiotic bacteria and eukaryotic cells. The host and its symbionts together are called a "holobiont," and their collective genome is known as "hologenome". Variation in the hologenome either by changes in the host genome or the microbiome may occur with reasonable fidelity maintaining plasticity of the holobiont [2]. In 2001, the human genome project was completed after which it was correctly argued that the "crowning achievement" in biology would be incomplete until the synergistic activities between human and microbes are understood [3–5]. Subsequently, several scientific efforts were initiated to understand the relationships between human and human-associated microbial communities. Discoveries of the Human Microbiome Project (HMP) and the Metagenome of Human Intestinal Tract (MetaHIT) opened new horizons in microbiome research for an enhanced understanding of host–microbe interactions at four major colonisation sites of the human body; viz. oral, gut, vagina and skin. Of these four sites, the human gut microbiota has drawn the attention of microbiologists for its clinical significance. Several gut microbiome projects including the Australian Gut Project, the American Gut project, the British gut project, the Canadian Microbiome Initiative, the Human MetaGenome Consortium Japan, the My NewGut project of the European Union and the International Human Microbiome Consortia, etc. were undertaken for a better understanding of the complex gut ecosystem and its role in health and diseases. The human gut (200–300 m^2 of mucosa) is the "secret garden" of ten trillion diverse symbionts (50 bacterial phyla and about 100–1000 bacterial species), collectively known as the 'microbiota'. Microbiota are ten times more abundant than our somatic and germ line cells of the body. The collective genes of microbiota are known as the 'microbiome' which is 150 times larger than the human genome [6, 7]. In an individual, 150–170 bacterial species predominate and get benefits from the warm nutrient rich environment of the gut and perform protective, metabolic and structural functions.

✉ Mojibur R. Khan
mojibur.khan@gmail.com

[1] Molecular Biology and Microbial Biotechnology Laboratory, Life Science Division, Institute of Advanced Study in Science and Technology (IASST), Guwahati, India

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Defining the Human Microbiome

Luke K Ursell[1], **Jessica L Metcalf**[1], **Laura Wegener Parfrey**[1], and **Rob Knight**[1,2,*]

[1]Department of Chemistry & Biochemistry, University of Colorado at Boulder, Boulder, CO, 80309, USA

[2]Howard Hughes Medical Institute, Boulder, CO, 80309, USA

Abstract

Rapidly developing sequencing methods and analytical techniques are enhancing our ability to understand the human microbiome, and, indeed, how we define the microbiome and its constituents. In this review we highlight recent research that expands our ability to understand the human microbiome on different spatial and temporal scales, including daily timeseries datasets spanning months. Furthermore, we discuss emerging concepts related to defining operational taxonomic units, diversity indices, core versus transient microbiomes and the possibility of enterotypes. Additional advances in sequencing technology and in our understanding of the microbiome will provide exciting prospects for exploiting the microbiota for personalized medicine.

Introduction

The human microbiota consists of the 10-100 trillion symbiotic microbial cells harbored by each person, primarily bacteria in the gut; the human microbiome consists of the genes these cells harbor[1]. Microbiome projects worldwide have been launched with the goal of understanding the roles that these symbionts play and their impacts on human health[2, 3]. Just as the question, "what *is* it to be human?", has troubled humans from the beginning of recorded history, the question, "what *is* the human microbiome?" has troubled researchers since the term was coined by Joshua Lederberg in 2001 [4]. Specifying the definition of the human microbiome has been complicated by confusion about terminology: for example, "microbiota" (the microbial taxa associated with humans) and "microbiome" (the catalog of these microbes and their genes) are often used interchangeably. In addition, the term "metagenomics" originally referred to shotgun characterization of total DNA, although now it is increasingly being applied to studies of marker genes such as the 16S rRNA gene. More fundamentally, however, new findings are leading us to question the concepts that are central to establishing the definition of the human microbiome, such as the stability of an individual's microbiome, the definition of the OTUs (Operational Taxonomic Units) that make up the microbiota, and whether a person has one microbiome or many. In this review, we cover progress towards defining the human microbiome in these different respects.

Studies of the diversity of the human microbiome started with Antonie van Leewenhoek, who, as early as the 1680s, had compared his oral and fecal microbiota. He noted the striking differences in microbes between these two habitats and also between samples from individuals in states of health and disease in both of these sites [5, 6].Thus, studies of the profound differences in microbes at different body sites, and between health and disease, are as old as microbiology itself. What is new today is not the ability to observe these obvious

*To whom correspondence should be addressed: Rob Knight, Department of Chemistry & Biochemistry, UCB 215, University of Colorado, Boulder, CO 80309, Tel: 303-492-1984, Fax: 303-492-7744, rob.knight@colorado.edu.

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Review

The oral microbiome and human health

Yoshihisa Yamashita[1)] and Toru Takeshita[1,2)]

[1)]Section of Preventive and Public Health Dentistry, Division of Oral Health, Growth and Development, Kyushu University Faculty of Dental Science, Fukuoka, Japan
[2)]OBT Research Center, Kyushu University Faculty of Dental Science, Fukuoka, Japan

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Abstract: In this brief review, we discuss our previous research on the relationship between the bacterial composition of salivary microbiota and periodontal disease. Analysis using a terminal restriction fragment length polymorphism method and an international comparison suggest that the predominance of the genera *Prevotella* and *Veillonella* in the salivary microbiota is attributable to periodontal disease conditions, and that the predominance of the genus *Neisseria* indicates healthy periodontal conditions. Furthermore, we recently used next-generation sequencing technology to perform a detailed large-scale analysis of the salivary microbiota. An important finding of that study was that high bacterial richness in the salivary microbiota was significantly associated with poor oral health, as indicated by decayed teeth, periodontitis, and poor oral hygiene. Another important result was that relative abundance of predominant bacteria in saliva was significantly associated with oral health-related conditions. Of the two different cohabiting groups of bacteria found in the salivary microbiota, a greater relative abundance of group I bacteria, which include *Prevotella* and *Veillonella* species, was associated with poor oral health, high body mass index, and old age. These findings suggest that the salivary microbiota reflects oral and systemic conditions.

Correspondence to Dr. Yoshihisa Yamashita, Section of Preventive and Public Health Dentistry, Division of Oral Health, Growth and Development, Kyushu University Faculty of Dental Science, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
Fax: +81-92-642-6354 E-mail: yoshi@dent.kyushu-u.ac.jp

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Keywords: oral microbiome; next generation sequencing; 16S rRNA; oral health; systemic health.

Introduction

The oral microbiome was first recognized by the Dutchman Antony van Leeuwenhoek, using a microscope of his own construction. In the late 1670s he reported to the British Royal Society that various forms of microbes were present in plaque found on tooth surfaces. This observation came at a time when bacteriology was not yet established, and he was immensely fascinated by the kineticism of the microbiome. His report described individual differences in the oral microbiome, and although he did not refer to it directly he realized that individual differences in the microbiome influenced the health of the oral cavity. Unfortunately, it would be 200 years before we developed a systematic theory that showed how the microbiome is related to disease in the oral cavity.

The American dentist W.D. Miller studied the association between oral microbes and oral diseases in a small laboratory in Berlin in the late 19th century. He was motivated by R. Koch, who, at about the same time, had been reporting cutting-edge results in studies of the association between microbes and infectious diseases. In his book, *The Micro-organisms of the Human Mouth*, Miller proposed the "chemicoparasitic theory", which held that the main cause of dental caries was acid metabolized from sugar in food by oral microbes. However, he failed to identify cariogenic bacteria and focused on acids as bacterial metabolites rather than on the bacteria that produced these metabolites. Until the mid-20th century, it is believed that his theory misled his successors, who categorized the Lactobacillus species as cario-

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INVITED MEDICAL REVIEW

The microbiome–systemic diseases connection

TA van der Meulen[1], HJM Harmsen[2], H Bootsma[3], FKL Spijkervet[1], FGM Kroese[3], A Vissink[1]

[1]*Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Groningen;* [2]*Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen;* [3]*Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

The human microbiome consists of all microorganisms occupying the skin, mucous membranes and intestinal tract of the human body. The contact of the mucosal immune system with the human microbiome is a balanced interplay between defence mechanisms of the immune system and symbiotic or pathogenic microbial factors, such as microbial antigens and metabolites. In systemic autoimmune diseases (SADs) such as rheumatoid arthritis, systemic lupus erythematosus and Sjögren's syndrome, the immune system is deranged to a chronic inflammatory state and autoantibodies are an important hallmark. Specific bacteria and/or a dysbiosis in the human microbiome can lead to local mucosal inflammation and increased intestinal permeability. Proinflammatory lymphocytes and cytokines can spread to the systemic circulation and increase the risk of inflammation at distant anatomical sites, such as the joints or salivary glands. Increased intestinal permeability increases antigen exposure and the risk of autoantibody production. If the human microbiome indeed plays such a critical role in SADs, this finding holds a great promise for new therapeutic strategies, such as diet interventions and probiotics and prebiotics. This review provides a background on the human microbiome and mucosal immunity in the gut and oral cavity and gives a summary of the current knowledge on the microbiome–SADs connection.

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Keywords: autoinflammatory diseases; inflammatory diseases; bacteria; microbiology; immunopathology; pathogenesis; rheumatology; immunology; autoimmune disease

Correspondence: Taco A. van der Meulen, MD, Department of Oral and Maxillofacial Surgery, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Tel: +31503613861, Fax: +31503612461, E-mail: t.a.van.der.meulen@umcg.nl
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Introduction

Despite the enormous effort from investigators over the world, the etiopathogenesis of systemic autoimmune diseases (SADs) such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), systemic sclerosis and vasculitis is only partially understood. These SADs have a multifactorial etiopathogenesis, meaning that a genetic background, environmental factors, hormones and a deranged immune system are all involved to a more or lesser extent.

The genetic contribution to RA and SLE has been well studied, but is yet understudied in SS (Lessard *et al*, 2012). Concordance rates for RA and SLE in monozygotic twins are 15% and 24%, respectively (Deapen *et al*, 1992; Silman *et al*, 1993). Thus, siblings with identical genomes often do not share a systemic disease phenotype. However, the heritability – which estimates the extent to which variation in liability to disease in a population can be explained by genetic variation – is estimated to be 60% and 44% for RA and SLE, respectively (MacGregor *et al*, 2000; Kuo *et al*, 2015). No data on twin concordance or heritability in SS are yet available (Bogdanos *et al*, 2012; Lessard *et al*, 2012).

Genomewide association studies (GWAS) have revealed that single nucleotide polymorphisms (SNPs) in the human leucocyte antigen (HLA) gene are the major genetic risk factor to develop a SAD. SNPs in the HLA gene locus account for maximum odds ratios (ORs) of 3.7 in RA, 2.9 in SLE and 3.5 in SS (Castaño-Rodríguez *et al*, 2008; Raychaudhuri *et al*, 2012; Lessard *et al*, 2013), but these ORs are low compared to other autoimmune diseases such as ankylosing spondylitis and type 1 diabetes (T1D) with HLA-related ORs of 41 and 11, respectively (Lin *et al*, 2011; Noble and Erlich, 2012). Many non-HLA encoding genes related to suspected pathogenic pathways are associated with RA, SLE and SS, but these ORs are seldom higher than 1.5 (Harley *et al*, 2008; Lessard *et al*, 2013; Okada *et al*, 2014). To summarize, although variations in the human genome explain only a small part of the aetiology of SADs, a relatively strong heritability and familial aggregation of SADs is noted (Cárdenas-Roldán *et al*, 2013). This apparent discrepancy in the role of genetics in

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Gut Homeostasis, Microbial Dysbiosis, and Opioids

Fuyuan Wang[1] and Sabita Roy[1,2]

Abstract

Gut homeostasis plays an important role in maintaining animal and human health. The disruption of gut homeostasis has been shown to be associated with multiple diseases. The mutually beneficial relationship between the gut microbiota and the host has been demonstrated to maintain homeostasis of the mucosal immunity and preserve the integrity of the gut epithelial barrier. Currently, rapid progress in the understanding of the host–microbial interaction has redefined toxicological pathology of opioids and their pharmacokinetics. However, it is unclear how opioids modulate the gut microbiome and metabolome. Our study, showing opioid modulation of gut homeostasis in mice, suggests that medical interventions to ameliorate the consequences of drug use/abuse will provide potential therapeutic and diagnostic strategies for opioid-modulated intestinal infections. The study of morphine's modulation of the gut microbiome and metabolome will shed light on the toxicological pathology of opioids and its role in the susceptibility to infectious diseases.

Keywords

gut microbiota, homeostasis, dysbiosis, opioid-related disorders

Gut Homeostasis and Health

The gut is a complex and dynamic network in which the interaction between the host and gut microbiota establishes a balanced, symbiotic, and mutually beneficial relationship (Kau et al. 2011). Gut homeostasis refers to the state of resilience and resistance to external and endogenous disturbances (Lozupone et al. 2012). Gut homeostasis is established and maintained by commensal microbiota, a functional barrier and a tolerant immune response (Brown, Sadarangani, and Finlay 2013). Gut microbiota include all microorganisms within the gastrointestinal (GI) tract, including bacteria, archaea, eukaryotes, fungi, and viruses (Gordon 2012). Microbiome refers to the entire collection of microbial genes in a particular environment (The Human Microbiome Project Consortium 2012). Recent rapid progress in metagenomics has provided powerful tools to determining perturbations of the human microbiome as contributors to diseases (Gordon 2012; Wang and Jia 2016). It is estimated that approximately 10^{13}–10^{14} bacteria inhabit the GI tract, which exceeds the total number of host cells by two orders of magnitude (Relman 2012). The unborn fetus lives in a basically sterile environment. During birth and thereafter, infants are exposed to the external environment whereby the gut microbial community is initialized, established, and gradually developed (Dominguez-Bello et al. 2010). The human gut microbiota become stable and adultlike at approximately 3–5 years of age (Rodríguez et al. 2015). It has been demonstrated that the gut microbiota play important roles in modulating host neural and immune development, morphogenesis, and resistance to diseases in both human beings and animals (Sommer and Bäckhed 2013). The mechanisms by which the gut microbiota maintain a healthy state and how microbial dysbiosis increases the susceptibility to diseases remain largely unknown (Figure 1).

Microbial Dysbiosis and Diseases

Microbial dysbiosis refers to a change in the structural and/or functional configuration of gut microbiota, which causes disruption of gut homeostasis and is associated with a variety of diseases, such as obesity, diabetes, autoimmune diseases, neurological disorders, allergies, and inflammatory and infectious diseases (Gordon 2012; Sommer and Bäckhed 2013). Changes in the composition or density of the microbiota have been shown to increase the susceptibility to a variety of pathogens and abnormal mucosal immune responses in humans and murine models (Stecher and Hardt 2008; Wells et al. 2011). For example, antibiotic-induced shifts in the mouse gut microbiome and metabolome increase the susceptibility to *Clostridium difficile* infections (Theriot et al. 2014). In addition, a

[1] Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA
[2] Department of Surgery, Medical School, University of Minnesota, Minneapolis, Minnesota, USA

Corresponding Author:
Sabita Roy, Department of Surgery, University of Miami, Miami, FL 33136, USA.
Email: sabita.roy@miami.edu

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Review

Human oral microbiota and its modulation for oral health

Yangheng Zhang[a,1], Xiang Wang[a,1], Houxuan Li[a], Can Ni[a], Zhibin Du[b,**], Fuhua Yan[a,*]

[a] Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, Jiangsu, China
[b] Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, Brisbane, Queensland, Australia

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ABSTRACT

The oral microbiome is an important part of the human microbiome. The oral cavity contains several significantly different niches with distinct microbial communities. A wide range of microorganisms inhabit the human oral cavity, including bacteria, fungi, viruses, archaea and protozoa. These microorganisms form a complex ecological community that influences oral and systemic health. The most prevalent oral diseases, dental caries and periodontal diseases, are microbiota-associated diseases. Moreover, increasing evidences have supported that many systemic diseases are associated with disturbances in the oral ecosystem, such as diabetes, cardiovascular diseases and tumors. The current control of dental plaque-related diseases is nonspecific and is centered on the removal of plaque by mechanical means. Due to this realization about the oral microbiome, several new methods based on the modulation of the microbiome that aim at maintaining and reestablishing a healthy oral ecosystem have been developed.

1. Introduction

Human are supraorganisms composed of both their own cells and microbial cells. The number of microorganisms residing on or in the human body is tenfold over that of the body's own cells [1]. These commensal microorganisms contribute to host health by resisting pathogens, maintaining homeostasis and modulating the immune system [2]. The National Institute of Health (NIH) of the United States (US) initiated the Human Microbiome Project (HMP) to characterize the human microbiome more completely and determine the association between changes of microbiome and health/disease [3]. The oral microbiome is one of the important parts of the human microbiome, and it refers specifically to the microorganisms residing in the human oral cavity [4].

The oral cavity has been considered to possess the second most complex microbiota in human body, only behind the colon [5]. The oral microbiome is highly diverse, including bacteria, fungi, viruses, archaea and protozoa. Approximately 700 species are present in the oral cavity, and most of them are indigenous [6]. Among them, approximately 54% have been cultivated and named, 14% are cultivated but unnamed, and 32% are known only as uncultivated phylotypes (from the Human Oral Microbiome Database). An increasing number of studies have demonstrated that the oral microbiota plays a vital role in the pathogenesis and development of many oral and systemic diseases.

In this review, we describe the microbial diversity of the oral cavity, expound microbial communities of different oral niches and present evidences that have confirmed the relationship between oral bacterial community shifts and oral or systemic diseases. Moreover, several prevention and treatment methods based on oral microbiota modulation are discussed.

2. Oral microbiome composition

2.1. Bacteria

Bacteria account for the main portion of oral microorganisms, and the major knowledge of the composition of oral bacteria comes from past culture-dependent methods. Culture-dependent techniques led to the identification of specific microorganisms thought to have a causative role in caries and periodontitis [5]. However, these data substantially underestimated the composition of the oral microbiome. The development of culture-independent methods, particularly targeting 16S ribosomal RNA, has expanded our awareness of the great richness and diversity of the oral microbiome. A list of oral bacteria with a description of their characteristics and genomic information are available from the Human Oral Microbiome Database website at www.homd.org. The oral bacterial community is dominated by the six major phyla, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes

* Corresponding author at: 30 Zhongyang Road, Nanjing, Jiangsu, 210008, China.
** Corresponding author at: Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove Campus, Brisbane, QLD 4059 Australia.
E-mail addresses: zhibin.du@qut.edu.au (Z. Du), yanfh@nju.edu.cn (F. Yan).
[1] These authors contributed equally to this work.

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Beyond microbial community composition: functional activities of the oral microbiome in health and disease

Ana E. Duran-Pinedo[a] and **Jorge Frias-Lopez**[a,b,*]

[a]Department of Microbiology, The Forsyth Institute, 245 First Street, Cambridge, MA 02142, USA

[b]Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, 188 Longwood Ave, Boston, MA 02115, USA

Abstract

The oral microbiome plays a relevant role in the health status of the host and is a key element in a variety of oral and non-oral diseases. Despite advances in our knowledge of changes in microbial composition associated with different health conditions the functional aspects of the oral microbiome that lead to dysbiosis remain for the most part unknown. In this review, we discuss the progress made towards understanding the functional role of the oral microbiome in health and disease and how novel technologies are expanding our knowledge on this subject.

Keywords

Microbiome; Oral; Omics; Dysbiosis; Periododontitis; Caries

1. Introduction: role of the human oral microbiome in health and disease

It is now common knowledge that the human microbiome plays an important role in the well-being and health status of the human host. A great effort has been placed in recent years on characterizing the different microbial communities colonizing the human body [1]. Among those sites, the oral cavity represents one of the most diverse microbial communities associated with any of the human sites studied [1]. It is a highly complex community with around 700 species identified to be associated with any of the different environments within the oral cavity [2]. To date, it is probably one of the best characterized communities of the human microbiome.

The oral cavity includes diverse structures and tissues, such as teeth, gingival sulcus, gingiva, tongue, cheeks, lips and palate, which provide different habitats, growth conditions and availability of nutrients. It has been shown that microbial profiles differ markedly depending on the intraoral location [2]. The microbiome of saliva is more similar to that of the dorsal and lateral surfaces of the tongue, and the soft tissues communities resemble each other more than the microbiota on the teeth above and below the gingival margin [3]. Hence, the oral microbiome can be seen as a group of diverse microbial biofilms.

*Corresponding author. 245 First Street, Cambridge, MA 02142, USA. Tel.: + 1 617 892 8576; fax: + 1 617 892 8510. jfriaslopez@forsyth.org (J. Frias-Lopez).

Le biofilm dentaire : composition, formation et propriétés

Elodie Houvion

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Oral microbiome: Unveiling the fundamentals

Priya Nimish Deo and Revati Deshmukh

Department of Oral Pathology and Microbiology, Bharati Vidyapeeth (Deemed to be University), Dental College and Hospital, Pune, Maharashtra, India

Address for correspondence: Dr. Priya Nimish Deo, "Devashish," Plot No. 378, Lane No. 16, Mahatma Society, Kothrud, Pune - 411 038, Maharashtra, India. E-mail: priyanimishdeo@gmail.com

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ABSTRACT

The oral cavity has the second largest and diverse microbiota after the gut harboring over 700 species of bacteria. It nurtures numerous microorganisms which include bacteria, fungi, viruses and protozoa. The mouth with its various niches is an exceptionally complex habitat where microbes colonize the hard surfaces of the teeth and the soft tissues of the oral mucosa. In addition to being the initiation point of digestion, the oral microbiome is crucial in maintaining oral as well as systemic health. Because of the ease of sample collection, it has become the most well-studied microbiome till date. Previously, studying the microbiome was limited to the conventional culture-dependent techniques, but the abundant microflora present in the oral cavity could not be cultured. Hence, studying the microbiome was difficult. The emergence of new genomic technologies including next-generation sequencing and bioinformatics has revealed the complexities of the oral microbiome. It has provided a powerful means of studying the microbiome. Understanding the oral microbiome in health and disease will give further directions to explore the functional and metabolic alterations associated with the diseased states and to identify molecular signatures for drug development and targeted therapies which will ultimately help in rendering personalized and precision medicine. This review article is an attempt to explain the different aspects of the oral microbiome in health.

Keywords: 16S rRNA, Human Oral Microbiome Database, microbiome, next-generation sequencing

INTRODUCTION

The community of microbial residents in our body is called the microbiome. The term "microbiome" is coined by Joshua Lederberg, a Nobel Prize laureate, to describe the ecological community of symbiotic, commensal and pathogenic microorganisms. These microorganisms literally share our body space.[1] The number of microbes present in our bodies is almost the same or even more as compared to that of our cells.[2]

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Structural studies of microcosm dental plaques grown under different nutritional conditions

Jonathan Pratten [a,*], Christopher S. Andrews [b], Duncan Q.M. Craig [b], Michael Wilson [a]

[a] *Department of Microbiology, Eastman Dental Institute for Oral Health Care Sciences, University College London, 256 Gray's Inn Road, London WC1X 8LD, UK*

[b] *School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK*

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Abstract

The aim of this study was to investigate the structure of intact oral biofilms using confocal laser scanning microscopy (CLSM). Mixed-species biofilms were grown on enamel discs in a constant depth film fermentor. The biofilms were fed with a mucin-containing artificial saliva with or without sucrose supplementation. Biofilms were examined using a Wild-Leitz CLSM, operating in reflected light mode. The microstructure of non-supplemented biofilms was revealed to be complex, with stacks of bacteria developing over time, separated by clear channels. Sucrose-supplemented biofilms appeared to colonise the substratum more rapidly. The results of this study have shown that using CLSM it is possible to examine the structure of oral biofilms grown under conditions similar to those which would exist in vivo.

Keywords: Confocal laser scanning microscopy; Microcosm dental plaque; Biofilm; Sucrose

1. Introduction

Dental plaque is a naturally occurring microbial film or biofilm which develops on the tooth surface [1]. Several methods have been used to view the microstructure of these biofilms including light microscopy, transmission electron microscopy, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) [2]. Traditional light microscopy has been shown to be useful only in the early stages of colonisation and plaque development, while electron microscopy usually requires specimen preparation involving dehydration, which may cause disruptive shrinkage and the loss of biofilm matrix which can comprise 73–98% of in vivo biofilms [3,4]. CLSM forms a bridge between optical and electron microscopy [5], affording penetrative views of specimens. It is possible to view hydrated biofilms of much greater thickness or age and the specimen can remain untouched. However, there are still problems associated with this approach, including beam quenching, auto-fluorescence of the specimen and the need, when using fluorescence labelling, for staining, washing and fixing [6,7].

Any physical handling will disrupt the structure of the biofilm [8] and therefore the object of this study was to investigate the structure of intact oral biofilms using CLSM grown under different nutritional conditions, without disturbing the biofilm structure.

2. Materials and methods

2.1. Inoculum and media

Saliva, collected from 10 healthy individuals, was used as an inoculum to provide a multi-species biofilm consisting of organisms found in the oral cavity. The nutrient source in all experiments was a mucin-containing artificial saliva, the composition of which has been described previously [9]. In some experiments, 330 ml of a 10% (w/v) aqueous solution of sucrose was pumped over the biofilms for 30 min via a second peristaltic pump. This was carried out thrice daily at 9 a.m., 1 p.m. and 5 p.m. to mimic the intake of sucrose in the diet and equating to the total mean daily intake of sucrose of an adult in the UK [10].

* Corresponding author. Tel.: +44 (20) 7915-1050; Fax: +44 (20) 7915-1127; E-mail: jpratten@eastman.ucl.ac.uk

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Review Article

Comparison of Next-Generation Sequencing Systems

Lin Liu, Yinhu Li, Siliang Li, Ni Hu, Yimin He, Ray Pong, Danni Lin, Lihua Lu, and Maggie Law

NGS Sequencing Department, Beijing Genomics Institute (BGI), 4th Floor, Building 11, Beishan Industrial Zone, Yantian District, Guangdong, Shenzhen 518083, China

Correspondence should be addressed to Lin Liu, linda.liu79@gmail.com

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With fast development and wide applications of next-generation sequencing (NGS) technologies, genomic sequence information is within reach to aid the achievement of goals to decode life mysteries, make better crops, detect pathogens, and improve life qualities. NGS systems are typically represented by SOLiD/Ion Torrent PGM from Life Sciences, Genome Analyzer/HiSeq 2000/MiSeq from Illumina, and GS FLX Titanium/GS Junior from Roche. Beijing Genomics Institute (BGI), which possesses the world's biggest sequencing capacity, has multiple NGS systems including 137 HiSeq 2000, 27 SOLiD, one Ion Torrent PGM, one MiSeq, and one 454 sequencer. We have accumulated extensive experience in sample handling, sequencing, and bioinformatics analysis. In this paper, technologies of these systems are reviewed, and first-hand data from extensive experience is summarized and analyzed to discuss the advantages and specifics associated with each sequencing system. At last, applications of NGS are summarized.

1. Introduction

(Deoxyribonucleic acid) DNA was demonstrated as the genetic material by Oswald Theodore Avery in 1944. Its double helical strand structure composed of four bases was determined by James D. Watson and Francis Crick in 1953, leading to the central dogma of molecular biology. In most cases, genomic DNA defined the species and individuals, which makes the DNA sequence fundamental to the research on the structures and functions of cells and the decoding of life mysteries [1]. DNA sequencing technologies could help biologists and health care providers in a broad range of applications such as molecular cloning, breeding, finding pathogenic genes, and comparative and evolution studies. DNA sequencing technologies ideally should be fast, accurate, easy-to-operate, and cheap. In the past thirty years, DNA sequencing technologies and applications have undergone tremendous development and act as the engine of the genome era which is characterized by vast amount of genome data and subsequently broad range of research areas and multiple applications. It is necessary to look back on the history of sequencing technology development to review the NGS systems (454, GA/HiSeq, and SOLiD), to compare their advantages and disadvantages, to discuss the various applications, and to evaluate the recently introduced PGM (personal genome machines) and third-generation sequencing technologies and applications. All of these aspects will be described in this paper. Most data and conclusions are from independent users who have extensive first-hand experience in these typical NGS systems in BGI (Beijing Genomics Institute).

Before talking about the NGS systems, we would like to review the history of DNA sequencing briefly. In 1977, Frederick Sanger developed DNA sequencing technology which was based on chain-termination method (also known as Sanger sequencing), and Walter Gilbert developed another sequencing technology based on chemical modification of DNA and subsequent cleavage at specific bases. Because of its high efficiency and low radioactivity, Sanger sequencing was adopted as the primary technology in the "first generation" of laboratory and commercial sequencing applications [2]. At that time, DNA sequencing was laborious and radioactive materials were required. After years of improvement, Applied Biosystems introduced the first automatic sequencing machine (namely AB370) in 1987, adopting capillary electrophoresis which made the sequencing faster and more accurate. AB370 could detect 96 bases one time, 500 K bases a day, and the read length could reach 600 bases.

microorganisms

MDPI

Review

The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems

Jesse R. Willis [1,2] and Toni Gabaldón [1,2,3,*]

[1] Barcelona Supercomputing Centre (BCS-CNS), Jordi Girona, 29., 08034 Barcelona, Spain; toni.gabaldon@bsc.es
[2] Institute for Research in Biomedicine (IRB), The Barcelona Institute of Science and Technology (BIST), 08034 Barcelona, Spain
[3] Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain
* Correspondence: toni.gabaldon.bcn@gmail.com

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Abstract: The human oral cavity is home to an abundant and diverse microbial community (i.e., the oral microbiome), whose composition and roles in health and disease have been the focus of intense research in recent years. Thanks to developments in sequencing-based approaches, such as 16S ribosomal RNA metabarcoding, whole metagenome shotgun sequencing, or meta-transcriptomics, we now can efficiently explore the diversity and roles of oral microbes, even if unculturable. Recent sequencing-based studies have charted oral ecosystems and how they change due to lifestyle or disease conditions. As studies progress, there is increasing evidence of an important role of the oral microbiome in diverse health conditions, which are not limited to diseases of the oral cavity. This, in turn, opens new avenues for microbiome-based diagnostics and therapeutics that benefit from the easy accessibility of the oral cavity for microbiome monitoring and manipulation. Yet, many challenges remain ahead. In this review, we survey the main sequencing-based methodologies that are currently used to explore the oral microbiome and highlight major findings enabled by these approaches. Finally, we discuss future prospects in the field.

Keywords: Oral microbiome; Next generation sequencing; oral diseases; systemic diseases; stomatotypes; microbiome perturbations

1. Introduction

Much like the various terrestrial biomes that make up the Earth, the human microbiome is a series of distinct communities of bacteria, fungi, viruses, archaea, protists, and other microorganisms, whose compositions are dependent upon environmental conditions [1]. Different sites of the human body can be seen as unique biomes, with drastically different environments and nutrient availabilities, which in turn promote different communities. Yet even within a particular body site, the microbiome composition can be highly variable between individuals in different states of health, with distinct lifestyles, or due to a number of other factors [2]. The focus of this review will be the human oral microbiome, techniques to approaching its analysis, and outlining its typical composition as we currently know it, as well as its deviations under atypical conditions.

The oral cavity contains one of the most diverse and unique communities of microbes in the human body [3,4], yet this niche is relatively understudied as compared to the gut—at the time of writing this review, a PubMed search with "oral microbiome" resulted in 746 articles, as compared to 5605 with "gut microbiome". A milliliter of saliva contains approximately 10^8 microbial cells [5], and an array of studies have detected up to 700 distinct prokaryotic taxa [6], with a typical healthy microbiome comprised of a range of about 100 to 200 distinct bacterial organisms [7]. The advent of

What is bioinformatics? An introduction and overview

Nicholas M Luscombe, Dov Greenbaum & Mark Gerstein*
Department of Molecular Biophysics and Biochemistry
Yale University, 266 Whitney Avenue
PO Box 208114, New Haven CT 06520-8114, USA
<mark.gerstein@yale.edu>

* - corresponding author

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1. Abstract

A flood of data means that many of the challenges in biology are now challenges in computing. Bioinformatics, the application of computational techniques to analyse the information associated with biomolecules on a large-scale, has now firmly established itself as a discipline in molecular biology, and encompasses a wide range of subject areas from structural biology, genomics to gene expression studies.

In this review we provide an introduction and overview of the current state of the field. We discuss the main principles that underpin bioinformatics analyses, look at the types of biological information and databases that are commonly used, and finally examine some of the studies that are being conducted, particularly with reference to transcription regulatory systems.

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2. Introduction

Biological data are flooding in at an unprecedented rate (1). For example as of August 2000, the GenBank repository of nucleic acid sequences contained 8,214,000 entries (2) and the SWISS-PROT database of protein sequences contained 88,166 (3). On average, the amount of information stored in these databases is doubling every 15 months (2). In addition, since the publication of the *H. influenzae* genome (4), complete sequences for over 40 organisms have been released, ranging from 450 genes to over 100,000. Add to this the data from the myriad of related projects that study gene expression, determine the protein structures encoded by the genes, and detail how these products interact with one another, and we can begin to imagine the enormous quantity and variety of information that is being produced.

PERSPECTIVE

OPEN

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The Integrative Human Microbiome Project

The Integrative HMP (iHMP) Research Network Consortium*

The NIH Human Microbiome Project (HMP) has been carried out over ten years and two phases to provide resources, methods, and discoveries that link interactions between humans and their microbiomes to health-related outcomes. The recently completed second phase, the Integrative Human Microbiome Project, comprised studies of dynamic changes in the microbiome and host under three conditions: pregnancy and preterm birth; inflammatory bowel diseases; and stressors that affect individuals with prediabetes. The associated research begins to elucidate mechanisms of host–microbiome interactions under these conditions, provides unique data resources (at the HMP Data Coordination Center), and represents a paradigm for future multi-omic studies of the human microbiome.

Although the 'omics era has accelerated all aspects of biological research, its effects have been particularly apparent in studies of microbial communities and the human microbiome. In the 18 years since the publication of the first human genome, studies of the microbiome have grown from culture-based surveys of the oral cavity and gut to molecular profiles of microbial biochemistry in all ecological niches of the human body[1–3]. Epidemiology and model systems have been used to identify associations between changes in the microbiome and conditions ranging from autism[4] to cancer[5–7], and microbial and immunological mechanisms have been identified that affect, for example, the efficacy of drugs used to treat cardiac conditions[8] or survival during graft-versus-host disease[9].

Contemporary studies of the human microbiome have also been a source of basic biological and translational surprises, exposing a compelling range of novel findings and open questions. Every human being appears to carry their own, largely individual, suite of microbial strains[10,11], which are acquired early in life[12–14], differ between environments and populations[15,16], and can persist for years[17] or undergo relatively rapid transitions[18]. Microbial diversity manifests differently in different ecological niches of the body; for example, greater diversity is generally expected in the gut, but can be associated with dysbiotic states and risk of adverse events in the female reproductive tract. The microbiome can be perturbed by conditions such as inflammatory bowel disease and diabetes, but a variety of microbiome-linked health states, and the underpinnings of these links, remain unexplored. How dynamic is the microbiome during processes such as pregnancy or viral infection? Which changes in the microbiome represent causes rather than effects of changes in health? Which molecular elements of a personalized microbiome might be responsible for health outcomes, and how do they integrate with and maintain physiological processes such as the immune system and metabolism? And what ecological elements dictate the success of a microbiota transplant, and why are they successful in treating some individuals and conditions, but not others?

The National Institutes of Health Human Microbiome Project was one of the first large-scale initiatives to address a subset of these questions[19] (Fig. 1). Launched in 2007[20], the first phase of the program sought to determine whether there were common elements to 'healthy' microbiomes, in the absence of overt disease. Studies of both a baseline adult population[21–23] and 'demonstration' populations with specific disease states established typical ranges (for some populations) of microbial membership and enzymatic repertoires across the body, combinations of metabolic functions that were either prevalent or strain-specific, and some of the host factors (such as race or ethnicity) that determine this variation. Studies of targeted populations identified ecological states of niches such as the vagina[24,25], skin[26–28], and gut[29–33], among many others (https://www.hmpdacc.org/health/projectdemos.php). This first phase of the HMP (HMP1) thus yielded a wealth of community resources: nucleotide sequences of microorganisms and communities from a large number of isolates, individuals, and populations (http://hmpdacc.org)[34–37]; protocols to support reproducible body-wide microbiome sampling and data generation[38–40]; and computational methods for microbiome analysis and epidemiology[41–47].

One of the main findings of the HMP1 was that the taxonomic composition of the microbiome alone was often not a good correlate with host phenotype—this tended to be better predicted by prevalent microbial molecular function or personalized strain-specific makeup[21]. This finding served as the foundation for the development of the second phase of the HMP, the Integrative HMP (iHMP or HMP2)[48], which was designed to explore host–microbiome interplay, including immunity, metabolism, and dynamic molecular activity, to gain a more holistic view of host–microbe interactions over time. This multi-omic program sought to expand the resource base available to the microbiome research community, to begin to address the relationship between host and microbiome mechanistically, and to address the questions introduced above. Disease-targeted projects within the HMP2 were therefore encouraged to use multiple complementary approaches in order to assess the mechanisms of human and microbial activity longitudinally and to provide protocols, data, and biospecimens for future work. These projects included three studies that followed the dynamics of human health and disease during conditions with known microbiome interactions, thus addressing important health outcomes directly while also serving as models of 'typical' microbiome-associated conditions of broad interest to the research community. These comprised pregnancy and preterm birth (PTB); inflammatory bowel diseases (IBD); and stressors that affect individuals with prediabetes. These studies, which have now reached the first stage of completion[49–51], together provide a wealth of information and insights about not only microbial dynamics, but also associated human host responses and microbial inter-relationships. A collection of more than 20 manuscripts to date describe some of these results at https://www.nature.com/collections/fiabfcjbfj, and together they provide a rich multi-omic data resource to be mined by future work (http://www.ihmpdcc.org).

*A list of participants and their affiliations appears at the end of the paper.

Minireview

Biomarker definitions and their applications

Robert M Califf[1,2,3]

[1]School of Medicine, Duke University, Durham, NC 27710, USA; [2]Verily Life Sciences (Alphabet), South San Francisco, CA 94043, USA; [3]Department of Medicine, Stanford University, Stanford, CA 94305, USA
Corresponding author: Robert M Califf. Email: robert.califf@duke.edu

Impact statement

Biomarkers are critical to the rational development of medical diagnostics and therapeutics, but significant confusion persists regarding fundamental definitions and concepts involved in their use in research and clinical practice. Clarification of the definitions of different biomarker classes and a better understanding of their appropriate application could yield substantial benefits. Biomarker definitions recently established in a joint FDA-NIH resource place different classes of biomarkers in the context of their respective uses in patient care, clinical research, or therapeutic development. Complex composite biomarkers and digital biomarkers derived from sensors and mobile technologies, together with biomarker-driven predictive toxicology and systems pharmacology, are reshaping development of diagnostic and therapeutic technologies. An approach to biomarker development that prioritizes the quality and reproducibility of the science underlying biomarker development and incorporates collaborative regulatory science involving multiple disciplines will lead to rational, evidence-based biomarker development that keeps pace with scientific and clinical need.

Abstract

Biomarkers are critical to the rational development of medical therapeutics, but significant confusion persists regarding fundamental definitions and concepts involved in their use in research and clinical practice, particularly in the fields of chronic disease and nutrition. Clarification of the definitions of different biomarkers and a better understanding of their appropriate application could result in substantial benefits. This review examines biomarker definitions recently established by the U.S. Food and Drug Administration and the National Institutes of Health as part of their joint *Biomarkers, EndpointS, and other Tools (BEST)* resource. These definitions are placed in context of their respective uses in patient care, clinical research, or therapeutic development. We explore the distinctions between biomarkers and clinical outcome assessments and discuss the specific definitions and applications of diagnostic, monitoring, pharmacodynamic/response, predictive, prognostic, safety, and susceptibility/risk biomarkers. We also explore the implications of current biomarker development trends, including complex composite biomarkers and digital biomarkers derived from sensors and mobile technologies. Finally, we discuss the challenges and potential benefits of biomarker-driven predictive toxicology and systems pharmacology, the need to ensure quality and reproducibility of the science underlying biomarker development, and the importance of fostering collaboration across the entire ecosystem of medical product development.

Keywords: Biomarkers, cardiovascular, epidemiology, medicine, monitoring, pharmacology/toxicology

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Introduction

Biomarkers are critical to the rational development of drugs and medical devices.[1] But despite their tremendous value, there is significant confusion about the fundamental definitions and concepts involved in their use in research and clinical practice. Further, the complexity of biomarkers has been identified as a limitation to understanding chronic disease and nutrition.[2]

Several years ago, this issue came to a head. At a joint leadership conference of the U.S. Food and Drug Administration (FDA) and the National Institutes of Health (NIH), it became apparent that leaders from each federal agency had differing impressions about the appropriate definitions of biomarkers in different contexts of use. A joint task force was therefore formed to forge common definitions and to make them publicly available through a continuously updated online document–the "Biomarkers, EndpointS, and other Tools" (BEST) resource.[3]

The importance of well-understood definitions and a shared understanding of how to apply them should not

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What are biomarkers?

Kyle Strimbu and Jorge A. Tavel

Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Correspondence to Jorge A. Tavel, MD, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, 6700B Rockledge Drive, Room #1127, MSC 7609, Bethesda, MD 20892, USA
Tel: +1 301 402 0564;
e-mail: Jtavel@niaid.nih.gov

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Purpose of review
This article provides working definitions and a conceptual framework to understand the roles of biomarkers in clinical research.
Recent findings
The definitions of the terms discussed in this article – medical signs, symptoms, biomarkers, surrogate endpoints, clinical endpoints, validation – are still under discussion, as are their relationships to each other, but broad consensus has developed in the past decade and a half about the necessity of distinguishing between, in particular, surrogate and clinical endpoints.
Summary
This article outlines the major definitions of the key terms in this field and considers select cases in which misunderstandings about the terms led to flawed research conclusions.

Keywords
biomarkers, clinical endpoints, surrogate endpoints

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Introduction

The use of biomarkers in basic and clinical research as well as in clinical practice has become so commonplace that their presence as primary endpoints in clinical trials is now accepted almost without question. In the case of specific biomarkers that have been well characterized and repeatedly shown to correctly predict relevant clinical outcomes across a variety of treatments and populations, this use is entirely justified and appropriate. In many cases, however, the 'validity' of biomarkers is assumed when, in fact, it should continue to be evaluated and reevaluated. This article will consider the current conceptual status of biomarkers as clinical and diagnostic tools and as surrogate endpoints in clinical research with the goal of providing context for interpreting studies that rely heavily on such biological measures.

What is a biomarker?

The term 'biomarker', a portmanteau of 'biological marker', refers to a broad subcategory of medical signs, that is, objective indications of medical state observed from outside the patient, which can be measured accurately and reproducibly. Medical signs stand in contrast to medical symptoms, which are limited to those indications of health or illness perceived by patients themselves. There are several more precise definitions of biomarkers in the literature, and they fortunately overlap considerably. In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' [1••]. A joint venture on chemical safety, the International Programme on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labour Organization, has defined a biomarker as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease' [2]. An even broader definition takes into account not just incidence and outcome of disease, but also the effects of treatments, interventions, and even unintended environmental exposure, such as to chemicals or nutrients. In their report on the validity of biomarkers in environmental risk assessment, the WHO has stated that a true definition of biomarkers includes 'almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction' [3]. Examples of biomarkers include everything from pulse and blood pressure through basic chemistries to more complex laboratory tests of blood and other tissues. Medical signs have a long history of use in clinical practice – as old as medical practice itself – and biomarkers are merely the most objective, quantifiable medical signs modern laboratory science allows us to measure reproducibly. The use of biomarkers, and in particular laboratory-measured

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Diagnostic Biomarkers for Oral and Periodontal Diseases

Mario Taba, Jr, DDS, PhD[a], Janet Kinney, RDH[a],
Amy S. Kim, DDS[b],
William V. Giannobile, DDS, DMSc[a,b,c,*]

[a]*Department of Periodontics/Prevention/Geriatrics, School of Dentistry, University of Michigan, 1011 North University Avenue, Ann Arbor, MI 48109-1078, USA*
[b]*Michigan Center for Oral Health Research, University of Michigan, 24 Frank Lloyd Wright Drive, Lobby M, Box 422, Ann Arbor, MI 48106, USA*
[c]*Department of Biomedical Engineering, College of Engineering, University of Michigan, Ann Arbor, MI, USA*

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth. It is widely accepted that the initiation and the progression of periodontitis are dependent on the presence of virulent microorganisms capable of causing disease. Although the bacteria are initiating agents in periodontitis, the host response to the pathogenic infection is critical to disease progression [1–3]. After its initiation, the disease progresses with the loss of collagen fibers and attachment to the cemental surface, apical migration of the junctional epithelium, formation of deepened periodontal pockets, and resorption of alveolar bone [4]. If left untreated, the disease continues with progressive bone destruction, leading to tooth mobility and subsequent tooth loss. Periodontal disease afflicts over 50% of the adult population in the United States, with approximately 10% displaying severe disease concomitant with early tooth loss [5].

A goal of periodontal diagnostic procedures is to provide useful information to the clinician regarding the present periodontal disease type, location, and severity. These findings serve as a basis for treatment planning

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* Corresponding author. Department of Periodontics/Prevention/Geriatrics, School of Dentistry, University of Michigan, 1011 North University Avenue, Ann Arbor, MI 48109-1078.

E-mail address: william.giannobile@umich.edu (W.V. Giannobile).

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Molecular Analysis of Bacterial Species Associated with Childhood Caries

Mitzi R. Becker,[1] Bruce J. Paster,[2,3] Eugene J. Leys,[4] Melvin L. Moeschberger,[5] Sarah G. Kenyon,[2] Jamie L. Galvin,[2] Susan K. Boches,[2] Floyd E. Dewhirst,[2,3] and Ann L. Griffen[1]*

Department of Pediatric Dentistry[1] and Department of Oral Biology,[4] College of Dentistry, and Division of Epidemiology and Biometrics, School of Public Health, College of Medicine and Public Health,[5] The Ohio State University, Columbus, Ohio, and Department of Molecular Genetics, The Forsyth Institute,[2] and Department of Oral Biology, Harvard School of Dental Medicine,[3] Boston, Massachusetts

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Although substantial epidemiologic evidence links *Streptococcus mutans* to caries, the pathobiology of caries may involve more complex communities of bacterial species. Molecular methods for bacterial identification and enumeration now make it possible to more precisely study the microbiota associated with dental caries. The purpose of this study was to compare the bacteria found in early childhood caries (ECC) to those found in caries-free children by using molecular identification methods. Cloning and sequencing of bacterial 16S ribosomal DNAs from a healthy subject and a subject with ECC were used for identification of novel species or uncultivated phylotypes and species not previously associated with dental caries. Ten novel phylotypes were identified. A number of species or phylotypes that may play a role in health or disease were identified and warrant further investigation. In addition, quantitative measurements for 23 previously known bacterial species or species groups were obtained by a reverse capture checkerboard assay for 30 subjects with caries and 30 healthy controls. Significant differences were observed for nine species: *S. sanguinis* was associated with health and, in order of decreasing cell numbers, *Actinomyces gerencseriae*, *Bifidobacterium*, *S. mutans*, *Veillonella*, *S. salivarius*, *S. constellatus*, *S. parasanguinis*, and *Lactobacillus fermentum* were associated with caries. These data suggest that *A. gerencseriae* and other *Actinomyces* species may play an important role in caries initiation and that a novel *Bifidobacterium* may be a major pathogen in deep caries. Further investigation could lead to the identification of targets for biological interventions in the caries process and thereby contribute to improved prevention of and treatment for this significant public health problem.

Dental caries is the single most common chronic disease of childhood, with a rate five times greater than that seen for the next most prevalent disease, asthma (37). Early childhood caries (ECC) results in a considerable direct burden of pain and suffering as well as poorer general health (1, 16). Caries is disproportionately present in low-income children (37), although it is by no means limited to this group. The overall prevalence of ECC in the United States is estimated at 1 to 5%, although among high-risk populations the prevalence has been reported to be as high as 60% (29). Dental care was recently shown to be the most common unmet health care need among children in the United States (22). Treatment for children with caries can be expensive, often requiring extensive restorative treatment under general anesthesia. Despite efforts in restorative therapy, children who experience ECC continue to be at a higher risk for new lesions in both the primary and the permanent dentition (36). Interventions which disrupt the pathobiology of caries are needed to prevent and treat this aggressive infectious disease. In order to develop these strategies, however, it is important that all bacteria associated with dental caries and dental health be identified.

Considerable epidemiologic evidence links *Streptococcus mutans* to caries (40), and numerous laboratory investigations have demonstrated the ability of strains of this species to produce the lactic acid which causes dental caries (40). A closely related lactate-producing species, *S. sobrinus*, has also been linked to caries, although the prevalence is distinctly lower and this species is seldom found without *S. mutans* (40). Various *Lactobacillus* species have also been consistently associated with caries and are thought to be important secondary pathogens in dental caries (40). *Actinomyces* species have also been suspected to play a role in caries, with most evidence linking them to root surface caries (40). Other bacteria have been investigated as potential contributors to caries, and several investigators have suggested that the pathobiology of caries may involve more complex communities of bacterial species than previously thought [(3, 40, 42); M. K. Russell, M. F. J. Maiden, J. Lopman, S. K. Boches, J. L. Galvin, F. E. Dewhirst, and B. J. Paster., J. Dent Res. **79**(IADR Abstr.)**:**465, 2000].

Nearly all investigations into the microbial pathogenesis of caries have been conducted by cultivation of bacteria. Molecular methods for bacterial identification and enumeration now make it possible to more precisely study the microbiota associated with dental caries (12, 24). DNA sequence-based assays can be used to identify closely related species that are difficult to differentiate by traditional, culture-based approaches. In addition, 16S ribosomal DNA (rDNA) sequence-based clonal analysis allows for the detection and identification of species that are refractory to detection by traditional methods. These bacteria may escape detection either because they do not grow

* Corresponding author. Mailing address: The Ohio State University, 305 W. 12th Ave., P.O. Box 182357, Columbus, OH 43218-2357. Phone: (614) 292-1150. Fax: (614) 292-1125. E-mail: griffen.1@osu.edu.

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Bacterial 16S Sequence Analysis of Severe Caries in Young Permanent Teeth▽

Erin L. Gross,[1] Eugene J. Leys,[1] Stephen R. Gasparovich,[2,5] Noah D. Firestone,[1] Judith A. Schwartzbaum,[3] Daniel A. Janies,[4] Kashmira Asnani,[1] and Ann L. Griffen,[2,5]*

The Ohio State University, College of Dentistry, Division of Oral Biology,[1] The Ohio State University, College of Dentistry, Division of Pediatric Dentistry,[2] The Ohio State University, College of Public Health, Division of Epidemiology,[3] The Ohio State University, College of Medicine, Department of Biomedical Informatics,[4] and Nationwide Children's Hospital, Department of Dentistry,[5] Columbus, Ohio

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Previous studies have confirmed the association of the acid producers *Streptococcus mutans* and *Lactobacillus* spp. with childhood caries, but they also suggested these microorganisms are not sufficient to explain all cases of caries. In addition, health-associated bacterial community profiles are not well understood, including the importance of base production and acid catabolism in pH homeostasis. The bacterial community composition in health and in severe caries of the young permanent dentition was compared using Sanger sequencing of the ribosomal 16S rRNA genes. *Lactobacillus* species were dominant in severe caries, and levels rose significantly as caries progressed from initial to deep lesions. *S. mutans* was often observed at high levels in the early stages of caries but also in some healthy subjects and was not statistically significantly associated with caries progression in the overall model. *Lactobacillus* or *S. mutans* was found either at low levels or not present in several samples. Other potential acid producers observed at high levels in these subjects included strains of *Selenomonas*, *Neisseria*, and *Streptococcus mitis*. *Propionibacterium* FMA5 was significantly associated with caries progression but was not found at high levels. An overall loss of community diversity occurred as caries progressed, and species that significantly decreased included the *Streptococcus mitis-S. pneumoniae-S. infantis* group, *Corynebacterium matruchotii*, *Streptococcus gordonii*, *Streptococcus cristatus*, *Capnocytophaga gingivalis*, *Eubacterium* IR009, *Campylobacter rectus*, and *Lachnospiraceae* sp. C1. The relationship of acid-base metabolism to 16S rRNA gene-based species assignments appears to be complex, and metagenomic approaches that would allow functional profiling of entire genomes will be helpful in elucidating the microbial pathogenesis of caries.

Dental caries is the most common chronic disease of childhood, affecting nearly three-fourths of all children by the age of 17 years (50). The majority of children experience mild caries in the permanent dentition that is easily managed, but nearly 20% of children suffer more aggressive caries (19) that is destructive and often recurrent. The cariogenicity of *Streptococcus mutans* and *Lactobacillus* species in tooth-associated biofilms has long been established based on culture studies (51), but this approach has provided a limited ability to study the role of other species present in biofilm communities. Recently DNA-based methods have been used to study early childhood caries (5, 11, 23), caries of the primary and permanent teeth in children and young adults (1), root caries in the elderly (42), and advanced dentin lesions (8, 10, 36). Taken together these studies have confirmed the association of *S. mutans* and *Lactobacillus* species with childhood caries, but they also suggest that these species are not sufficient to explain all cases of caries. In addition, health-associated bacterial community profiles are not well understood, including the importance of species that produce basic compounds that lower pH and species that metabolize lactic acid to lower-pK_a acids.

The purpose of the present study was to compare bacterial community profiles associated with severe dental caries and health in the young permanent dentition. This was accomplished by using an open-ended molecular approach, 16S rRNA gene cloning, and Sanger sequencing. A previously established clinical model was used (5) in which samples representing the full range from early- to late-stage caries were collected to reconstruct the natural history of caries. These samples were compared within individuals and to samples from healthy control subjects. The data indicated that caries has a heterogeneous etiology, with multiple profiles of acid-producing species observed. An overall loss of community diversity occurred as caries progressed, and species that are part of a health-associated ecosystem were identified.

MATERIALS AND METHODS

Clinical methods. (i) Subject recruitment. Subjects with dental caries and a dentally healthy control group were recruited at the Nationwide Children's Hospital Dental Clinic in Columbus, OH. General exclusionary criteria for either group included (i) age greater than 16 years, (ii) indications for infective endocarditis prophylaxis, (iii) antibiotic use in the past 30 days, and (iv) professional cleaning in the past 30 days. Only one child per family was included in each group. The inclusion requirement for the caries group was the presence of at least three permanent teeth with cavitated, multisurface lesions involving a smooth surface and with at least one of these three teeth with a vital pulp. Age-, race-, and gender-matched healthy control subjects who were caries-free and had no existing restorations were also recruited. Institutional Review Board approval was obtained for this protocol, consent was obtained from the parents of all subjects, and assent was obtained from subjects who were at least 9 years old.

* Corresponding author. Mailing address: College of Dentistry, 4126 Postle Hall, 305 West 12th Avenue, Columbus, OH 43210. Phone: (614) 292-1150. Fax: (614) 292-1125. E-mail: griffen.1@osu.edu.
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Dental plaque as a biofilm

PD Marsh and DJ Bradshaw

Department of Microbial Pathogenicity, CAMR, Porton Down, Salisbury SP4 0JG, UK

Dental plaque is the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin. Once a tooth surface is cleaned, a conditioning film of proteins and glycoproteins is adsorbed rapidly to the tooth surface. Plaque formation involves the interaction between early bacterial colonisers and this film (the acquired enamel pellicle). To facilitate colonisation of the tooth surface, some receptors on salivary molecules are only exposed to bacteria once the molecule is adsorbed to a surface. Subsequently, secondary colonisers adhere to the already attached early colonisers (co-aggregation) through specific molecular interactions. These can involve protein–protein or carbohydrate–protein (lectin) interactions, and this process contributes to determining the pattern of bacterial succession. As the biofilm develops, gradients in biologically significant factors develop, and these permit the co-existence of species that would be incompatible with each other in a homogenous environment. Dental plaque develops naturally, but it is also associated with two of the most prevalent diseases affecting industrialised societies (caries and periodontal diseases). Future strategies to control dental plaque will be targeted to interfering with the formation, structure and pattern of development of this biofilm.

Keywords: dental plaque; biofilm; adhesion; co-aggregation

Introduction

The recognition that surface-associated bacteria can have novel properties compared to their planktonic counterparts was probably first made from studies of dental plaque. Antonie van Leeuwenhoek is reported to have failed to kill plaque bacteria on his teeth by prolonged rinsing with strong wine-vinegar, while plaque was 'killed' when the bacteria were first removed from his molars and mixed with vinegar *in vitro* [cited by 19].

The mouth is unique in the human body in that it provides non-shedding surfaces (teeth) for natural microbial colonisation. This can result in the accumulation of large masses of bacteria and their products at stagnant sites between teeth (approximal surfaces), in the pits and fissures on the biting (occlusal) surfaces of molars and premolars, and around the gums (gingival crevice). Plaque found above or below the gum margins is described as supra- or sub-gingival plaque, respectively. Elsewhere, desquamation ensures that the bacterial load is light on mucosal surfaces.

Dental plaque forms naturally on teeth and acts as part of the defences of the host by helping to prevent colonisation by exogenous, and often pathogenic microorganisms [28]. However, if plaque is allowed to accumulate beyond levels that are compatible with health, then disease can occur. Plaque is associated with two of the most prevalent diseases affecting industrialised societies, namely dental caries and periodontal diseases. The widespread nature of these diseases, together with their huge treatment costs, has provided great impetus for research into improved means of controlling plaque formation. The aim of this paper is to review recent advances in the microbiology of dental plaque, with particular emphasis on the mechanisms by which bacteria adhere to the tooth surface and produce a biofilm.

Correspondence: Dr PD Marsh, Department of Microbial Pathogenicity, CAMR, Porton Down, Salisbury SP4 0JG, UK
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Definition of dental plaque

Dental plaque has been defined as the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin [31]. Plaque that becomes calcified is termed calculus or tartar. The resident plaque microflora consists of a wide range of Gram-positive and Gram-negative bacteria, including facultatively anaerobic and obligately anaerobic species (Table 1). The composition of plaque varies at different sites over the tooth surface due to differences in their local biological properties, and a detailed description of the microflora of

Table 1 Bacterial genera found in dental plaque

Gram-positive	Gram-negative
Cocci:	Cocci:
Streptococcus	*Neisseria*
Peptostreptococcus	*Veillonella*
Rods:	Rods:
Actinomyces	*'Bacteroides'*[a]
Bifidobacterium	*Campylobacter*
Corynebacterium	*Eikenella*
Eubacterium	*Fusobacterium*
Lactobacillus	*Haemophilus*
Propionibacterium	*Leptotrichia*
Rothia	*Prevotella*
	Porphyromonas
	Selenomonas
	Treponema

Most genera contain more than one species. Some genera are found rarely and only in low numbers at healthy sites
[a]The genus '*Bacteroides*' has been redefined. Eventually, oral bacteria included in this genus will be reclassified

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Relationship between oral microbiota and periodontal disease: a systematic review

R. PATINI[1], E. STADERINI[1], C. LAJOLO[1], L. LOPETUSO[2], H. MOHAMMED[3], L. RIMONDINI[3,4], V. ROCCHETTI[3], F. FRANCESCHI[5], M. CORDARO[1], P. GALLENZI[1]

[1]Institute of Dentistry and Maxillofacial Surgery, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Università Cattolica del Sacro Cuore
[2]Internal Medicine and Gastroenterology, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "Agostino Gemelli", Rome, Italy
[3]Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy
[4]Interdisciplinary Research Center of Autoimmune Diseases, Università del Piemonte Orientale, Novara, Italy
[5]Head of Emergency Medicine Division. Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "Agostino Gemelli", Rome, Italy

Abstract. – OBJECTIVE: In recent years metagenomic analysis has become more accessible for the characterization of biological specimens. There has been an important increase of studies using this technique for subgingival human samples. To date, there are no updated systematic reviews on the relationship between oral microbiota and periodontal disease. The aim of the present systematic review was to update data about studies concerning the influences of changes in oral microbiota composition on the periodontal status in human subjects.

MATERIALS AND METHODS: An electronic search was conducted in four databases (MEDLINE, Scopus, CENTRAL and Web of Science) for articles published in English from January 2014 to April 2018. *In vitro* or animal studies, case reports, case series, retrospective studies, review articles, abstracts and discussions were excluded. Also, studies that evaluated less than 5 microbial species, only viruses or already known periodontal pathogens were excluded. Two independent researches selected the studies and extracted the data. The quality of evidence was assessed as high, moderate or low for each microorganism.

RESULTS: Eight studies and three additional publications recovered from the bibliography search of the selected articles were included in the review. The Bacteria domain was the main detected among the others and it included 53 species. The review confirmed the presence of recognized periodontal pathogens such as the members of the red complex but also identified, with high weight of evidence, the presence of new pathogens.

CONCLUSIONS: The results of this systematic review support high evidence for the association of 3 new species/genera with the etiology of periodontitis. Future investigations on the actual role of these new pathogens in the onset and progression of the disease are needed.

Key Words
Oral microbiota, Periodontal disease, Metagenomic analysis, Pathogens bacteria, Systematic review.

Introduction

Severe periodontitis is the 6th most prevalent disease worldwide, with an overall prevalence of 11.2% and around 743 million people affected. The global burden of periodontal disease increased by 57.3% from 1990 to 2010[1-4]. Periodontal diseases are multifactorial infections induced by a complex of bacterial species that interact with host tissues to determine the destruction of periodontal structures, including the supporting tissues of the teeth, alveolar bone and periodontal ligament. It has recently been shown that some systemic diseases and syndromes are related to an increase on the activity of the cells of the immune system and a worsening of periodontal clinical conditions[5-7]. The importance of bacteria in dental plaque and the key role of plaque in the etiopathogenesis of periodontal disease are already well known[8]. Therefore, the control of oral infection

Corresponding Author: Edoardo Staderini, DDS; e-mail: edoardo.staderini@yahoo.it

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ORIGINAL ARTICLE

Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing

Ann L Griffen[1,6], Clifford J Beall[2,6], James H Campbell[3,6], Noah D Firestone[2], Purnima S Kumar[4], Zamin K Yang[3], Mircea Podar[3,5] and Eugene J Leys[2]

[1]*Division of Pediatric Dentistry and Community Oral Health, The Ohio State University College of Dentistry, Columbus, OH, USA;* [2]*Division of Oral Biology, The Ohio State University College of Dentistry, Columbus, OH, USA;* [3]*Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA;* [4]*Division of Periodontology, The Ohio State University College of Dentistry, Columbus, OH, USA and* [5]*Genome Science and Technology Program, University of Tennessee, Knoxville, TN, USA*

Periodontitis has a polymicrobial etiology within the framework of a complex microbial ecosystem. With advances in sequencing technologies, comprehensive studies to elucidate bacterial community differences have recently become possible. We used 454 sequencing of 16S rRNA genes to compare subgingival bacterial communities from 29 periodontally healthy controls and 29 subjects with chronic periodontitis. Amplicons from both the V1-2 and V4 regions of the 16S gene were sequenced, yielding 1 393 579 sequences. They were identified by BLAST against a curated oral 16S database, and mapped to 16 phyla, 106 genera, and 596 species. 81% of sequences could be mapped to cultivated species. Differences between health- and periodontitis-associated bacterial communities were observed at all phylogenetic levels, and UniFrac and principal coordinates analysis showed distinct community profiles in health and disease. Community diversity was higher in disease, and 123 species were identified that were significantly more abundant in disease, and 53 in health. *Spirochaetes*, *Synergistetes* and *Bacteroidetes* were more abundant in disease, whereas the *Proteobacteria* were found at higher levels in healthy controls. Within the phylum Firmicutes, the class *Bacilli* was health-associated, whereas the *Clostridia*, *Negativicutes* and *Erysipelotrichia* were associated with disease. These results implicate a number of taxa that will be targets for future research. Some, such as *Filifactor alocis* and many *Spirochetes* were represented by a large fraction of sequences as compared with previously identified targets. Elucidation of these differences in community composition provides a basis for further understanding the pathogenesis of periodontitis.

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Subject Category: microbe–microbe and microbe–host interactions
Keywords: oral microbiome; pyrosequencing; 16S rRNA; periodontitis; bacteria

Introduction

Chronic periodontitis is a common disease of adults, and leads to significant treatment costs and to loss of teeth. It has been associated with a number of systemic diseases and conditions (Scannapieco *et al.*, 2010), such as cardiovascular disease and diabetes, and interactions may contribute to these systemic diseases. Chronic periodontitis has a complex etiology dependent on the bacterial community residing in the gingival sulcus. Bacteria may be directly pathogenic or may stimulate damaging host inflammatory responses. The core oral microbiome appears to consist of <1000 species-level taxa (Dewhirst *et al.*, 2010; Griffen *et al.*, 2011), although any number of species may appear transiently in a site so open to the environment.

Periodontitis is thought to have a polymicrobial etiology, but comprehensive studies to elucidate differences between health and disease for the complex communities that make up the oral microbiome have only recently become possible. Early studies based on cultivation were extremely difficult to perform and yielded confusing and contradictory results. Community members had variable growth requirements and rates, many taxa did not grow well in culture, and nearly all were difficult to identify definitively. Targeted approaches such as DNA hybridization and PCR-based assays provided more power to track variation in levels of individual species, but did not provide a comprehensive view

Correspondence: AL Griffen, Division of Pediatric Dentistry and Community Oral Health, The Ohio State University College of Dentistry, 305 W. 12th Ave. Columbus, Ohio 43210, USA.
E-mail: griffen.1@osu.edu
[6]These authors contributed equally to this work.
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ORAL MICROBIOLOGY AND IMMUNOLOGY

Bacterial diversity in aphthous ulcers

L. Marchini[1,2,3], M. S. Campos[1], A. M. Silva[1], L. C. Paulino[4], F. G. Nobrega[1,3]
[1]Laboratory of Molecular Genetics and Genomics, University of Vale do Paraíba, São José dos Campos, São Paulo, Brazil, [2]Department of Dentistry, University of Taubaté, Taubaté, São Paulo, Brazil, [3]Microbiology Graduate Program, Biomedical Sciences Institute, University of São Paulo, São Paulo, Brazil, [4]Department of Medicine, New York University School of Medicine, New York, NY, USA

Marchini L, Campos MS, Silva AM, Paulino LC, Nobrega FG. Bacterial diversity in aphthous ulcers.
Oral Microbiol Immunol 2007: 22: 225–231. © 2007 The Authors. Journal compilation © 2007 Blackwell Munksgaard.

Introduction: Recurrent aphthous ulcers are common lesions of the oral mucosa of which the etiology is unknown. This study aimed to estimate the bacterial diversity in the lesions and in control mucosa in pooled samples using a culture-independent molecular approach.
Methods: Samples were collected from ten healthy individuals and ten individuals with a clinical history of recurrent aphthous ulcers. After DNA extraction, the 16S ribosomal RNA bacterial gene was amplified by polymerase chain reaction with universal primers; amplicons were cloned, sequenced and matched to the GenBank database.
Results: A total of 535 clones were analyzed, defining 95 bacterial species. We identified 62 putative novel phylotypes. In recurrent aphthous ulcer lesions 57 phylotypes were detected, of which 11 were known species. Control samples had 38 phylotypes, five of which were already known. Only three species or phylotypes were abundant and common to both groups (*Gemella haemolysans*, *Streptococcus mitis* strain 209 and *Streptococcus pneumoniae* R6). One genus was found only in recurrent aphthous ulcer samples (*Prevotella*) corresponding to 16% of all lesion-derived clones.
Conclusion: The microbiota found in recurrent aphthous ulcers and in the control groups diverged markedly and the rich variety of genera found can provide a new starting point for individual qualitative and quantitative analyses of bacteria associated with this oral condition.

Key words: 16S RNA gene sequence; aphthous ulcers; microbial diversity

F.G. Nobrega, Laboratório de Genética Molecular e Genomas, IP UNIVAP, Avenida Shishima Hifumi, 2911, Urbanova, SJ Campos, São Paulo, 12244-000, Brazil
Tel./fax: +55 12 39471119;
e-mail: fgdnobre@univap.br
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Recurrent aphthous ulcers are one of the most common oral mucosal lesions in the general population (6). The incidence of this pathology can affect 50% of the population, depending on the sample evaluated (22). Clinical manifestations include minor (ulcers ≤10 mm in diameter), major (ulcers >10 mm in diameter) and herpetiform (showing multiple small pinpoint ulcers) recurrent aphthous ulcers (27).

Minor recurrent aphthous ulcers are the most prevalent form (80% of all recurrent aphthous ulcers) and their clinical features include round or oval shallow ulcers, with a grayish white pseudomembrane in the center, enveloped by a thin erythematous halo (14). Minor recurrent aphthous ulcers occur on non-keratinized mucosal surfaces and generally appear as a single ulcer, although multiple ulcers have been found in some cases. These ulcers usually heal within 10–14 days without scarring (23). However, they generally cause considerable pain and discomfort, and can interfere with many oral functions such as speaking, eating and swallowing (5).

Regardless of its clinical significance, the primary cause these ulcers remains unknown. Consequently, the treatment is still palliative (14). Recent hypotheses postulate that recurrent aphthous ulcers are a consequence of an autoimmune reaction against oral epithelium. It has been suggested that this autoimmune reaction could be a cross-reaction immune response, activated by heat-shock proteins released by oral bacteria and targeting similar peptides in the oral epithelium (11, 29). However, the micro-organisms present in these lesions have so far only been investigated with culture-based techniques (4), which are known to underestimate bacterial diversity (16).

DNA sequencing was suggested as a powerful tool for a better understanding of the participation of micro-organisms in the etiology of recurrent aphthous ulcers (26). Modern molecular methods that allow organism identification without cultivation would disclose the real diversity of micro-organisms, from pathogenic to commensal bacteria (24). By sequencing the 16S ribosomal RNA gene, the presence of many previously unidentified bacteria was revealed in the gingival sulcus, an exhaustively studied microbial niche (16). Broad-range 16S ribosomal DNA analysis

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CLINICAL INVESTIGATIONS

Mucosal Microbiome in Patients with Recurrent Aphthous Stomatitis

K. Hijazi[1]*, T. Lowe[2], C. Meharg[3], S.H. Berry[4], J. Foley[1†], and G.L. Hold[4]

Abstract: *Recurrent aphthous stomatitis (RAS) is the most common disease affecting oral mucosae. Etiology is unknown, but several factors have been implicated, all of which influence the composition of microbiota residing on oral mucosae, which in turn modulates immunity and thereby affects disease progression. Although no individual pathogens have been conclusively shown to be causative agents of RAS, imbalanced composition of the oral microbiota may play a key role. In this study, we sought to determine composition profiles of bacterial microbiota in the oral mucosa associated with RAS. Using high-throughput 16S rRNA gene sequencing, we characterized the most abundant bacterial populations residing on healthy and ulcerated mucosae in patients with RAS (recruited using highly stringent criteria) and no associated medical conditions; we also compared these to the bacterial microbiota of healthy controls (HCs). Phylum-level diversity comparisons revealed decreased Firmicutes and increased Proteobacteria in ulcerated sites, as compared with healthy sites in RAS patients, and no differences between RAS patients with healthy sites and HCs. Genus-level analysis demonstrated higher abundance of total Bacteroidales in RAS patients with healthy sites over HCs. Porphyromonadaceae comprising species associated with periodontal disease and Veillonellaceae predominated in ulcerated sites over HCs, while no quantitative differences of these families were observed between healthy sites in RAS patients and HCs. Streptococcaceae comprising species associated with oral health predominated in HCs over ulcerated sites but not in HCs over healthy sites in RAS patients. This study demonstrates that mucosal microbiome changes in patients with idiopathic RAS—namely, increased Bacteroidales species in mucosae of RAS patients not affected by active ulceration. While these changes suggest a microbial role in initiation of RAS, this study does not provide data on causality. Within this limitation, the study contributes to the understanding of the potential role of mucosal microbiome changes in oral mucosal disease.*

Key Words: oral ulcer, oral mucosa, chronic disease, microbiota, host-pathogen relations, high-throughput DNA sequencing.

Introduction

Recurrent aphthous stomatitis (RAS) is the most common oral mucosal disease in the general population (5% to 60% in different study groups). RAS is characterized by multiple recurrent round or ovoid inflammatory ulcerations with circumscribed margins, erythematous haloes, and yellow or gray floors (Jurge et al. 2006). RAS causes considerable pain, can interfere with oral functions (eating, speech, toothbrushing), and can thereby have a negative impact on quality of life (Al-Omiri et al. 2014). Current treatment is based on topical corticosteroids and systemic immunosuppressants depending on severity but is still palliative, as it only reduces the severity of the ulceration and does not stop recurrence (Altenburg et al. 2007; Sheikh et al. 2013). Understanding the etiology and pathogenesis of RAS, currently unknown, will aid the development of more effective therapeutic strategies.

Host genetics, nutritional deficiencies, as well as a number of systemic conditions (including chronic inflammatory disorders and immunodeficiencies) have been recognized as systemic modulating factors of RAS (Jurge et al. 2006; Slebioda et al. 2013; Sun et al. 2014). While not directly involved in the etiology of RAS, all these factors influence the composition of the community of microorganisms that colonize the oral mucosa (oral microbiota), which can in

DOI: 10.1177/0022034514565458. [1]School of Medicine and Dentistry, Division of Medical and Dental Education, University of Aberdeen, Aberdeen, UK; [2]Maxillofacial Unit, Aberdeen Royal Infirmary, Aberdeen, UK; [3]School of Biological Sciences, Institute for Global Food Security, Queen's University Belfast, Belfast, UK; and [4]School of Medicine and Dentistry, Division of Applied Medicine, University of Aberdeen, Aberdeen, UK; †present address, Edinburgh Dental Institute, Edinburgh, UK; *corresponding author, k.hijazi@abdn.ac.uk

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Research

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The salivary microbiota as a diagnostic indicator of oral cancer: A descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects

DL Mager*[1], AD Haffajee[1], PM Devlin[2], CM Norris[3], MR Posner[3] and JM Goodson[1]

Address: [1]The Forsyth Institute, 140 The Fenway, Boston, MA, USA, [2]Brigham and Women's Hospital, 27 Francis Street, Boston, MA, USA and [3]Dana Farber Cancer Institute, 44 Binney Street, Boston, MA, USA

Email: DL Mager* - dmager@forsyth.org; AD Haffajee - ahaffajee@forsyth.org; PM Devlin - pdevlin@lroc.harvard.edu; CM Norris - Charles_Norris@dfci.harvard.edu; MR Posner - marshall_posner@dfci.harvard.edu; JM Goodson - mgoodson@forsyth.org

* Corresponding author

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Abstract

Background: The purpose of the present investigation was to determine if the salivary counts of 40 common oral bacteria in subjects with an oral squamous cell carcinoma (OSCC) lesion would differ from those found in cancer-free (OSCC-free) controls.

Methods: Unstimulated saliva samples were collected from 229 OSCC-free and 45 OSCC subjects and evaluated for their content of 40 common oral bacteria using checkerboard DNA-DNA hybridization. DNA counts per ml saliva were determined for each species, averaged across subjects in the 2 subject groups, and significance of differences between groups determined using the Mann-Whitney test and adjusted for multiple comparisons. Diagnostic sensitivity and specificity in detection of OSCC by levels of salivary organisms were computed and comparisons made separately between a non-matched group of 45 OSCC subjects and 229 controls and a group of 45 OSCC subjects and 45 controls matched by age, gender and smoking history.

Results: Counts of 3 of the 40 species tested, *Capnocytophaga gingivalis*, *Prevotella melaninogenica* and *Streptococcus mitis*, were elevated in the saliva of individuals with OSCC ($p < 0.001$). When tested as diagnostic markers the 3 species were found to predict 80% of cancer cases (sensitivity) while excluding 83% of controls (specificity) in the non-matched group. Diagnostic sensitivity and specificity in the matched group were 80% and 82% respectively.

Conclusion: High salivary counts of *C. gingivalis*, *P. melaninogenica* and *S. mitis* may be diagnostic indicators of OSCC.

Background

Each year nearly 30,000 Americans are diagnosed with oral cancer. 90% of these lesions are oral squamous cell carcinomas [1]. Despite advances in surgery, radiation

Critical Reviews in Oral Biology & Medicine

The Oral Microbiota Is Modified by Systemic Diseases

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D.T. Graves[1], J.D. Corrêa[2], and T.A. Silva[2]

Abstract

Periodontal diseases are initiated by bacteria that accumulate in a biofilm on the tooth surface and affect the adjacent periodontal tissue. Systemic diseases such as diabetes, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) increase susceptibility to destructive periodontal diseases. In human studies and in animal models, these diseases have been shown to enhance inflammation in the periodontium and increase the risk or severity of periodontitis. All 3 systemic diseases are linked to a decrease in bacterial taxa associated with health and an increase in taxa associated with disease. Although there is controversy regarding the specific oral bacterial changes associated with each disease, it has been reported that diabetes increases the levels of *Capnocytophaga, Porphyromonas*, and *Pseudomonas*, while *Prevotella* and *Selenomonas* are increased in RA and *Selenomonas, Leptotrichia*, and *Prevotella* in SLE. In an animal model, diabetes increased the pathogenicity of the oral microbiome, as shown by increased inflammation, osteoclastogenesis, and periodontal bone loss when transferred to normal germ-free hosts. Moreover, in diabetic animals, the increased pathogenicity could be substantially reversed by inhibition of IL-17, indicating that host inflammation altered the microbial pathogenicity. Increased IL-17 has also been shown in SLE, RA, and leukocyte adhesion deficiency and may contribute to oral microbial changes in these diseases. Successful RA treatment with anti-inflammatory drugs partially reverses the oral microbial dysbiosis. Together, these data demonstrate that systemic diseases characterized by enhanced inflammation disturb the oral microbiota and point to IL-17 as key mediator in this process.

Keywords: bacteria, biofilm, dysbiosis, periodontitis, periodontium, inflammation

Oral Microbiome in Health

The microbiome has a significant impact on the host, as germ-free mice have increased immune diseases, such as asthma and inflammatory bowel disease, indicating a dynamic relationship between them (Olszak et al. 2012). The oral microbiome includes bacteria, fungi, archaea, viruses, and protozoa (Dewhirst et al. 2010). The bacterial component is the best understood and is the focus of this review. The formation of dental plaque is affected by the mode of delivery (vaginal or caesarean), breast or bottle-feeding, and proximity to siblings and pets (Dewhirst et al. 2010). Bacteria can be found on all oral tissues, and there is overlap in the bacteria found on each. The most abundant bacteria are *Streptococcus oralis, Streptococcus mitis*, and *Streptococcus peroris*. Bacteria associated with periodontal health include *Streptococcus, Granulicatella, Neisseria, Haemophilus, Corynebacterium, Rothia, Actinomyces, Prevotella*, and *Capnocytophaga* (Segata et al. 2012). A biofilm forms on the tooth surface, initiated by a pellicle that promotes bacterial adhesion, with *Streptococcus* and *Actinomyces* as early colonizers (Socransky and Haffajee 2005). The latter facilitate formation of a multispecies biofilm that is spatially organized and depends on coaggregation among bacterial taxa (Socransky and Haffajee 2005). The subgingival biofilm is typically more anaerobic than the supragingival biofilm (Socransky et al. 1998).

Changes in the Oral Microbiota Caused by Periodontal Disease

In a National Health and Nutrition Examination Study, 47% of US adults had evidence of periodontitis, and 10% to 15% had advanced periodontitis (Kinane et al. 2017). Periodontal diseases are thought to result from opportunistic infections. The specific factors leading to changes in bacteria that cause periodontal diseases are unknown, although it is recognized that nonideal restorations, genetic conditions that alter the host response, and systemic diseases, such as diabetes and rheumatoid arthritis (RA), predispose to disease (Kinane et al. 2017). The relationships between the biofilm and the host immune response are dynamic, and the ecologic interactions between them determine local homeostasis or transition to a state of disease (Dewhirst 2010; Griffen 2012). Inflammation occurs when bacteria or their products encounter leukocytes in the

[1]Department of Periodontics, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA
[2]Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Brazil

Corresponding Author:
D.T. Graves, Department of Periodontics, School of Dental Medicine, University of Pennsylvania, 240 S 40th St, Philadelphia, PA 19104, USA.
Email: dtgraves@upenn.edu

Diabetes and Periodontal Disease: A Case-Control Study

Guglielmo Campus,* Abeer Salem,* Sergio Uzzau,† Edoardo Baldoni,* and Giancarlo Tonolo‡

Background: Periodontitis is often associated with diabetes and might be considered one of the chronic complications of diabetes mellitus, both in Type 1 (T1DM) and Type 2 (T2DM). This case-control study was designed to evaluate the possible association between non–insulin-dependent diabetes (T2DM) and clinical and microbiological periodontal disease among adult Sardinians.

Methods: A total of 212 individuals participated in this study: 71 T2DM patients aged 61.0 ± 11.0 years and 141 non-diabetic controls in good general health aged 59.1 ± 9.2 years. All subjects were given a clinical periodontal examination for probing depth, attachment level, presence of calculus, bleeding on probing, and assessment of plaque. Subgingival plaque samples were obtained, and *P. gingivalis, P. intermedia*, and *T. forsythensis* were identified using multiplex polymerase chain reaction.

Results: T2DM patients showed a significantly lower number of teeth present ($P = 0.002$); a significant increase in number of probing depths >4 mm, and percent of pocket depths >4 mm ($P = 0.04$ and $P = 0.05$, respectively); periodontitis ($P = 0.046$); bleeding on probing ($P = 0.02$); and plaque index ($P = 0.01$). A significant association with diabetes was detected for plaque ($\chi^2 = 4.46$; $P < 0.05$) and bleeding on probing ($\chi^2 = 3.60$; $P < 0.05$). Concerning bacteria prevalence, a positive association was detected for *P. gingivalis* ($\chi^2 = 2.80$; $P < 0.05$) and *T. forsythensis* ($\chi^2 = 3.87$; $P < 0.05$). Presence of plaque was positively associated with case status (odds ratio [OR] = 1.3; 95% confidence interval [CI]: 1.2, 3.6) and with prevalence of *P. gingivalis* and *T. forsythensis* (OR = 1.2, 95% CI: 1.3, 2.2; and 1.2, 95% CI: 1.2, 1.8, respectively).

Conclusion: Patients with T2DM undoubtedly have a susceptibility for more severe periodontal disease. *J Periodontol 2005;76: 418-425.*

KEY WORDS

Diabetes, non-insulin dependent; periodontal diseases; risk factors; Sardinia.

* Dental Institute, University of Sassari, Sassari, Italy.
† Diabetology Unit, Clinica Medica and Patologia Medica, University of Sassari.
‡ Department of Biomedical Sciences, Center for Biotechnology Development and Biodiversity Research, University of Sassari.

The criteria for diagnosing diabetes have undergone significant changes since the early 1960s; consequently, the diagnosis of periodontal diseases has been better defined.[1-3] Using refined standards for diagnosing these two disease states, several general trends are apparent.

Diabetes prevalence is increasing worldwide and it is estimated that more than 300 million subjects will be affected by the year 2025;[4] thus, all diabetes complications will increase. Uncontrolled or poorly controlled diabetes is associated with increased susceptibility to oral infections, including periodontitis. The incidence of periodontitis increases with age among diabetic subjects after puberty.[5,6] Periodontal disease may be more frequent and severe in diabetic individuals with more advanced systemic complications.[5] The increased susceptibility does not correlate with increased levels of plaque and calculus. Collectively, the data support the hypothesis that periodontal disease could affect diabetics, especially those with poorly controlled disease.[5,7-9] Since type 2 diabetes mellitus (T2DM) is debuting earlier in patients, increasing their length of exposure to the disease, periodontal disease might become a serious health and social problem. Type 2 diabetes mellitus (T2DM) patients had a higher prevalence of periodontal disease as determined by using either periodontal attachment loss or radiographic bone loss parameters, indicating that T2DM is a risk factor for periodontal disease.[10] The United States Adult National Survey[11] found significantly more missing teeth and sextants

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ORIGINAL ARTICLE

A tale of two risks: smoking, diabetes and the subgingival microbiome

Sukirth M Ganesan[1], Vinayak Joshi[2], Megan Fellows[1], Shareef M Dabdoub[1], Haikady N Nagaraja[3], Benjamin O'Donnell[4], Neeta Rohit Deshpande[5] and Purnima S Kumar[1]

[1]*Division of Periodontology, College of Dentistry, The Ohio State University, Coloumbus, OH, USA;* [2]*Maratha Mandal's NathajiRao G. Halgekar Institute of Dental Sciences and Research Center, Rajiv Gandhi University, Belgaum, India;* [3]*College of Public Health, The Ohio State University, Coloumbus, OH, USA;* [4]*Division of Endocrinology, Diabetes and Metabolism, The Wexner Medical Center, The Ohio State University, Coloumbus, OH, USA and* [5]*Department of General Medicine, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Centre, Rajiv Gandhi University, Belgaum, India*

Although smoking and diabetes have been established as the only two risk factors for periodontitis, their individual and synergistic impacts on the periodontal microbiome are not well studied. The present investigation analyzed 2.7 million 16S sequences from 175 non-smoking normoglycemic individuals (controls), smokers, diabetics and diabetic smokers with periodontitis as well as periodontally healthy controls, smokers and diabetics to assess subgingival bacterial biodiversity and co-occurrence patterns. The microbial signatures of periodontally healthy smokers, but not diabetics, were highly aligned with the disease-associated microbiomes of their respective cohorts. Diabetics were dominated by species belonging to *Fusobacterium, Parvimonas, Peptostreptococcus, Gemella, Streptococcus, Leptotrichia, Filifactor, Veillonella, TM7* and *Terrahemophilus*. These microbiomes exhibited significant clustering based on HbA1c levels (pre-diabetic (<6.5%), diabetic (6.5–9.9%), diabetics >10%). Smokers with periodontitis evidenced a robust core microbiome (species identified in at least 80% of individuals) dominated by anaerobes, with inter-individual differences attributable largely to the 'rare biosphere'. Diabetics and diabetic smokers, on the other hand, were microbially heterogeneous and enriched for facultative species. In smokers, microbial co-occurrence networks were sparse and predominantly congeneric, while robust inter-generic networks were observed in diabetics and diabetic smokers. Smoking and hyperglycemia impact the subgingival microbiome in distinct ways, and when these perturbations intersect, their synergistic effect is greater than what would be expected from the sum of each effect separately. Thus, this study underscores the importance of early intervention strategies in maintaining health-compatible microbiomes in high-risk individuals, as well as the need to personalize these interventions based on the environmental perturbation.

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Introduction

Periodontitis, a polymicrobial disease that causes destruction of the structures that anchor the tooth to the jawbone, is the sixth most prevalent disease in the world, affecting over 750 million people (Eke *et al.*, 2012; Kassebaum *et al.*, 2014). Smoking and diabetes are the only known risk factors for this disease, increasing the extent and severity of periodontitis by 3–10-fold and hastening periodontal destruction exponentially (1996; Bergstrom *et al.*, 2000; Tomar and Asma, 2000). Periodontitis has been called the sixth complication of diabetes (Loe, 1993), with one-third of diabetics suffering from severe periodontitis (CDC, 2014). Nearly 42% of periodontitis cases can be attributed to smoking (Tomar and Asma, 2000), and critically, 24% of diabetics smoke (Ford *et al.*, 2004), thus creating three high-risk groups for periodontal diseases: smokers, diabetics and diabetic smokers. With nearly 30 million Americans suffering from adult-onset diabetes (CDC, 2014) and over 1 billion smokers worldwide (Ng *et al.*, 2014), preventing periodontitis in these high-risk populations will be a highly cost effective healthcare strategy.

Although it is established that dysbiosis of the indigenous periodontal microbiome is the primary etiological trigger for periodontitis

Correspondence: PS Kumar, Division of Periodontology, College of Dentistry, The Ohio State University, 4111 Postle Hall, 305, W 12th Avenue, Columbus, OH 43210, USA.
E-mail: kumar.83@osu.edu
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associated with pain and numbness for a week and antibiotic use for 7 days". During clinical examination, it was observed a discreet edema in the left mandibular region, compatible with sialoadenitis. The initial occlusal radiography suggested poorly defined radiolucency in the damaged area. In a 3-week period, it was found refractory osteomyelitis, which resulted in intraoral node and a full deletion of the mandibular sulcus, as well as high-intensity pain, blood cell count with left deviation, and the new X-ray revealed pathologic fracture and the patient reported the use of melphalan. The incisional biopsy revealed undifferentiated malignant neoplasia, being necessary immunohistochemical exams, which allowed the diagnostic conclusion. The patient was sent for treatment to a hematologist and oncologist for chemotherapy, and leading to death in the period of 5 months.

CPP284 - NECROTIZING ULCERATIVE MUCOSITIS IN PEDIATRIC CANCER PATIENTS. *BRENDA DE SOUZA MOURA, MARIA ELISA RANGEL JANINI, TAÍSA DOMINGUES BERNARDES SILVA, VALDIR MEIRELLES JUNIOR, RAFAEL NETTO, BRUNNA AGUIAR DA SILVA, CAROLINE GOSSELEN DE SOUZA.*

The necrotizing ulcerative mucositis is closely related to the patient's immune response. Herein we report the case of a child diagnosed with LLA presenting relapse in right testis (isolated), being treated with BFM 2002. During the second cycle, the child began to complain of pain in the maxillary upper and lower anterior region. At this time, the child was neutropenic. The clinical examination showed an erythematosus area with yellowish-white pseudomembrane. At first, the picture was associated with a systemic condition and started Cefepime and systemic antifungal. As there was no improvement, metronidazole was initiated. At this time, the diagnosis was changed to Ulcerative Mucositis necrotizing. The scheme has been lying for twenty-one days. The exfoliation teeth deciduous begins and permanent initiated the eruption process. Currently, the patient has permanent teeth arch and slightly erythematous gums. The final diagnosis was Ulcerative Mucositis necrotizing infection due the exfoliation process associated with neutropenia.

CPP285 - IDIOPATHIC CERVICAL RESORPTION OF THE ENTIRE PERMANENT DENTITION: CASE REPORT. *ELIZA LEANDRO GANZAROLI, INARA CARNEIRO COSTA REGE, MARÍLIA OLIVEIRA MORAIS, ALLISSON FILIPE LOPES MARTINS, ARTHUR WILSON FLORENCIO, TESSA DE LUCENA BOTELHO, ELISMAURO FRANCISCO DE MENDONÇA.*

Idiopathic root resorptions are uncommon alterations and usually occur in the middle third and apical root. In the last 10 years, only eleven cases of multiple root resorption have been described in the English literature, and of these, only 2 cases were classified as cervical multiple reabsorption of idiopathic nature. An 18-year-old male patient sought dental care complaining of "teeth soften and fall". Patient reported severe pain. Medical history found mild cerebral palsy after birth. The intraoral physical examination showed upper teeth with severe mobility and various dental absence. All the lower teeth were present and amalgam restorations were observed in the teeth 36 and 46. Radiographic examinations showed multiple cervical root resorption in upper teeth however, the lower teeth were unchanged. The patient underwent multiple extraction of all the upper teeth with cervical resorption. Patient was rehabilitated with upper dentures.

CPP286 - ORAL CICATRICIAL PEMPHIGOID IN A YOUNG WOMAN: A CASE REPORT. *TARSILA DE CARVALHO FREITAS RAMOS, MARCIO CAMPOS OLIVEIRA, JENER GONÇALVES FARIAS, VALERIA SOUZA FREITAS, MICHELLE MIRANDA LOPES FALCÃO, JOANA DOURADO MARTINS, MARIA EMILIA PEREIRA RAMOS.*

Cicatricial pemphigoid is a vesiculobullous disease of the skin that may be found in the oral cavity. It is a chronic autoimmune disease that affects mucous membranes. Tissue-bound autoantibodies are directed against components of the basement membrane. The lesions occur more frequently in women in the fifth to seventh decades of life. This study reports a clinical case of this pathology in an 18-year-old female patient that was referred to the Reference Center with a painful gingival ulceration with 8 months duration. The patient had ulceration in the maxillary and mandibular gingival mucous, painful and bleeding. The ulcerations were preceded by vesiculobullous lesions. The microscopic diagnosis was cicatricial pemphigoid and the findings were characterized by a smooth, linear split between the surface epithelium and the underlying connective tissue at the level of the basement membrane. The patient was referred to an ophthalmologist and center for autoimmune diseases treatment.

CPP287 - ORAL MANIFESTATION OF TUBERCULOSIS - A CHALLENGING DIAGNOSIS. *RAFAEL NETTO, MARIA ELISA RANGEL JANINI, VALDIR MEIRELLES JÚNIOR, ARLEY SILVA JÚNIOR, ELIANE PEDRA DIAS, THAYLLA NÚÑEZ AMIN DICK, THAMYRES CAMPOS FONSECA.*

Tuberculosis (TB) is an infectious disease usually caused by the Mycobacterium tuberculosis. It generally affects the lungs, but can also affect other sites, such as the oral cavity. The classic symptoms of active TB are a chronic cough with blood-containing sputum, fever, night sweats and weight loss. Diagnosis of active TB is based on chest X-rays, as well as microscopic examination and culture of body fluids. Treatment requires the use of multiple antibiotics over a long period of time. Herein we present a case of a patient who sought clinical care with a granulomatous ulcer in the palate. Two biopsies were made, but no diagnosis could be achieved. The patient underwent internment and after chest X-ray and 2 bacilloscopy, a diagnosis of tuberculosis was made. The correct treatment was instituted and the oral lesion healed well in approximately 2 months.

CPP288 - ORAL MANIFESTATION OF SYSTEMIC LUPUS ERYTHEMATOSUS. *RAFAEL NETTO, MARIA ELISA RANGEL JANINI, VALDIR MEIRELLES JÚNIOR, BRUNO AUGUSTO BENEVENUTO DE ANDRADE, BRENDA DE SOUZA MOURA, CAROLINE GOSSELEN DE SOUZA, BRUNNA AGUIAR DA SILVA.*

Systemic lupus erythematosus (SLE), or lupus, is an autoimmune connective tissue disease. There are many kinds of lupus,

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Dysbiosis of oral microbiota is associated with systemic lupus erythematosus

Bao-Zhu Li[a,b,*], Hao-Yue Zhou[a,b], Biao Guo[c], Wen-Jun Chen[d], Jin-Hui Tao[e], Nv-Wei Cao[a,b], Xiu-Jie Chu[a,b], Xiang Meng[f]

[a] Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui, China
[b] Anhui Province Key Laboratory of Major Autoimmune Diseases, 81 Meishan Road, Hefei, Anhui, China
[c] Department of Human Resource, The Second Affiliated Hospital of Anhui Medical University, Anhui, Hefei, China
[d] Department of Nutrition and Food Hygiene, School of Public Health, Anhui Medical University, Hefei, Anhui, China
[e] Department of Rheumatology & Immunology, Anhui Provincial Hospital, Anhui, Hefei, China
[f] School of Stomatology, Anhui Medical University, Hefei, Anhui, China

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ABSTRACT

Objective: The important role of intestinal microbiota in systemic lupus erythematosus (SLE) has been recognized. Oral-gut microbiome axis is a crucial link in human health and disease, but few researches indicated the relationship between oral microorganisms and SLE. This study mainly explored the composition and changes of oral microorganisms in SLE patients with different stages, clinical manifestations and biomarkers.

Design: Oral microbiota was detected by 16S ribosomal RNA gene sequencing from 20 SLE patients and 19 healthy controls (HCs). The evenness, diversity and composition of oral microbiota were analyzed. Moreover, receiver-operating characteristic analysis was conducted. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to investigate microbiota functions.

Results: The oral microbiota of SLE patients was imbalanced and the diversity was decreased, but no difference was found between new-onset and treated SLE patients. Families Lactobacillaceae, Veillonellaceae and Moraxellaceae were enriched in SLE patients. Families like Corynebacteriaceae, Micrococcaceae, Defluviitaleaceae, Caulobacteraceae, Phyllobacteriaceae, Methylobacteriaceae, Hyphomicrobiaceae, Sphingomonadaceae, Halomonadaceae, Pseudomonadaceae, Xanthomonadaceae, etc. were decreased in SLE patients. After multiple testing adjustment, families Sphingomonadaceae, Halomonadaceae, and Xanthomonadaceae were significantly decreased in SLE patients. And area under the curve was 0.953 (95% confidence intervals 0.890–1.000) to distinguish SLE patients from HCs. There were differences in metabolic pathways between SLE and HCs ($P = 0.025$).

Conclusions: These findings collectively support that oral microbiota dysbiosis and aberrant metabolic pathways were observed in patients with SLE. Our findings may provide suggestive evidences for the diagnosis and treatment of SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is a severe autoimmune disease with a worldwide prevalence of 0.02–0.24 %. The clinical manifestations of SLE are diverse, such as skin rash, oral ulcer, lupus arthritis, nephritis, etc. Many organs are also damaged with the onset of disease (Lisnevskaia, Murphy, & Isenberg, 2014). The etiology of SLE is complex and remains unclear. Most scholars believe that environmental, infectious, and genetic factors contribute to the pathogenesis of SLE (Illescas-Montes, Corona-Castro, Melguizo-Rodríguez, Ruiz, & Costela-Ruiz, 2019).

One of the factors involved in the development of SLE is the body's

Abbreviations: SLE, systemic lupus erythematosus; ACR, American College of Rheumatology; SLICC, Systemic Lupus International Collaborating Clinics; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; BMI, body mass index; PCR, polymerase chain reaction; OUT, operational taxonomic unit; PCoA, principal coordinate analysis; LEfSe, Linear Discriminant Analysis Effect Size; LDA, linear discriminant analysis; ROC, receiver-operating characteristic; PICRUSt, phylogenetic investigation of communities by reconstruction of unobserved states; KEGG, Kyoto Encyclopedia of Genes and Genomes; Kos, KEGG orthologs; AUC, area under the curve; HCs, healthy controls; pSS, Sjögren's syndrome; RA, rheumatoid arthritis

* Corresponding author at: Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Anhui, Hefei, 230032, China.
E-mail address: lbz88730@163.com (B.-Z. Li).

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The Human Microbiota: Composition, Functions, and Therapeutic Potential

Rick Conrad
EF Alexander V. Vlassov

Department of Research and Development, Thermo Fisher Scientific, Austin TX, U.S.A.

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

Corresponding Author: Alexander V. Vlassov, e-mail: sasha.vlassov@thermofisher.com
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The human body – primarily (but not solely) the gut – is populated by 100 trillion bacteria and other members of the microbiota community, which play a fundamental role in our well-being. Deviations from healthy microbial compositions have been linked with many human diseases, including inflammatory bowel disease, obesity, cancer, asthma, diabetes, and allergies. This review provides a high-level summary of human microbiome composition and known health effects, and highlights the typical workflows and tools used in microbiome research – from sample collection and storage to isolation and analysis of DNA. We particularly focus on multiple novel microbiota-based therapeutic approaches, including fecal microbiota transplantation (FMT) and targeted bacteriophage engineering. Although our understanding of the microbiome and its interaction with the host is still in the nascent stages, it is becoming increasingly clear that we need to treat it as a sophisticated system, much like the circulatory and immune systems, that exists in harmony with homeostasis, playing multiple roles within the human body.

MeSH Keywords: Bacteriophages • DNA Sequencing • Microbiome • Microbiota

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CHAPTER FOUR

The Oral Microbiome in Health and Its Implication in Oral and Systemic Diseases

B. Sampaio-Maia*,[1], I.M. Caldas*,§,¶, M.L. Pereira*, D. Pérez-Mongiovi§ and R. Araujo*,||

*Universidade do Porto, Portugal
§Institute of Research and Advanced Training in Health Sciences and Technologies, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal
¶Universidade de Coimbra, Portugal
||Flinders University, Adelaide, SA, Australia
[1]Corresponding author: E-mail: mbsmaia@gmail.com

Contents

Abstract

The oral microbiome can alter the balance between health and disease, locally and systemically. Within the oral cavity, bacteria, archaea, fungi, protozoa, and viruses may all be found, each having a particular role, but strongly interacting with each other and with the host, in sickness or in health. A description on how colonization occurs and

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ESSAY

Revised Estimates for the Number of Human and Bacteria Cells in the Body

Ron Sender[1], Shai Fuchs[2¤]*, Ron Milo[1]*

1 Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel,
2 Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

¤ Current address: Division of Endocrinology, The Hospital for Sick Children and Department of Pediatrics, The University of Toronto, Toronto, Canada
* shai.fuchs@sickkids.ca (SF); ron.milo@weizmann.ac.il (RM)

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Abbreviations: CV, coefficient of variation; FISH, fluorescent in situ hybridization; SD, standard deviation; SA, surface area; SEM, Standard Error of the Mean.

Abstract

Reported values in the literature on the number of cells in the body differ by orders of magnitude and are very seldom supported by any measurements or calculations. Here, we integrate the most up-to-date information on the number of human and bacterial cells in the body. We estimate the total number of bacteria in the 70 kg "reference man" to be $3.8 \cdot 10^{13}$. For human cells, we identify the dominant role of the hematopoietic lineage to the total count (≈90%) and revise past estimates to $3.0 \cdot 10^{13}$ human cells. Our analysis also updates the widely-cited 10:1 ratio, showing that the number of bacteria in the body is actually of the same order as the number of human cells, and their total mass is about 0.2 kg.

Introduction

How many cells are there in the human body? Beyond order of magnitude statements that give no primary reference or uncertainty estimates, very few detailed estimates have been performed (the one exception [1] is discussed below). Similarly, the ubiquitous statements regarding 10^{14}–10^{15} bacteria residing in our body trace back to an old back-of-the-envelope calculation [2–4].

The aim of this study is to critically revisit former estimates for the number of human and bacterial cells in the human body. We give up-to-date detailed estimates where the calculation logic and sources are fully documented and uncertainty ranges are derived. By updating the cell counts in the body, we also revisit the 10:1 value that has been so thoroughly repeated as to achieve the status of an established common knowledge fact [4]. This ratio was criticized recently in a letter to the journal *Microbe* [5], but an alternative detailed estimate that will give concrete values and estimate the uncertainty range is needed. Here, we provide an account of the methodologies employed hitherto for cell count and revise past estimates. Doing so, we repeat and reflect on the assumptions in previous back-of-the-envelope calculations, also known as Fermi problems. We find such estimates as effective sanity checks and a way to improve our quantitative understanding in biology.

A major part of the available literature used in the derivation of human cell numbers was based on cohorts of exclusively or mostly men, and as we use these sources, our analysis starts

RESOURCE REPORT
Host-Microbe Biology

New Insights into Human Nostril Microbiome from the Expanded Human Oral Microbiome Database (eHOMD): a Resource for the Microbiome of the Human Aerodigestive Tract

Isabel F. Escapa,[a,b] Tsute Chen,[a,b] Yanmei Huang,[a,b] Prasad Gajare,[a] Floyd E. Dewhirst,[a,b] Katherine P. Lemon[a,c]

[a]The Forsyth Institute (Microbiology), Cambridge, Massachusetts, USA
[b]Department of Oral Medicine, Infection & Immunity, Harvard School of Dental Medicine, Boston, Massachusetts, USA
[c]Division of Infectious Diseases, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA

ABSTRACT The expanded Human Oral Microbiome Database (eHOMD) is a comprehensive microbiome database for sites along the human aerodigestive tract that revealed new insights into the nostril microbiome. The eHOMD provides well-curated 16S rRNA gene reference sequences linked to available genomes and enables assignment of species-level taxonomy to most next-generation sequences derived from diverse aerodigestive tract sites, including the nasal passages, sinuses, throat, esophagus, and mouth. Using minimum entropy decomposition coupled with the RDP Classifier and our eHOMD V1-V3 training set, we reanalyzed 16S rRNA V1-V3 sequences from the nostrils of 210 Human Microbiome Project participants at the species level, revealing four key insights. First, we discovered that *Lawsonella clevelandensis*, a recently named bacterium, and *Neisseriaceae* [G-1] HMT-174, a previously unrecognized bacterium, are common in adult nostrils. Second, just 19 species accounted for 90% of the total sequences from all participants. Third, 1 of these 19 species belonged to a currently uncultivated genus. Fourth, for 94% of the participants, 2 to 10 species constituted 90% of their sequences, indicating that the nostril microbiome may be represented by limited consortia. These insights highlight the strengths of the nostril microbiome as a model system for studying interspecies interactions and microbiome function. Also, in this cohort, three common nasal species (*Dolosigranulum pigrum* and two *Corynebacterium* species) showed positive differential abundance when the pathobiont *Staphylococcus aureus* was absent, generating hypotheses regarding colonization resistance. By facilitating species-level taxonomic assignment to microbes from the human aerodigestive tract, the eHOMD is a vital resource enhancing clinical relevance of microbiome studies.

IMPORTANCE The eHOMD (http://www.ehomd.org) is a valuable resource for researchers, from basic to clinical, who study the microbiomes and the individual microbes in body sites in the human aerodigestive tract, which includes the nasal passages, sinuses, throat, esophagus, and mouth, and the lower respiratory tract, in health and disease. The eHOMD is an actively curated, web-based, open-access resource. eHOMD provides the following: (i) species-level taxonomy based on grouping 16S rRNA gene sequences at 98.5% identity, (ii) a systematic naming scheme for unnamed and/or uncultivated microbial taxa, (iii) reference genomes to facilitate metagenomic, metatranscriptomic, and proteomic studies and (iv) convenient cross-links to other databases (e.g., PubMed and Entrez). By facilitating the assignment of species names to sequences, the eHOMD is a vital resource for enhancing the clinical

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Address correspondence to Katherine P. Lemon, klemon@forsyth.org.

T.C. and Y.H. contributed equally to this work.

The oral microbiota – a mechanistic role for systemic diseases

G. Jia,[1] A. Zhi,[2] P. F. H. Lai,[1] G. Wang,[1] Y. Xia,[1] Z. Xiong,[1] H. Zhang,[1] N. Che[3] and L. Ai*[1]

Key points

- Provides an overview on basic composition and distribution of oral microbiota.
- Elucidates the underlying mechanisms of endogenous and exogenous factors on oral microbiota and oral health.
- Reviews oral microbiota and its implications for systemic diseases.
- Summarises the improvement of clinical diagnosis and treatment based on microbial community information.

Human oral microbiota is the ecological community of commensal, symbiotic, and pathogenic microorganisms found in the oral cavity. Oral microbiota generally exists in the form of a biofilm and plays a crucial role in maintaining oral homeostasis, protecting the oral cavity and preventing disease development. Human oral microbiota has recently become a new focus research for promoting the progress of disease diagnosis, assisting disease treatment, and developing personalised medicines. In this review, the scientific evidence supporting the association that endogenous and exogenous factors (diet, smoking, drinking, socioeconomic status, antibiotics use and pregnancy) modulate oral microbiota. It provides insights into the mechanistic role in which oral microbiota may influence systemic diseases, and summarises the challenges of clinical diagnosis and treatment based on the microbial community information. It provides information for noninvasive diagnosis and helps develop a new paradigm of personalised medicine. All these benefit human health in the post-metagenomics era.

Introduction

The oral cavity is a connection channel between outside environments and the respiratory tract and digestive tract. It provides an appropriate temperature, humidity, and nutrition for microorganism colonisation. The human oral microbiome has been extensively studied as part of the Human Microbiome Project. The oral microbiome has an essential role in maintaining a normal oral ecological balance and in the development of oral diseases. There is abundant evidence supporting the theory that endogenous and exogenous factors are closely related to oral microbiota and systemic diseases.[1,2] Studies on dietary behaviours demonstrate a fundamental aspect of the oral disease paradigm.[3] Lifestyles and diets including smoking, alcohol drinking and consuming spicy food, and antibiotic treatments can persistently alter commensal microbial communities.[4] The resultant microbial disturbances may increase pathogen susceptibility.[5]

The disturbance of the oral microbiota–ecology balance in the host usually causes a series of oral infectious diseases including dental caries, apical periodontitis, periodontal diseases, pericoronitis, and craniofacial bone osteomyelitis. Oral microbiota is also associated with several systemic diseases, namely cardiovascular disease, pneumonia, heart disease, rheumatoid arthritis, pancreatic cancer, colorectal cancer, oesophageal cancer, stroke, and adverse pregnancy outcomes. Accordingly, oral microbiota has been considered as a potential biomarker for human diseases. Relationships between oral microbiota and systemic diseases are essential and need to be elucidated, in order to provide a reasonable diagnosis basis for disease prevention and treatments.

This article mainly discusses the mechanisms for how endogenous and exogenous factors modulate oral microbiota, provides insights into their roles in the influence of oral microbiota on systemic diseases, and summarises the challenges for clinical diagnosis and treatment.

[1]Shanghai Engineering Research Centre of Food Microbiology, School of Medical Instrument and Food Engineering, University of Shanghai for Science and Technology, Shanghai 200093, People's Republic of China; [2]Chemical Technology and Food Science College, Zhengzhou Institute of Engineering and Technology, Zhengzhou 450044, People's Republic of China; [3]Department of Otolaryngology, Tongji Hospital, Tongji University, Shanghai 200065, PR China
*Correspondence to: Lianzhong Ai
Email: ailianzhong@hotmail.com

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Basic composition and distribution of oral microbiota

The oral microbiome can be classified into core microbiome and variable microbiome. The core microbiome is similar for all individuals and comprised of the predominant species at different sites of the healthy body. The variable microbiome is different between individuals in response to unique lifestyles and phenotypic and genotypic determinants.

For newborns, within five minutes of birth, bacterial communities in the oral cavity and other body habitats are very similar to each other.[6] Types of microorganisms are closely decided by the delivery mode.[7] In addition, the mother's oral microbiota is the most important source of infants' and young children's oral microbiota by successful vertical transmission.[7,8] As ageing continues, babies and children form a wide variety of oral microorganisms in response to different diets, lifestyles, environments and so on.[9]

The oral cavity contains over 700 microbial species as well as commensal

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Overview of Next Generation Sequencing Technologies

Barton E. Slatko, **Andrew F. Gardner**, and **Frederick M. Ausubel**

Department of Molecular Biology, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114

Abstract

High throughput DNA sequencing methodology (next generation sequencing; NGS) has rapidly evolved over the past 15 years and new methods are continually being commercialized. As the technology develops, so do increases in the number of corresponding applications for basic and applied science. The purpose of this review is to provide a compendium of NGS methodologies and associated applications. Each brief discussion is followed by web links to the manufacturer and/or web-based visualizations. Keyword searches, such as with Google, may also provide helpful internet links and information.

"The greatest adventure is what lies ahead.

Today and tomorrow are yet to be said.

The chances, the changes are all yours to make.

The mold of your life is in your hands to break."

J. R. R. TOLKIEN, *The Hobbit*

Founding Methodology

The founding methods in DNA sequencing were the Sanger dideoxy synthesis (Sanger & Coulson, 1975; Sanger, Nicklen, & Coulson, 1977) (UNIT 7.4) and Maxam-Gilbert chemical cleavage (Maxam & Gilbert, 1980) (UNIT 7.5) methods. The Maxam-Gilbert method is based on chemical modification of DNA and subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides. Sanger sequencing uses specific chain-terminating nucleotides (dideoxy nucleotides) that lack a 3′-OH group. Thus no phosphodiester bond can be formed by DNA polymerase, resulting in termination of the growing DNA chain at that position. The ddNTPs are radioactively or fluorescently labeled for detection in "sequencing" gels or automated sequencing machines, respectively. Although the chemistry of the original Maxam-Gilbert method has been modified to help eliminate toxic reagents, the Sanger sequencing by synthesis (SBS) dideoxy method has become the sequencing standard.

The Sanger sequencing method was developed in 1977 and is described in detail in Unit 7.4. Although relatively slow by current NGS standards, improvements in the Sanger chain

Tel: (617) 726-5969, ausubel@molbio.mgh.harvard.edu.

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THE DENTAL
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Oral manifestations of patients with lupus erythematosus

Michael T. Brennan, DDS, MHS*,
Manuel A. Valerin, DDS, Joel J. Napeñas, DDS,
Peter B. Lockhart, DDS

Department of Oral Medicine, Carolinas Medical Center, 1000 Blythe Boulevard, Charlotte, NC 28232, USA

Epidemiology

The prevalence of lupus erythematosus (LE) in the United States has been estimated at 500,000 to 1 million cases. Cutaneous LE is two to three times more common than systemic LE (SLE) [1]. Numerous factors seem to predispose patients to the cutaneous variants of LE; among them are genetic factors, environmental factors (sun exposure), and immune dysregulation [2].

In the United States the prevalence of SLE is 500/million, and the annual incidence is 70/million population [3]. Women are more commonly affected, with a ratio of 6 to 10:1 and a peak incidence between 15 and 40 years of age [4]. A genetic predisposition is one important factor in the development of SLE, because disease concordance in identical twins is 24%, compared with approximately 2% in dizygotic twins [5]. Other risk factors include hormonal and immune dysregulation; environmental factors such as infectious agents, stress, diet, and toxins; and physical agents such as sunlight [4,6].

Pathogenesis

The characteristic disease findings in LE include inflammation, blood vessel changes such as vasculopathy, and immune-complex deposition. Generalized autoantibody production in SLE is a hallmark immunologic

* Corresponding author.
E-mail address: mike.brennan@carolinas.org (M.T. Brennan).

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ORIGINAL RESEARCH
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Association between Systemic Lupus Erythematosus and Periodontitis: A Systematic Review and Meta-analysis

*Zoe Rutter-Locher[1], Toby O. Smith[2], Ian Giles[3] and Nidhi Sofat[1]**

[1] Musculoskeletal Research Group, Institute of Infection and Immunity, St George's University of London, London, United Kingdom, [2] Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, United Kingdom, [3] Center for Rheumatology Research, Rayne Institute, University College London, London, United Kingdom

Background: Systemic lupus erythematosus (SLE) is a chronic systemic inflammatory autoimmune disease, the etiology of which remains only partially characterized. Strong evidence implicates chronic infections in the development and chronicity of autoimmune conditions. Recently, an association has been demonstrated between periodontitis and rheumatoid arthritis. Such observations have led to the investigation of the possible role of periodontitis and oral dysbiosis in other systemic inflammatory conditions, including SLE. The aim of this study was to examine whether there is an association between SLE and periodontitis.

Methods: MEDLINE *via* OVID, EMBASE *via* OVID, and PsycINFO *via* OVID databases were searched to identify eligible studies, screened by two independent authors and verified by a third. Studies comparing presence of periodontitis in SLE cases to controls without SLE were included. Data were extracted using a predefined table and papers were appraised using Down's and Black tool. Mantel–Haenszel meta-analysis was performed using RevMan.

Results: Eight case–control studies were included, with 487 SLE cases and a total of 1,383 participants. On meta-analysis of four studies, risk of periodontitis in SLE cases compared to controls was significantly greater with a risk ratio of 1.76 (95% CI 1.29–2.41, $p = 0.0004$). No statistical difference was found in individual measures of periodontitis, such as probing depth or clinical attachment loss, between SLE cases and controls.

Conclusion: Our study found a statistically significant increased risk of periodontitis in patients with SLE compared to controls. This finding suggests a possible association between these two conditions. Larger longitudinal studies are needed to confirm this possible association.

Keywords: systemic lupus erythematosus, autoimmune and inflammatory diseases, microorganisms, periodontitis, periodontal disease, meta-analysis

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****Correspondence:***
Nidhi Sofat
n.sofat@sgul.ac.uk

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic, chronic inflammatory condition with diverse clinical manifestations, primarily affecting the joints, internal organs, and the skin (1).

The etiology of SLE is incompletely understood, but it is thought to occur in genetically primed individuals in whom the inflammatory response is triggered by an environmental stimulus.

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The oral microbiome diversity and its relation to human diseases

Jinzhi He · Yan Li · Yangpei Cao · Jin Xue · Xuedong Zhou

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Abstract As one of the most clinically relevant human habitats, the human mouth is colonized by a set of microorganisms, including bacteria, archaea, fungi, and viruses. Increasing evidence has supported that these microbiota contribute to the two commonest oral diseases of man (dental caries and periodontal diseases), presenting significant risk factors to human health conditions, such as tumor, diabetes mellitus, cardiovascular diseases, bacteremia, preterm birth, and low birth weight in infants. It is widely accepted that oral microorganisms cause diseases mainly by a synergistic or cooperative way, and the interspecies interactions within the oral community play a crucial role in determining whether oral microbiota elicit diseases or not. Since a comprehensive understanding of the complex interspecies interactions within a community needs the knowledge of its endogenous residents, a plenty of research have been carried out to explore the oral microbial diversity. In this review, we focus on the recent progress in this field, including the oral microbiome composition and its association with human diseases.

Introduction

Only about 10 % of cells in our bodies are truly from the human host, and the rest are from human microbiota (Savage 1977; Wilson 2008). These commensal microorganisms help us resist pathogens, educate immune system, and provide some traits humans do not originally evolve with the body (Dethlefsen et al. 2007; Gill et al. 2006; Turnbaugh et al. 2007). For instance, the plant polysaccharides commonly consumed in the diet are rich in xylan-, pectin-, and arabinose-containing carbohydrate structures. Although the human genome lacks most of the enzymes required for degrading these compounds, the distal gut microbiota provides us with this capacity (Gill et al. 2006). In fact, the human genetic landscape is a blend of the human genome and the metagenome of microorganisms colonizing in/on the human bodies (Turnbaugh et al. 2007). Therefore, the genetic diversity of humans resides not only in the allele frequencies of shared *Homo sapiens* genes but also in the genes within our microbial communities (Bäckhed et al. 2005; Li et al. 2008). To fully understand the human genetic and physiological variations, the composition and structure of human microbiota in major parts (e.g., mouth, skin, and gut) of the body and their influencing factors must be characterized (Gill et al. 2006; Heijtz et al. 2011).

As one of the most clinically relevant microbial habitats, the oral cavity is colonized by a personalized set of microorganisms, including bacteria, archaea, fungi, and viruses. If the term "human microbiome" is used to describe the sum of microbes that live in symbiosis or commensalism with us and elicit various human diseases under certain conditions (Lederberg and McCray 2001), the "oral microbiome" is suitable to refer specifically to the microorganisms inhabiting the human mouth (Dewhirst et al. 2010). The oral microbiome not only greatly contributes to the two commonest human oral diseases (i.e., dental caries and periodontal diseases) but also has been proven to present a significant risk factor to human health, such as tumor (Farrell et al. 2011), diabetes mellitus (Löe 1993), cardiovascular diseases (Figuero et al. 2011), bacteremia (Bahrani-Mougeot et al. 2008), and preterm birth and low birth weight in infants (Mitchell-Lewis et al. 2001;

Jinzhi He and Yan Li have equal contribution to this work.

J. He · Y. Li · Y. Cao · J. Xue · X. Zhou
State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan Province, China

J. He · Y. Cao · J. Xue · X. Zhou (✉)
Department of Endodontics and Operative Dentistry, West China Hospital of Stomatology, Chengdu, China
e-mail: zhouxd_scu@163.com

Systemic Diseases and Oral Health

Mary Tavares, DMD, MPH[a,b,*], Kari A. Lindefjeld Calabi, DMD[c], Laura San Martin, DDS, PhD, MDPH[d]

KEYWORDS

• Chronic illnesses • Diabetes mellitus • Cardiovascular diseases
• Systemic complications

KEY POINTS

- Oral disease management is more complex in patients with several systemic diseases.
- Severe periodontitis adversely affects diabetes control.
- Additional considerations exist for diabetic patients in a dental office setting.
- Osteoarthritis of the hands reduces manual dexterity and constrains the patient's capability of maintaining adequate oral hygiene.

INTRODUCTION

Several new studies have shown that an association exists between oral diseases and systemic chronic diseases. Inflammation has additionally been recognized as the key factor that connects many of these diseases.[1] Chronic diseases are defined as long-lasting illnesses, with duration of more than 3 months that affect a person's life and require constant medical treatment. Chronic diseases more frequently affect aging individuals; 80% have one chronic condition, and 50% have at least 2 conditions.[2] Chronic conditions are the leading cause of death and disability in the United States. According to the National Vital Statistics, the 10 leading causes of death among the 65-years-and-over age group are heart diseases, malignant neoplasm, chronic lower respiratory diseases, cerebrovascular diseases, Alzheimer diseases, diabetes mellitus (DM), influenza and pneumonia, nephritis, unintentional accidents, and septicemia.[3] The authors have chosen to select cardiovascular diseases (CADs), hypertension,

[a] Dental Public Health, Oral Health Policy and Epidemiology, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, MA 02115, USA; [b] Department of Applied Oral Sciences, The Forsyth Institute, 245 First Street, Cambridge, MA 02142, USA; [c] Oral Health Policy and Epidemiology, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, MA 02115, USA; [d] Department of Stomatology, School of Dentistry, University of Seville, Avicena, Seville 41009, Spain
* Corresponding author. Dental Public Health, Oral Health Policy and Epidemiology, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, MA 02115.
E-mail address: mary_tavares@hsdm.harvard.edu

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ORIGINAL ARTICLE

The Oral Microbiota: Living with a Permanent Guest

Maria Avila,[1] David M. Ojcius,[1] and Özlem Yilmaz[2,3]

The oral cavity of healthy individuals contains hundreds of different bacterial, viral, and fungal species. Many of these can associate to form biofilms, which are resistant to mechanical stress or antibiotic treatment. Most are also commensal species, but they can become pathogenic in responses to changes in the environment or other triggers in the oral cavity, including the quality of an individual's personal hygiene. The complexity of the oral microbiome is being characterized through the newly developed tools of metagenomics. How the microbiome of the oral cavity contributes to health and disease is attracting the interest of a growing number of cell biologists, microbiologists, and immunologists.

"No man is an island, entire of itself"
—John Donne (1572–1631)

Introduction

We have more prokaryotic organisms on or in our bodies than we have eukaryotic cells. In fact, only one out of 10 cells in our bodies is human. These prokaryotic guests perform many biological functions that we could not perform on our own and protect us from invasion by pathogenic microorganisms.

In the early 1990s, scientists were confident that the sequencing of the human genome would be sufficient for understanding the basis for human function and disease, but analysis of the human genome was only an introduction to the genetic composition of our bodies. Humans and their commensal organisms have evolved together over the last two million years and have gradually become dependent on one another (O'Connell *et al.*, 1998; Turnbaugh *et al.*, 2007; Ley *et al.*, 2008). The various commensals include eubacteria, archaebacteria, and fungi, which together comprise the human microbiome (Gill *et al.*, 2006; Turnbaugh *et al.*, 2007). To complicate matters further, the microbial communities within the body respond to different environmental conditions by modifying their species composition and population size. The effects of microbial metabolism in different locations on the human body also correspond to the physiological needs of the sites, which can vary from vitamin K production to the renewal of epithelial cells lining the gut.

While most organisms colonizing our bodies are beneficial to our health, some of them can transition from a commensal relationship to one of pathogenicity, for reasons that are still not understood. According to one view, the disease-causing bacteria are always present in a pathogenic state, but the commensal bacteria, which are more abundant, prevent the dangerous microbes from establishing a foothold (Pennisi, 2005). According to another view, some elusive trigger from the environment, or some temporal cue, stimulates the activity of the bacteria, resulting in infection or disease. Most likely, both possibilities are right. In the case of mucosal biofilms, a confounding issue is the relationship between inflammation and disease, and it is still not clear which comes first: the immune response, or the change in integrity of the mucosal biofilm (Dongari-Bagtzoglou, 2008).

The microbial communities are bound to impact the health of individual humans, and a better understanding of their dynamic complexity may contribute to the next level in medical diagnostic tools. Ideally, this should also lead to more specific treatment, by providing the potential to manipulate the microbiome to optimize personal health. These goals gave rise to the Human Microbiome Project, which aims to identify a core human microbiome, a common set of commensal species that can be defined as a healthy microbiota (Turnbaugh *et al.*, 2007; National Institutes of Health, 2009). This project is being conducted around the world and includes the National Institutes of Health in the United States (Turnbaugh *et al.*, 2007). Technology is now providing advanced sequencing techniques that are more cost effective and faster than ever, allowing for metagenomic analysis of microbial communities found in samples as varied as the human gut and ocean water (National Research Council, 2007). The field of metagenomics allows genomic analysis to be applied to entire communities of microbes, circumventing the need to isolate and culture individual bacterial community members. The new technologies include 454 pyrosequencing, which rapidly and inexpensively obtains genomic sequences without cloning bias, and proteomics (National Research Council, 2007; Keller and Hettich, 2009; Mitreva and Mardis, 2009).

Multiple projects are currently in progress, but the best-described microbiota to date reside in the human gut, which

[1]School of Natural Sciences, University of California, Merced, California.
[2]Department of Periodontology, College of Dentistry; [3]Emerging Pathogens Institute; University of Florida, Gainesville, Florida.

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Purnima S. Kumar*

Division of Periodontology, College of Dentistry, The Ohio State University, 4111 Postle Hall, 305 W. 12th Avenue, Columbus, OH 43210, USA

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Periodontal medicine

ABSTRACT

It is well known that bacteria are the primary cause of infectious diseases, however, evidence is emerging that these organisms are also indirectly responsible for several diseases including cancer and rheumatoid arthritis. The oral cavity is home to several million bacteria that can cause two major diseases-periodontitis and caries. The relationship between periodontopathic bacteria and systemic diseases has been explored for several years. The concept of the oral cavity as a source of distant infection has been debated for at least a century. This review will discuss the historic aspects of the development of the focal infection theory, the reasons for its demise, its re-emergence and current status.

1. Introduction

During the Anaerobe 2012 meeting, a session entitled "Oral Microbiota and Systemic Disease " reviewed current knowledge about the inter-relationships between oral bacteria and susceptibility to systemic diseases. This article will discuss the historic aspects of the development of the focal infection theory, the reasons for its demise, its re-emergence and current status.

2. Rise, fall and rise of the focal infection theory

A focus of infection is best described as a circumscribed lesion that is clinically asymptomatic and contains pathogenic bacteria. According to the theory of focal infection, bacteria and/or bacterial products are disseminated from this nidus to distant parts, leading to disease in these organ systems. Several foci of infection have been described in the literature, including tonsils, sinuses, prostate, appendix, bladder, gall bladder, and kidney. Several diseases have been attributed to focal infections, including arthritis, neuritis, myalgia, nephritis, osteomyelitis, endocarditis, pneumonia, asthma, emphysema, gastritis, pancreatitis, colitis, diabetes, goiter, thyroiditis and Hodgkin's disease.

The tooth as a focus of infection: The theory of the oral cavity as a focus of infection is not new; Hippocrates reported arthritis being cured following extraction of a tooth [1]. The term oral focal sepsis was introduced by W.D. Miller in his 1890 article "*The Micro-Organisms of the Human Mouth: The Local and General Diseases Which Are Caused by Them*" and recommended removing decayed parts of a tooth and replacing them with fillings or root canal fillings [2]. However, in 1900, British physician William Hunter ascribed a plethora of systemic diseases to the preservation of a carious tooth by building 'a veritable mausoleum of gold fillings, crowns and bridges over a mass of sepsis' [3]. In 1940, Fish published an article on teeth as a source of systemic infections [4]. He described a state where teeth affected by periodontitis (a bacterially-induced disease that affects the structures that support the tooth and anchor it to the jawbone) "shower bacteria into the blood stream" even during the simple process of chewing or tooth brushing. He cited evidence from his own and other studies where dental bacteria could be detected in proximal and distant blood vessels (median basilic and peri-apical veins) following tooth extraction or chewing on hard candy. He proposed that the bacteria or their toxins stagnate in areas where tissues of mesenchymal origin predominate, namely joints, muscle and nerve sheaths. The purported susceptibility of these tissues was due to their 'unique functions of repair, regeneration and scavenging of waste products'. Thus, he hypothesized that dissemination of bacteria of oral origin to tissues of mesenchymal origin led to the pathogenesis of diverse diseases like osteomyelitis, fasciitis of the sciatic nerve, fibromyalgia and endocarditis.

Therapeutic impact of the oral focal sepsis theory: The focal sepsis theory gained momentum in the 19th century and the early 20th century based on the recommendations of prominent

* Tel.: +1 614 247 4532; fax: +1 614 292 4612.
E-mail address: kumar.83@osu.edu.

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Review

Fundamentals of DNA Hybridization Arrays for Gene Expression Analysis

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Willard M. Freeman, Daniel J. Robertson and Kent E. Vrana
Wake Forest University School of Medicine, Winston-Salem, NC, USA

ABSTRACT

DNA hybridization arrays [also known as macroarrays, microarrays and/or high-density oligonucleotide arrays (Gene Chips™)] bring gene expression analysis to a genomic scale by permitting investigators to simultaneously examine changes in the expression of literally thousands of genes. For hybridization arrays, the general approach is to immobilize gene-specific sequences (probes) on a solid state matrix (nylon membranes, glass microscope slides, silicon/ceramic chips). These sequences are then queried with labeled copies of nucleic acids from biological samples (targets). The underlying theory is that the greater the expression of a gene, the greater the amount of labeled target, and hence, the greater output signal. In spite of the simplicity of the experimental design, there are at least four different platforms and several different approaches to processing and labeling the biological samples. Moreover, investigators must also determine whether they will utilize commercially available arrays or generate their own. This review will cover the status of the hybridization array field with an eye toward underlying principles and available technologies. Future developments and technological trends will also be evaluated.

INTRODUCTION

As investigators work on more complete and annotated copies of the human genome project, their attention turns to methods for using this wealth of information. Two questions generated by sequencing the entire complement of human genetic material are (*i*) how differential expression of that information is associated with health and disease and (*ii*) how mutations or small natural variations in that sequence produce genetic disorders and/or increased risk for disease. The former of these, gene expression, is the cornerstone of functional genomics. In the present context, functional genomics is defined as the study of all the genes expressed by a specific cell or group of cells and the changes in their expression pattern during development, disease or environmental exposure (Figure 1). While sequence polymorphisms are sometimes included as part of the functional genomics field, this review will place such work under genomics because it represents variations in DNA sequence. Specifically, this review will look at the use of hybridization arrays to study gene expression.

Hybridization arrays have created a wave of interest and skepticism in the past five years. While many scientists view the use of arrays to monitor gene expression for thousands of genes as the dawn of functional genomics (13,19,27), others see the technology as expensive nonhypothesis-driven descriptive research—the ultimate "fishing experiment" (9,47). Both views have valid points, and this controversy can be typical of any new field of study. Although gene expression studies using multiplex hybridization arrays have been performed on a wide range of research topics including cell biology, aging, cancer, environmental toxicity and drug abuse (20,29,37,42,54,75), performing these experiments in a manner that yields accurate results presents a unique technical challenge.

This review will elaborate the technical underpinnings of hybridization arrays and describe potential problems that must be addressed for reliable determination of gene expression changes. The basics of multiplex hybridization arrays will be presented first, followed by the descriptions of the four different types of array platforms: macroarrays, microarrays, high-density oligonucleotide arrays (Gene Chips™; Affymetrix, Santa Clara, CA, USA) and microelectronic arrays. Next, probe selection and design will be discussed. Subsequently, because all arrays employ the same four basic components: target labeling, target-probe hybridization, detection and data analysis (Figure 2), these steps will be individually discussed. Finally, central aspects of experimental design will be reviewed. This presentation is not intended to advocate a specific experimental approach or any single version of the technology. Neither will this commentary address uses of arrays for multiplex sequencing or polymorphism detection (21,32) (important topics worthy of independent discussion in their own right; "genomics" in Figure 1). Rather, this discussion focuses on the "good practice" use of arrays for monitoring differential gene expression with the realization that the best choice of experimental options depends on the specific application.

ARRAY BASICS

In the past, analysis of gene expression (through measurement of steady-state levels of mRNA) was conducted one gene at a time. Northern blotting, dot blots and quantitative RT-PCR were

PRODUCT APPLICATION FOCUS

...a forum for manufacturers to describe the current and potential applications of new research instruments or products.

"Checkerboard" DNA-DNA Hybridization

S.S. Socransky, C. Smith, L. Martin, B.J. Paster, F.E. Dewhirst and A.E. Levin[1]
Forsyth Dental Center, Boston, MA, and [1]Immunetics, Cambridge, MA, USA

ABSTRACT

A method is introduced for hybridizing large numbers of DNA samples against large numbers of DNA probes on a single support membrane. Denatured DNA from up to 43 samples was fixed in separate lanes on a single membrane mounted in a Miniblotter® 45. The membrane was then rotated 90° in the same device, which enabled simultaneous hybridization with 43 different DNA probes. Hybridizations were also performed on lysates of bacterial cells blotted to membranes. A MiniSlot™ device allowed lysates loaded in parallel channels to be aspirated through the membrane, depositing horizontal lanes on the membrane surface. Hybridizations were performed in vertical lanes with either digoxigenin-labeled whole genomic probes or 16S rRNA-based oligonucleotide probes directly conjugated to alkaline phosphatase. The method permits the simultaneous determination of the presence of multiple bacterial species in single or multiple dental plaque samples, thus suggesting its usefulness for a range of clinical or environmental samples.

INTRODUCTION

A major technical barrier in DNA-DNA hybridization assays is the limitation in the number of DNA probes that may be simultaneously hybridized with large numbers of DNA samples. Previous methods, e.g., "reverse hybridization" techniques, have been used to evaluate small numbers of samples against large numbers of target DNAs (8,9). Our aim was to examine large numbers of dental plaque samples for their content of a wide range of bacterial species. A new assay method was devised to accomplish this goal, and it appears to have general applicability to the hybridization of multiple samples with multiple DNA probes. The method was based on use of MiniSlot™ and Miniblotter® devices (Figure 1) in the "checkerboard" format previously employed for the detection of multiple antigen-antibody reactions on a single solid-support membrane (References 5 and 7; see schematic, Figure 2).

MATERIALS AND METHODS

Purified DNAs

DNA was isolated from 7 reference and 36 fresh isolates of *Campylobacter* and related species using the method of Smith et al. (10). The DNA was quantified using a spectrofluorimeter and Hoechst 33258 dye (6). Each DNA sample was adjusted to 1 ng/μL and loaded into the channels of a Miniblotter 45 (Immunetics, Cambridge, MA, USA). The membranes were incubated at 4°C overnight resulting in the deposition of approximately 1 ng DNA per mm^2 on the surface of Hybond®-N+ nylon membranes (Amersham, Arlington Heights, IL, USA). The membranes were removed from the device and the DNA denatured and fixed in a solution of 0.4 M NaOH and 1.5 M NaCl. After fixation, the membranes were rinsed in 2× SSC (1× = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). The membranes were pre-hybridized at 42°C for 1 h in 50 mL of a solution consisting of 50% formamide, 5× SSC, 1% casein (Sigma Chemical, St. Louis, MO, USA), 5× Denhardt's solution (1× = 0.02% Ficoll®, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 25 mM sodium phosphate (pH 6.5) and 0.5 mg/mL denatured herring sperm DNA. The membrane was then placed in the Miniblotter 45, rotated 90 degrees from its original orientation. Digoxigenin-labeled, whole chromosomal DNA probes were prepared from each of

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Distribution of selected bacterial species on intraoral surfaces

Donna L. Mager, Laurie Ann Ximenez-Fyvie, Anne D. Haffajee and Sigmund S. Socransky
Department of Periodontology, The Forsyth Institute, Boston, MA, USA

Mager DL, Ximenez-Fyvie LA, Haffajee AD, Socransky SS: Distribution of selected bacterial species on intraoral surfaces. J Clin Periodontol 2003; 30: 644–654.

Abstract

Background/aim: To examine the proportions of 40 bacterial species in samples from 8 oral soft tissue surfaces and saliva in systemically healthy adult subjects and to compare these microbiotas with those of supra- and subgingival plaque.

Methods: Microbial samples were taken from 8 oral soft tissue surfaces of 225 systemically healthy subjects using a ''buccal brush''. Saliva was taken by expectoration. Forty-four of these subjects provided additional supra- and subgingival plaque samples. Samples were individually evaluated for their content of 40 bacterial species using checkerboard DNA–DNA hybridization. The percentage of total DNA probe count was determined for each species, at each sample location and averaged across subjects. The significance of differences among the proportions of the 40 test species at different sample locations was sought in the 225 and 44 subjects separately using the Quade test and adjusted for multiple comparisons. Cluster analysis was performed using the proportions of the 40 species at the different sample locations using the minimum similarity coefficient and an average unweighted linkage sort. The proportions of each species were averaged across subjects in the resulting cluster groups and the significance of differences was tested using the *t*-test and ANOVA.

Results: Microbial profiles differed markedly among sample locations in the 225 subjects, with 34 of 40 species differing significantly. Proportions of *Veillonella parvula* and *Prevotella melaninogenica* were higher in saliva and on the lateral and dorsal surfaces of the tongue, while *Streptococcus mitis* and *S. oralis* were in significantly lower proportions in saliva and on the tongue dorsum. Cluster analysis resulted in the formation of 2 clusters with $>85\%$ similarity. Cluster 1 comprised saliva, lateral and dorsal tongue surfaces, while Cluster 2 comprised the remaining soft tissue locations. *V. parvula, P. melaninogenica, Eikenella corrodens, Neisseria mucosa, Actinomyces odontolyticus, Fusobacterium periodonticum, F. nucleatum* ss *vincentii* and *Porphyromonas gingivalis* were in significantly higher proportions in Cluster 1 and *S. mitis, S. oralis* and *S. noxia* were significantly higher in Cluster 2. These findings were confirmed using data from the 44 subjects providing plaque samples. The microbial profiles of supra- and subgingival plaque differed from the other sample locations, particularly in the increased proportions of the *Actinomyces* species. Species of different genera exhibited different proportions on the various intraoral surfaces, but even within the genus *Streptococcus*, there were differences in colonization patterns. *S. oralis, S. mitis* and *S. constellatus* colonized the soft tissues and saliva in higher proportions than the samples from the teeth, while the other 4 streptococcal species examined colonized the dental surfaces in proportions comparable to the soft tissue locations and saliva.

Conclusions: Proportions of bacterial species differed markedly on different intraoral surfaces. The microbiota of saliva was most similar to that of the dorsal and lateral surfaces of the tongue. The microbiotas of the soft tissues resembled each other more than the microbiotas that colonized the teeth both above and below the gingival margin.

Key words: soft tissue microbiota; saliva; periodontal disease; supra- and subgingival plaque; systemically healthy

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The mean surface area of the adult oral cavity is approximately 215 cm^2 (Collins & Dawes 1987). The teeth, keratinized and nonkeratinized soft tissues comprise about 20%, 30% and 50% of this surface area respectively. While a great deal is known about the microbial composition of hard tissue biofilms, surprisingly little is known about the microbiotas that colonize approximately 80% of the surface area of the oral cavity. Most

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The Human Microbiota in Health and Disease

Baohong Wang, Mingfei Yao, Longxian Lv, Zongxin Ling, Lanjuan Li*

National Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China

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ABSTRACT

Trillions of microbes have evolved with and continue to live on and within human beings. A variety of environmental factors can affect intestinal microbial imbalance, which has a close relationship with human health and disease. Here, we focus on the interactions between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases. We also review important advances in techniques associated with microbial research, such as DNA sequencing, metabonomics, and proteomics combined with computation-based bioinformatics. Current research on the human microbiota has become much more sophisticated and more comprehensive. Therefore, we propose that research should focus on the host-microbe interaction and on cause-effect mechanisms, which could pave the way to an understanding of the role of gut microbiota in health and disease, and provide new therapeutic targets and treatment approaches in clinical practice.

1. Introduction

More than 100 trillion symbiotic microorganisms live on and within human beings and play an important role in human health and disease. The human microbiota, especially the gut microbiota, has even been considered to be an "essential organ" [1], carrying approximately 150 times more genes than are found in the entire human genome [2]. Important advances have shown that the gut microbiota is involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity [3–6]. Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota [7,8] (Fig.1).

In recent decades, a tremendous amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease [7,9–23] via several mechanisms. First, the microbiota has the potential to increase energy extraction from food [24], increase nutrient harvest [9,10], and alter appetite signaling [25,26]. The microbiota contains far more versatile metabolic genes than are found in the human genome, and provides humans with unique and specific enzymes and biochemical pathways [9]. In addition, a large proportion of the metabolic microbiotic processes that are beneficial to the host are involved in either nutrient acquisition or xenobiotic processing, including the metabolism of undigested carbohydrates and the biosynthesis of vitamins [10]. Second, the human microbiota also provides a physical barrier, protecting its host against foreign pathogens through competitive exclusion and the production of antimicrobial substances [11–13]. Finally, the microbiota is essential in the development of the intestinal mucosa and immune system of the host [14,16]. For example, germ-free (GF) animals have abnormal numbers of several immune cell types, deficits in local and systemic lymphoid structures, poorly formed spleens and lymph

* Corresponding author.
E-mail address: ljli@zju.edu.cn

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Microbial Diversity in Saliva of Oral Squamous Cell Carcinoma

Smruti Pushalkar[1], **Shrinivasrao P. Mane**[2], **Xiaojie Ji**[1], **Yhiong Li**[1], **Clive Evans**[2], **Oswald R. Crasta**[2], **Douglas Morse**[3], **Robert Meagher**[4], **Anup Singh**[4], and **Deepak Saxena**[1,*]

[1] Department of Basic Science and Craniofacial Biology, NYU College of Dentistry, NY 10010, USA

[2] Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061, USA

[3] Department of Epidemiology and Health Promotion, NYU College of Dentistry, NY 10010, USA

[4] Sandia National Laboratories, Livermore, CA 94551, USA

Abstract

In oral cavity chronic inflammation has been observed at various stages of oral squamous cell carcinomas (OSCC). This inflammation could result from persistent mucosal or epithelial cell colonization by microorganisms. There is an increasing evidence of the involvement of oral bacteria in inflammation and warrant further studies on the association of bacteria in the progression of OSCC. The objective of this study was to evaluate the diversity and relative abundance of bacteria in the saliva of subjects with OSCC. Using 454 parallel DNA sequencing, ~58,000 PCR amplicons that span the V4-V5 hypervariable region of ribosomal RNAs from 5 subjects were sequenced. Members of 8 phyla (divisions) of bacteria were detected. The majority of classified sequences belonged to phyla, *Firmicutes* (45%) and *Bacteroidetes* (25%). Further, a total of 52 different genera containing approximately 860 (16.51%) known species were identified, 1077 (67%) sequences belonged to various uncultured bacteria or unclassified group. The species diversity estimates obtained with abundance-based coverage estimators (ACE) and Chao1 were greater than published analyses of other microbial profiles from the oral cavity. Fifteen unique phylotypes were present in all three OSCC subjects.

Keywords

oral squamous cell carcinoma; microbial diversity; denaturing gradient gel electrophoresis; 454 pyrosequencing

Introduction

Microbes induce an estimated 20% of all the fatal cancers in human beings (Blaser, 2008) and numerous bacterial species are associated with different cancers (Lax & Thomas, 2002, Vogelmann & Amieva, 2007, Kurago, *et al.*, 2008). The best documented relationship between a bacterial infection and cancer is that of *Helicobacter pylori* and two different forms of gastric cancer: MALT lymphoma and the more common gastric adenocarcinoma (Marshall & Windsor, 2005). It is estimated that *H. pylori* is causally related to 60 to 90% of all gastric cancers (Malfertheiner, *et al.*, 2005). Other known associations between bacterial infections and human cancer include *Salmonella typhi* infection and gall bladder cancer in people that develop chronic carriage after typhoid fever (Shukla, *et al.*, 2000); *Streptococcus*

*Corresponding Author: Deepak Saxena, Ph.D., Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, 345 E. 24th Street Room: 921B, New York, NY 10010, Ph. no.: 212-998-9256, ds100@nyu.edu.

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REVIEW

Role of the normal gut microbiota

Sai Manasa Jandhyala, Rupjyoti Talukdar, Chivkula Subramanyam, Harish Vuyyuru, Mitnala Sasikala, D Nageshwar Reddy

Sai Manasa Jandhyala, Rupjyoti Talukdar, Chivkula Subramanyam, Harish Vuyyuru, Mitnala Sasikala, Department of Gastroenterology, Asian Institute of Gastroenterology, Asian Healthcare Foundation, Hyderabad 500082, India

Rupjyoti Talukdar, D Nageshwar Reddy, Asian Institute of Gastroenterology, Hyderabad 500082, India

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Correspondence to: Dr. Rupjyoti Talukdar, Department of Gastroenterology, Asian Institute of Gastroenterology, Asian Healthcare Foundation, 6-3-661 Somajiguda, Hyderabad 500082, India. rup_talukdar@yahoo.com
Telephone: +91-40-23378888
Fax: +91-40-23324255

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Abstract

Relation between the gut microbiota and human health is being increasingly recognised. It is now well established that a healthy gut flora is largely responsible for overall health of the host. The normal human gut microbiota comprises of two major phyla, namely Bacteroidetes and Firmicutes. Though the gut microbiota in an infant appears haphazard, it starts resembling the adult flora by the age of 3 years. Nevertheless, there exist temporal and spatial variations in the microbial distribution from esophagus to the rectum all along the individual's life span. Developments in genome sequencing technologies and bioinformatics have now enabled scientists to study these microorganisms and their function and microbe-host interactions in an elaborate manner both in health and disease. The normal gut microbiota imparts specific function in host nutrient metabolism, xenobiotic and drug metabolism, maintenance of structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens. Several factors play a role in shaping the normal gut microbiota. They include (1) the mode of delivery (vaginal or caesarean); (2) diet during infancy (breast milk or formula feeds) and adulthood (vegan based or meat based); and (3) use of antibiotics or antibiotic like molecules that are derived from the environment or the gut commensal community. A major concern of antibiotic use is the long-term alteration of the normal healthy gut microbiota and horizontal transfer of resistance genes that could result in reservoir of organisms with a multidrug resistant gene pool.

Key words: Normal gut microbiota; Bioinformatics; Health; Immunomodulation; Metabolic function

Dysbiosis and the immune system

Maayan Levy, Aleksandra A. Kolodziejczyk*, Christoph A. Thaiss* and Eran Elinav*

Abstract | Throughout the past century, we have seen the emergence of a large number of multifactorial diseases, including inflammatory, autoimmune, metabolic, neoplastic and neurodegenerative diseases, many of which have been recently associated with intestinal dysbiosis — that is, compositional and functional alterations of the gut microbiome. In linking the pathogenesis of common diseases to dysbiosis, the microbiome field is challenged to decipher the mechanisms involved in the *de novo* generation and the persistence of dysbiotic microbiome configurations, and to differentiate causal host–microbiome associations from secondary microbial changes that accompany disease course. In this Review, we categorize dysbiosis in conceptual terms and provide an overview of immunological associations; the causes and consequences of bacterial dysbiosis, and their involvement in the molecular aetiology of common diseases; and implications for the rational design of new therapeutic approaches. A molecular-level understanding of the origins of dysbiosis, its endogenous and environmental regulatory processes, and its downstream effects may enable us to develop microbiome-targeting therapies for a multitude of common immune-mediated diseases.

Xenobiotics
Small chemical compounds that enter an organism unnaturally, such as drugs or pollutants.

The incidence of many common multifactorial human diseases, such as diabetes and obesity, allergy and asthma, neurodegeneration and inflammatory bowel disease (IBD), has substantially increased during the past two centuries. The short duration of this period, which encompasses only a limited number of human generations, makes it unlikely that these disorders can be explained by genetic factors alone[1]. Instead, changes in lifestyle and environmental factors, which are broadly adopted by post-industrial revolution societies, compared with the conditions prevalent during the preceding evolution of the human gene pool are probably associated with the increasing incidence of these autoimmune, inflammatory and metabolic diseases[2]. These lifestyle and environmental factors include alterations in diet, physical activity, hygiene, longevity, exposure to xenobiotics and a newly acquired human ability to control light and temperature. In the quest to better understand the origin of these pandemics, it has recently been recognized that another gene pool needs to be considered when evaluating the impact of such environmental factors on human health, namely the metagenome of the entirety of microorganisms that colonize the human body, which is collectively termed the microbiome[3]. The microbiome has co-evolved with the eukaryotic genome of its host and colonizes the host's interfaces with the outside world, including the gastrointestinal tract, skin, respiratory tract and urogenital tract. Both the human and microbial genomes have been subject to dietary and environmental pressures, including the rapid environmental changes that characterized the industrial revolution that has occurred in the past two centuries. The substantially shorter generation times of commensal microorganisms, relative to humans, make the microbiome amenable to rapid evolutionary changes on a much shorter timescale and may suggest that adaptation of the metagenome to changes in environmental conditions is more rapid than that of the host genome. In recent years, many of the modern multifactorial diseases that show an increasing incidence have been associated with an abnormal microbiome structure, termed dysbiosis, which affects the taxonomical composition as well as the metagenomic function of the microbial community. The microbiome consists of complex bacterial, archaeal, fungal, viral and protozoan communities that colonize multiple body sites. In this Review, we focus primarily on the bacterial part of the gastrointestinal tract microbiome, and its effects on immune homeostasis and the risk of immune-mediated and immune-associated diseases.

The healthy intestinal microbial community can be characterized in terms of diversity, stability and resistance, and resilience[4], which are defined, respectively, as the richness of the ecosystem, its amenability to perturbation and its ability to return to the pre-perturbation state. Data from large human cohort studies suggest that multiple stable states of the microbial ecosystem can colonize a host in the absence of overt signs of disease[5–7] (FIG. 1). A common definition of dysbiosis describes it as a compositional and functional alteration in the microbiota that is driven by a set of environmental and host-related

Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel.

Correspondence to E.E.
eran.elinav@weizmann.ac.il

**These authors contributed equally to this work.*

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Reviews

Next-Generation Sequencing: From Basic Research to Diagnostics

Karl V. Voelkerding,[1,2*] Shale A. Dames,[1†] and Jacob D. Durtschi[1†]

BACKGROUND: For the past 30 years, the Sanger method has been the dominant approach and gold standard for DNA sequencing. The commercial launch of the first massively parallel pyrosequencing platform in 2005 ushered in the new era of high-throughput genomic analysis now referred to as next-generation sequencing (NGS).

CONTENT: This review describes fundamental principles of commercially available NGS platforms. Although the platforms differ in their engineering configurations and sequencing chemistries, they share a technical paradigm in that sequencing of spatially separated, clonally amplified DNA templates or single DNA molecules is performed in a flow cell in a massively parallel manner. Through iterative cycles of polymerase-mediated nucleotide extensions or, in one approach, through successive oligonucleotide ligations, sequence outputs in the range of hundreds of megabases to gigabases are now obtained routinely. Highlighted in this review are the impact of NGS on basic research, bioinformatics considerations, and translation of this technology into clinical diagnostics. Also presented is a view into future technologies, including real-time single-molecule DNA sequencing and nanopore-based sequencing.

SUMMARY: In the relatively short time frame since 2005, NGS has fundamentally altered genomics research and allowed investigators to conduct experiments that were previously not technically feasible or affordable. The various technologies that constitute this new paradigm continue to evolve, and further improvements in technology robustness and process streamlining will pave the path for translation into clinical diagnostics.

[1] ARUP Institute for Experimental and Clinical Pathology, Salt Lake City, Utah; [2] Department of Pathology, University of Utah, Salt Lake City, Utah.
* Address correspondence to this author at: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, Utah 84108. Fax (801) 584-5207; e-mail voelkek@aruplab.com.
† S.A. Dames and J.D. Durtschi contributed equally to the review.
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In 1977, 2 landmark articles describing methods for DNA sequencing were published. Allan Maxam and Walter Gilbert reported an approach in which terminally labeled DNA fragments were subjected to base-specific chemical cleavage and the reaction products were separated by gel electrophoresis *(1)*. In an alternative approach, Frederick Sanger and colleagues described the use of chain-terminating dideoxynucleotide analogs that caused base-specific termination of primed DNA synthesis *(2)*. Refinement and commercialization of the latter method led to its broad dissemination throughout the research community and, ultimately, into clinical diagnostics. In an industrial, high-throughput configuration, Sanger technology was used in the sequencing of the first human genome, which was completed in 2003 through the Human Genome Project, a 13-year effort with an estimated cost of $2.7 billion. In 2008, by comparison, a human genome was sequenced over a 5-month period for approximately $1.5 million *(3)*. The latter accomplishment highlights the capabilities of the rapidly evolving field of "next-generation" sequencing (NGS)[3] technologies that have emerged during the past 5 years. Currently, 5 NGS platforms are commercially available, with additional platforms on the horizon. To add to this pace, the US National Human Genome Research Institute (NHGRI) announced funding in August 2008 for a series of projects as part of its Revolutionary Genome Sequencing Technologies program, which has as its goal the sequencing of a human genome for $1000 or less (http://www.genome.gov/27527585). This review describes NGS technologies, reviews their impact on basic research, and explores how they have the translational potential to substantially impact molecular diagnostics.

Fundamentals of NGS Platforms

NGS platforms share a common technological feature—massively parallel sequencing of clonally amplified or single DNA molecules that are spatially separated in a flow cell. This design is a paradigm shift from that of

[3] Nonstandard abbreviations: NGS, next-generation sequencing; NHGRI, National Human Genome Research Institute; dNTP, deoxynucleoside triphosphate; Mb, million base pairs; Gb, billion base pairs; miRNA, microRNA.

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Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis

R. C. V. Casarin[1,3], A. Barbagallo[2], T. Meulman[3], V. R. Santos[4], E. A. Sallum[3], F. H. Nociti[3], P. M. Duarte[4], M. Z. Casati[1,3], R. B. Gonçalves[2]

[1]Division of Periodontics, Paulista University, São Paulo, Brazil, [2]Groupe de Recherche em Ecologie Bucale, Université Laval, Quebec, Canada, [3]Department of Periodontics, State University of Campinas, Piracicaba, Brazil and [4]Division of Periodontics, Guarulhos University, Guarulhos, Brazil

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Background and Objective: There is a bidirectional relationship between periodontal disease and type-2 diabetes mellitus (DM). Inflammatory mediators may negatively affect glycemic control, and increased glucose levels and resultant glycation end-products may alter the host response against bacterial infection. However, no agreement has been reached regarding the effect of DM on periodontal subgingival microbiota. Therefore, the purpose of the present study was to compare the subgingival biodiversity in deep periodontal pockets of subjects with chronic periodontitis and either uncontrolled type-2 diabetes or no diabetes using 16S rRNA gene cloning and sequencing.

Material and methods: Twelve subjects with uncontrolled type-2 diabetes (glycated hemoglobin > 8%) and eleven nondiabetic subjects presenting severe and generalized chronic periodontitis were selected. Subgingival biofilm from periodontal pockets > 5 mm were assessed using the 16S rRNA gene cloning and sequencing technique.

Results: Significant differences were observed in subgingival microbiota between diabetic and nondiabetic subjects. Diabetic subjects presented higher percentages of total clones of *TM7, Aggregatibacter, Neisseria, Gemella, Eikenella, Selenomonas, Actinomyces, Capnocytophaga, Fusobacterium, Veillonella* and *Streptococcus* genera, and lower percentages of *Porphyromonas, Filifactor, Eubacterium, Synergistetes, Tannerella* and *Treponema* genera than nondiabetic individuals ($p < 0.05$). Moreover, some phylotypes, such as *Fusobacterium nucleatum, Veillonella parvula, V. dispar* and *Eikenella corrodens* were detected significantly more often in diabetic subjects than in nondiabetic subjects ($p < 0.05$).

Conclusion: Subjects with uncontrolled type-2 diabetes and chronic periodontitis presented significant dissimilarities in subgingival biodiversity compared with nondiabetic subjects.

Renato Corrêa Viana Casarin, UNIP – Paulista University, 1212 Dr Bacelar, São Paulo 04026-002, Brazil
Tel: +(11) 5586 4000
Fax: +(11) 2275 1541
e-mail: casarinrcv@yahoo.com.br

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Chronic periodontitis is caused by complex microbial communities present in the subgingival environment. Moreover, subjects presenting with diabetes mellitus (DM), particularly with a poorly controlled glycemic status, have a more severe and generalized periodontitis (1). Hyperglycemia and the resultant formation of advanced

antibiotics

MDPI

Review

Multimodal Role of Amino Acids in Microbial Control and Drug Development

Muhammad Idrees [1], Afzal R. Mohammad [2], Nazira Karodia [1] and Ayesha Rahman [1,*]

[1] Faculty of Science and Technology, University of Wolverhampton, Wolverhampton WV1 1LY, UK; M.Idrees@wlv.ac.uk (M.I.); nazira.karodia@wlv.ac.uk (N.K.)
[2] Aston Pharmacy School, Aston University, Birmingham B4 7ET, UK; a.u.r.mohammed@aston.ac.uk
* Correspondence: A.Rahman5@wlv.ac.uk

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Abstract: Amino acids are ubiquitous vital biomolecules found in all kinds of living organisms including those in the microbial world. They are utilised as nutrients and control many biological functions in microorganisms such as cell division, cell wall formation, cell growth and metabolism, intermicrobial communication (quorum sensing), and microbial-host interactions. Amino acids in the form of enzymes also play a key role in enabling microbes to resist antimicrobial drugs. Antimicrobial resistance (AMR) and microbial biofilms are posing a great threat to the world's human and animal population and are of prime concern to scientists and medical professionals. Although amino acids play an important role in the development of microbial resistance, they also offer a solution to the very same problem i.e., amino acids have been used to develop antimicrobial peptides as they are highly effective and less prone to microbial resistance. Other important applications of amino acids include their role as anti-biofilm agents, drug excipients, drug solubility enhancers, and drug adjuvants. This review aims to explore the emerging paradigm of amino acids as potential therapeutic moieties.

Keywords: amino acids; antimicrobial resistance; quorum sensing; microbial biofilm; solubility; excipients; adjuvants

1. Introduction

Amino acids are important biomolecules and the very basic constituents or building blocks of proteins [1]. They have at least one amino group, one carboxyl group, and a side chain in their structures [2,3]. There are 20 different amino acids, represented by a general formula H2N-CαH(R)-CO2H (where -R is a side chain with an exception of glycine, in which the side chain -R has been replaced by -H), which are responsible for protein formation in all living organisms [4,5].

Several amino acids (in different proportions) are joined together by amide bonding (also known as peptide bonding) in such a way that the carboxyl group of one amino acid links to the amino group of another amino acid with the loss of a water molecule (per each peptide bond) to form protein polymers [6]. Figure 1a,b illustrates a general chemical structure for 19 of the 20 amino acids and glycine, respectively.