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Review article

The utility of microbiome (microbiota) and exosomes in dentistry

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Abstract The concept of the oral-systemic link is important in both basic and clinical dentistry. The microbiome (microbiota) and exosomes are two prevalent issues in the modern medical researches. The common advent of oral and general microbiological investigation originated from the initial observations of oral bacteria within the dental plaque known as oral microbiome. In addition to oral diseases related to oral microbiome, the disruption of the oral and intestinal microbiome could result in the onset of systemic diseases. In the past decade, the exosomes have emerged in the field of the medical researches as they play a role in regulating the transport of intracellular vesicles. However, with the rapid advancement of exosomes researches in recent years, oral tissues (such as dental pulp stem cells and salivary gland cells) are used as the research materials to further promote the development of regenerative medicine. This article emphasized the importance of the concept of the oral-systemic link through the examples of microbiome (microbiota) and exosomes. Through the researches related to microbiome (microbiota) and exosomes, many evidences showed that as the basic dentistry developed directly from the assistance of the basic medicine, indirectly the progress

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of the basic dentistry turns back to promote the development of the basic medicine, indicating the importance of the concept of the oral-systemic link. The understanding of the oral-systemic link is essential for both clinicians and medical researchers, regardless of their dental backgrounds.

Introduction

In clinical dentistry, the concept of the oral-systemic link has been emphasized repeatedly, which includes awareness of problems and connections between the oral and general health, avoiding the physical distress caused by the dental treatment, and understanding the risk of treating dental patients with systemic diseases.[1] However, in addition to clinical dentistry, the concept of the oral-systemic link is equally applicable in various dental fields (especially basic dentistry). By the same token, we could say that in basic dentistry, the concept of the oral-systemic link includes awareness of problems and connections between the oral and general medicine, paying attention to systemic performance caused by dental origins, and understanding the issues of dental research with systemic relationships. Due to the separation of medical and dental educations, the teaching and learning of dentistry seems to focus on the oral cavity rather than the whole body. The same phenomenon also occurs between the basic researches in medicine and in dentistry.[1–3] Therefore, the separation of general health education and research from dental education and research indeed has serious negative implications for the delivery of oral health care and the development of oral health medicine.

The common advent of oral and general microbiological investigation originated from the initial observations of oral bacteria within the dental plaque by Antony van Leeuwenhoek (1632–1723) using his primitive microscopes in 1680. In the modern era, oral microbiology knowledge has developed rapidly because oral researchers adopt new concepts of systemic thinking, such as microbiome, interspecies interactions, microbial communities, biofilms, and polymicrobial diseases. This knowledge has dramatically changed into the oral microbiome, commonly known as the dental plaque, which is one of the most complex floras associated with the human body.[4,5] Oral microbiology has been developed for more than 300 years. However, after the first dental college (Baltimore College of Dental Surgery) was established in the United States in 1840, it accompanied the development path of dentistry to an independent profession and became an important basic discipline in dentistry.[6] Unfortunately, the teaching and research of oral microbiology seems to be confined to dental schools and dental research institutions. In fact, we firmly believe oral microbiology should not be independent from general microbiology.

The exosomes were first discovered in sheep reticulocytes in 1983. They are a type of extracellular vesicles.[7,8] At that time, the exosomes were thought to be just cell metabolites.[9] It was not until the mid-1990s that the exosomes were shown to have an immunological function.[10] In the past decade, the exosomes have emerged in the field of medical research as they play a role in regulating the transport of intracellular vesicles, such as lactation, inflammation, cell proliferation, immune response, and neuronal function and have the clinical application potential in disease diagnosis and treatment.[11–13] Although exosomes have shown promising effects in various medical applications, and 293 clinical trials have been conducted up to December 2022 (NIH ClinicalTrials.gov), their researches in the treatment of oral and dental diseases is still in the early stages. By the end of 2022, according to NIH ClinicalTrials.gov, there was only one clinical trial about exosomes used as an adjunctive therapy of periodontitis.[14]

Therefore, we consider that microbiome (microbiota) and exosomes are two classic examples related to the concept of the oral-systemic link in the history of medical development, and they are still ongoing. Recently, imbalances in the interactions between the microbiota and immunity may play a role in the development of a range of immune-mediated diseases.[15] The idea of an oral-systemic interconnecting related to microbiome has been gaining traction.[16–18] The effects of exosomes on oral diseases, such as periodontitis, oral potentially malignant disorders (OPMDs), and oral cancer, have received increasing attention in recent years, giving us a better understanding of the functions that exosomes play important roles in oral diseases for assisting clinical diagnosis, treatment, and determining prognosis.[19–21] In this review, we briefly summarized the current state of knowledge on microbiome (microbiota) and exosomes in dentistry (Fig. 1).

Definition of microbiota, microbiome, and exosomes

Microbiota

The origin of microbiota can be dated back to early 1900s. It was found that a vast number of microorganisms, including bacteria, yeasts, and viruses, coexist in various sites of the human body.[22] The microbiota refers specifically to the collection of microorganisms (bacteria, viruses, fungi, etc.) that inhabit a particular ecological niche or host organism. It focuses on the actual microorganisms themselves. In the context of the human body, the human microbiota refers to the diverse community of microorganisms that reside in various parts of the body, such as the skin, mouth, gastrointestinal tract, lung, vagina, and other

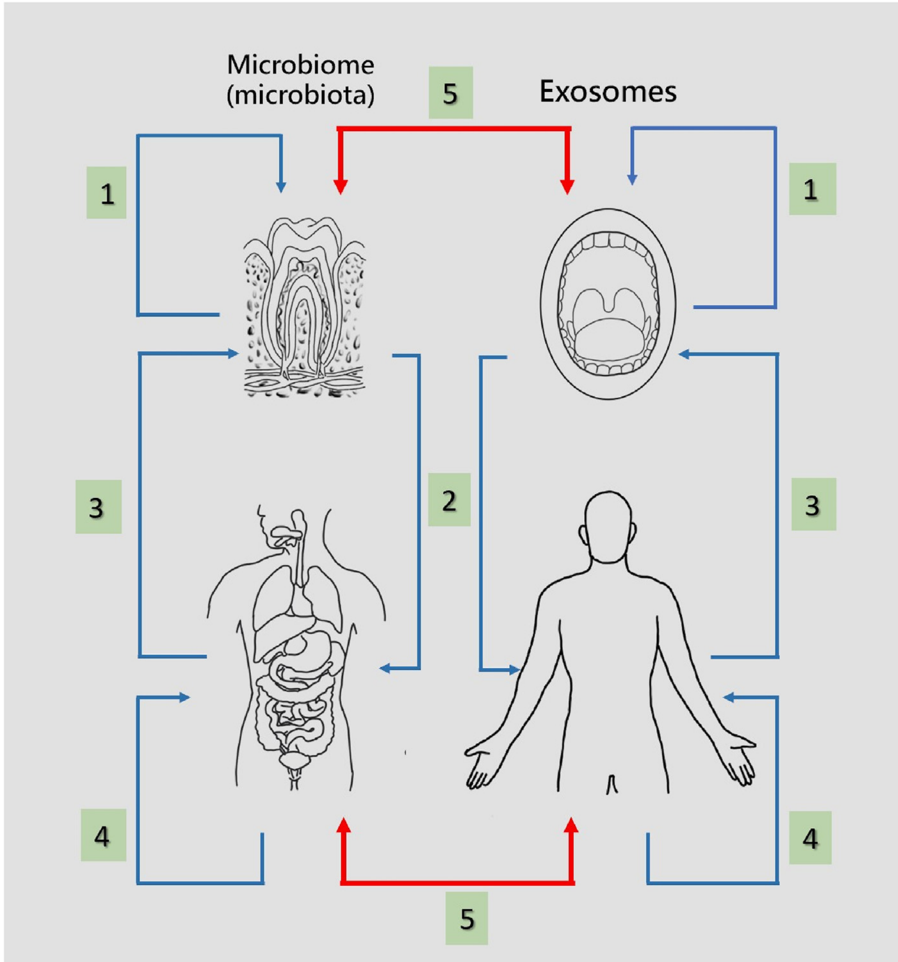

Figure 1 The oral-systemic link of human microbiome (microbiota) and exosomes. Left side: Microbiome (microbiota) from the oral cavity or the digestive/respiratory tract outside of the oral cavity. Connecting pathway: (1) The disruption of the oral microbiome affecting the onset of the oral diseases. (2) The disruption of the oral microbiome affecting the onset of the systemic diseases. (3) The disruption of the systemic microbiome affecting the onset of the oral diseases. (4) The disruption of the systemic microbiome affecting the onset of the systemic diseases. (5) The interactions between the microbiome and exosomes in the oral cavity (upper side). Right: Exosomes from the oral cavity or the systemic tissues. Connecting pathway: (1) The application of exosomes from the oral tissues for diagnosis and treatment of the oral diseases. (2) The application of exosomes from the oral tissues for diagnosis and treatment of the systemic diseases. (3) The application of exosomes from the systemic tissues for diagnosis and treatment of the oral diseases. (4) The application of exosomes from the systemic tissues for diagnosis and treatment of the systemic diseases. (5) The interactions between the microbiome and exosomes in the digestive/respiratory tract outside of the oral cavity (lower side).

mucosal surfaces. The microbiota can vary in composition and abundance across different body sites.[23]

Microbiome

The microbiome encompasses not only the microorganisms (microbiota) but also their genomes and the overall genetic information associated with the microbial community. It includes the collective genetic material of all the microorganisms present in a particular environment or on/in a specific organism. The microbiome provides insights into the functional capabilities of the microbial community and how it interacts with the host organism. The microbiota and microbiome are often interchangeable. However, there are certain differences between the two terms. The microbiota describes the living microorganisms found in a particular environment, such as the oral and intestinal microbiota. The microbiome refers to the genome collection of all microorganisms in the environment, including not only the microbial community, but also the microbial structural elements, metabolites, and the environmental conditions of the microorganisms.[23,24] Therefore, the microbiome covers a broader spectrum than the microbiota. In summary, while the microbiota refers to the actual microorganisms living in a particular habitat or organism, the microbiome extends the concept to include the genetic material (genomes) of these microorganisms.

Exosomes

The exosomes are small extracellular vesicles that are secreted by cells, surrounded by lipid bilayer, and released by cells into the extracellular environment. These vesicles are involved in the cell-to-cell communication and the transport of various molecules between cells. The exosomes are part of the broader category of extracellular vesicles, which also includes micro-vesicles and apoptotic bodies.[25] They contain a variety of biomolecules, including proteins, lipids, and nucleic acids (such as RNA and DNA) to target recipient cells. They are produced by the inward budding of the endosomal membrane, resulting in the formation of multivesicular bodies (MVBs). When these MVBs fuse with the cell membrane, the exosomes are released into the extracellular space. The exosomes play important roles in intercellular communication, immune response modulation, and the transfer of genetic materials between cells.[9] They have been implicated in various physiological processes and are of interest in researches related to cancer, neurodegenerative diseases, and other medical conditions.[9,26–28] The study of exosomes has expanded in recent years due to their potential diagnostic and therapeutic applications.

Microbiome (microbiota) and exosomes from the oral cavity

The oral cavity is home to a diverse array of microorganisms, with bacterial species being the most abundant. The composition of the oral microbiota can vary between individuals and different oral sites. The microorganisms in the oral cavity often form biofilms, which are the communities of microorganisms embedded in a matrix of extracellular polymeric substances. The dental plaque, for example, is a biofilm that forms on the tooth surfaces.[4] In addition, various factors can influence the composition and balance of the oral microbiota, including oral hygiene practices, diet, genetics, and systemic health.

The exosomes are characterized as nanometer-sized extracellular vesicles and secreted by virtually all types of cells. In oral health field, as the research continues, the complex roles of these intracellular-derived extracellular vesicles from dental pulp stem cells or salivary gland cells in biological processes have gradually unfolded.[19,29,30] The exosomes from the oral tissue have attracted attention as valuable diagnostic and therapeutic tools for their ability to transfer abundant biological cargos and their intricate involvement in multiple cellular functions, especially focused on angiogenic and osteogenic properties for accelerating bone formation and cutaneous wound healing.[29,30]

Microbiome (microbiota) and exosomes from the systemic tissues

The human intestinal microbiome is almost an organ of the human body,[31] symbiotic with the human body, and closely related to various physiology and diseases throughout the body. The number of genes in the intestinal microbiome is 150 times that of the human body. There are more than 1000 species of microbiota in the intestine, and the total number of bacterial cells is 10 times that of the human cells. The intestinal microbiome is equivalent to the human endocrine organ, and the secreted metabolites affect digestive system-related and other systemic diseases.[24,31]

The exosomes are extracellular vesicles secreted by cells into the extracellular environment with a crucial role in cell-to-cell communication in both physiological and pathological conditions. Almost all eukaryotic cells can secrete exosomes. The exosomes secreted by the mesenchymal stem cells, which are widely found in the bone marrow, fat, and dental pulp, are the most common. The stem cell exosomes with the function of repairing abnormal cell defects can be isolated from the mesenchymal stem cells. However, the exosomes are not only found in the eukaryotic cells, but also in various types of cells, such as the prokaryotic cells, fungi, protozoa, and plant cells.[32]

Microbiome (microbiota) and exosomes from the oral cavity related to the oral diseases

The oral cavity contains one of the most diverse and unique communities of microbiota in the human body.[33] The oral microbiota plays a crucial role in maintaining oral health by contributing to several body processes such as the digestion, immune system modulation, and protection against harmful pathogens. However, an imbalance in the oral microbiota can lead to conditions such as dental caries, gingivitis, and periodontal disease. Therefore, the oral cavity may be affected by a variety of diseases with a high incidence in the human population, including dental caries, gingivitis, periodontal disease, and even oral cancer and esophageal cancer, all of which are clearly related to the changes in the oral microbiome.[34–36]

By diagnosis markers correlated with the oral diseases (such as periodontitis, oral lichen planus, oral cancer, and Sjögren's syndrome), in vivo applications of exosomes can be used as the potential cell-free therapeutic agents in the regeneration of craniofacial bone, skin, temporomandibular joint (TMJ), periodontal tissue, and dental pulp, as well as the treatment of oral cancer, OPMDs, and other craniofacial and dental diseases.[37–40] The exosomes of the mesenchymal stromal cell (MSC) from dental pulp have the functions to control or induce bone, cartilage, dentin, mucosa, and pulp tissue formation. In brief, the exosomes from oral tissue can be used as potentially in oral and maxillofacial tissue repair and regeneration (such as craniofacial bone regeneration, TMJ regeneration, periodontal and dental pulp regeneration, and skin and wound healing), and even OPMDs and oral cancer treatment.[37]

Microbiome (microbiota) and exosomes from the oral cavity related to the systemic diseases

The oral microbiota may have implications beyond oral health and may influence systemic health. The links have been explored between oral health and conditions. Because the oral cavity is an entry point to the respiratory and digestive systems and is highly vascularized. The oral microbiome has the potential impact on other systemic diseases. In fact, a growing number of studies are showing the links between other diseases (such as respiratory infections, cardiovascular disease, diabetes, colorectal cancer, pancreatic cancer, cystic fibrosis, rheumatoid arthritis, and Alzheimer's disease) and the changes in the oral microbiome.[34,41—47] This suggests that the oral microbiota may provide the potential biomarkers for the diagnosis of certain systemic diseases.[34]

Researches in recent years have concluded that the salivary exosomes have a novel role as the potential biomarkers for oral and other systemic diseases, such as inflammatory bowel disease (IBD), pancreatic cancer, pancreatobiliary tract cancer, and lung cancer.[48—51] The aberrant expression and underlying mechanisms of the salivary exosomes may be considered as the potential diagnostic and therapeutic biomarkers for systemic diseases.[52] Given the increasing roles of the salivary exosomes, understanding their functions and specific mechanisms may provide new insights into possible applications of the salivary exosomes in the diagnosis and treatment of systemic diseases. In comparison to other bodily fluids, salivary exosomes are probably a better and accessible tool to examine the function of exosomes in the diagnosis and treatment of disease.[53]

Microbiome (microbiota) and exosomes from the systemic tissues related to the oral diseases

In the presence of oral intestinal barrier dysfunction, the oral microbiota can translocate to the intestinal mucosa. Conversely, gut dysbiosis can also have an impact on the composition of the oral microbiome. The microbial transmission between the oral cavity and intestines can reshape the microbial ecosystems in both habitats, ultimately regulating physiological functions and pathological processes in the body. Theoretically, the oral gut microbial interactions may influence the pathogenesis of oral diseases and thereby promote the occurrence of dental caries and oral diseases.[54]

Presently, a total of 293 clinical trials involving exosome-related treatments and diagnoses of various diseases have been conducted up to December 2022 (NIH ClinicalTrials.gov).[14] Although the researches in the treatment of oral and dental diseases are still in the early stages, the speed of the clinical translation of exosome-based diagnosis and therapeutics has far exceeded initial expectations. For example, there are many exosomes from the immune cells (such as neutrophil, macrophage, dendritic cell, and lymphocyte) involved in the effects related to periodontitis diagnosis and treatment. In the future, exosome-mediated drug delivery strategy is expected to make a major breakthrough in the treatment of inflammatory diseases, including periodontitis.[55,56]

Microbiome (microbiota) and exosomes from the systemic tissues related to the systemic diseases

The impact of the microbiome on systemic health and diseases is underestimated. The changes in the structure and composition of the microbiome have a strong impact on the individual's health and diseases. The intestinal mucosal barrier is one of the primary body defense barriers, protecting the body from bacterial invasion, foreign antigens and toxins entering the circulatory system, and preventing the loss of water and nutrients. The dysregulation in the composition, prevalence, and vitality of the microbiota can induce various infectious and non-infectious diseases, including emerging infectious diseases, allergy, immune system diseases, neurodegenerative disease, cardiovascular and liver diseases, depression, metabolic syndrome, diabetes mellitus, gout, obesity, and cancer. Therefore, the disruption of this balanced equilibrium or the occurrence of leaky gut has serious consequences.[57] Controlling external factors such as the diet and lifestyle can re-establish the balance between the microbiota to prevent or even treat such diseases. The use of probiotics, dietary supplements, and microbiota transplantation from healthy individuals is a future treatment trend for these infectious and non-infectious diseases.[58]

Clinically, the exosomes are considered ideal candidates for applications related to biomarker development for the early detection of different diseases. In addition, they may be of interest as the potential drug delivery vehicles that can improve factors such as bioavailability of loaded molecular cargo, side effects, off-target effects, and pharmacokinetics of drug molecules. The various in-vitro studies have demonstrated the safety, efficacy, and therapeutic potential of exosomes in the various cancers, neurodegenerative diseases, tissue or organ injuries, brain and spinal cord trauma, cardiovascular diseases, and orthopedic diseases.[59]

Special issues of interactions between the microbiome (microbiota) and exosomes

The exosomes are small extracellular vesicles released by the prokaryotic and eukaryotic cells with a crucial role in cell-to-cell communication in both physiological and pathological conditions.[32] The exosomes derived from intestinal microbiota can be involved in the pathophysiological conditions of the body by transporting a variety of substances, while the exosome-like nanoparticles derived from the diet can act as bacterial modulators beyond microRNA.[60,61]

Based on the gut—liver axis theory, intestinal microorganisms are an important part of the human body. Their derived extracellular vesicles contain rich microbial DNA, proteins, and lipids, which have gradually been considered to contribute to the pathological mechanisms of the various liver diseases, especially metabolic dysfunction-associated fatty liver disease (MAFLD). There is a theoretical basis for the development of gut microbiome-based therapies by discussing their potential relationships with the insulin resistance, intestinal barrier, inflammation, lipid metabolism, and liver fibrosis, as well as improving MAFLD through probiotic colonization.[60]

Furthermore, the food is an important factor affecting the composition of the intestinal microbiota. Although all the attention is usually focused on the nutrients such as lipids, proteins, vitamins or polyphenols, a critical role in these processes is associated with the dietary-derived exosome-like nanoparticles (DELN). Due to the function of the DELN as a bacterial regulator, the DELN has a direct effect on bacterial growth. Many studies have highlighted the role of microbiota in health and the importance of maintaining ecological balance. Therefore, there is an urgent need to better understand how the DELNs interact with bacteria and induce microbiota modulations useful for the disease treatment.[61]

Conclusion

In the history of the medical research development, too many stories about the oral-systemic link could be said. This article emphasized the importance of the concept of the oral-systemic link through the examples of microbiome (microbiota) and exosomes. The origins of the contemporary microbiome (microbiota) research could be traced back to the discovery of oral microbiota in the 17th century. With the rapid advancement of exosome researches in recent years, the oral tissues (such as dental pulp stem cells and salivary gland cells) are used as the research materials to further promote the development of regenerative medicine. Thereby, as the basic dentistry developed directly from the assistance of the basic medicine, indirectly the progress of the basic dentistry turns back to promote the development of the basic medicine. This is becoming increasingly important as we strive to improve our understanding of the concept of the oral-systemic link between dentistry and medicine. The understanding of the oral-systemic link is essential for both clinicians and medical researchers, regardless of their dental backgrounds.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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