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Oral Microbiome Transplantation: A Novel Therapeutic Approach for Restoring Oral and Systemic Health

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ABSTRACT

Oral Microbiome Transplantation (OMT) is a novel therapeutic strategy designed to restore microbial equilibrium in the oral cavity to address diseases linked to oral dysbiosis, such as dental caries and periodontitis, as well as associated systemic conditions like diabetes and cardiovascular disease. In contrast to traditional therapies that mostly focus on alleviating symptoms by mechanical plaque removal or restoration procedures, OMT aims to rectify the root problem by reinstalling advantageous microorganisms from healthy donors into the oral ecosystem. This work investigated the feasibility and potential of OMT using an innovative hydrogel-based delivery method made from dopamine-grafted oxidized sodium alginate, aimed at improving microbial viability and ensuring steady application. The hydrogel exhibited advantageous characteristics, such as mechanical stability, biocompatibility, and the capacity to maintain microbial functioning, making it a suitable medium for microbial transfer. A major problem in OMT is the identification of appropriate donors, attributed to the interpersonal variability of oral microbiota; the notion of a "super donor" with a highly diversified, stable, and low-pathogenic microbiome is highlighted as crucial for therapeutic effectiveness. Initial findings from canine models indicated that OMT decreased inflammation and obstructed the development of pathogenic biofilms, corroborating its efficacy as both a therapeutic and preventative intervention. Nonetheless, more study is necessary to convert these discoveries into human applications, including thorough safety evaluations, rigorous donor screening procedures, and extensive investigations to assess the longevity and integration of transplanted microbiota. If proven, OMT might signify a transformative change in dental care, providing a comprehensive, root-cause-oriented alternative to traditional therapies for oral and systemic health management.

Keywords: Oral microbiome, Microbiome transplantation, Oral dysbiosis, Periodontitis, Dental caries, Super donor, Hydrogel delivery system, Oral health, Systemic disease.

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INTRODUCTION

The microorganisms present in the human mouth cavity are termed oral microflora, oral microbiota, or oral microbiome. Joshua Lederberg coined the term "microbiome" to denote the biological community of commensal, symbiotic, and pathogenic microorganisms inhabiting human bodies, which had largely been overlooked as factors influencing health and illness. The oral cavity encompasses many distinct microbial environments, including the teeth, gingival sulcus, attached gingiva, tongue, cheeks, lips, hard palate, and soft palate. The tonsils, pharynx, esophagus, Eustachian tube, middle ear, trachea, lungs, nasal passages, and sinuses function in unison with the oral cavity.[1] The oral microbiome comprises around 700 distinct species, including bacteria, fungi, viruses, archaea, and protozoa, forming a complex ecological system. The oral cavity is segmented into many niches, each harbouring a unique microbial life. The Human Oral Microbiome Database (HOMD) has documented more than 600 prevalent species, illustrating the diversity and intricacy of the oral microbiome. The oral microbiota has a role in both oral and systemic health.[2]

It protects against detrimental environmental factors and promotes oral homeostasis. The commensal microbiota is essential for maintaining oral and systemic health. Commensal organisms in the gastrointestinal tract are essential for the development of gut architecture and the proper maturation of both local and systemic immunity.[3] Lymphoid follicles are absent in the small intestines of germ-free mice, and mucosal IgA is

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generated only in the presence of microbiota. The oral cavity serves as a crucial ingress to the human body. Food is ingested via the mouth, where it is masticated and combined with saliva before proceeding to the stomach and digestive system. Air traverses the nasal passages and oral cavity on route to the trachea and lungs.[4] Microorganisms residing in one area of the oral cavity are very likely to disseminate to neigh boring epithelial surfaces and adjacent locations. Microorganisms in the oral cavity have been associated with several infectious conditions, including caries, periodontitis, endodontic infections, alveolar osteitis, and tonsillitis. Increasing evidence ties oral bacteria with several systemic illnesses,[5] including cardiovascular disease,[6] stroke,[7] preterm birth, diabetes mellitus, and pneumonia. An imbalance in microbial flora may lead to oral problems such as dental caries and periodontal disease, as well as systemic conditions like diabetes, cardiovascular disease, and cancer. This condition is referred to as Oral Dysbiosis. Particular infections, such as *Porphyromonas gingivalis*, are associated with both oral and systemic diseases, including cancer. Oral dysbiosis is often induced by inflammation, dietary carbohydrates, and environmental factors. Dysbiosis in the oral cavity has been linked to systemic illnesses, including cardiovascular disease, diabetes, and rheumatoid arthritis, illustrating the impact of dental hygiene on overall health.[8]

Preliminary animal studies indicate that diabetes enhances the pathogenicity of formerly commensal bacteria, now referred to as pathobionts. The increased inflammation, osteoclastogenesis, and periodontal bone loss suggest that these pathobionts are more dangerous. IL-17, a cytokine that boosts pro-inflammatory mediators including IL-6 and RANKL, has been linked to an increase in pathogenicity. Blocking IL-17 makes the oral microbiome less harmful in diabetic animal models. The link to IL-17 shows that inflammation in the host may indirectly activate pathobionts and cause oral dysbiosis. People with diabetes who have oral dysbiosis have higher levels of *Capnocytophaga*, *Porphyromonas gingivalis*, and *Tannerella forsythia*. Long-term studies have demonstrated that uncontrolled diabetes and high blood sugar levels cause an increase in Proteobacteria and Firmicutes. There is a decrease in the variety of bacteria in the oral microbiome at the same time as levels of pathobionts rise.[9,10]

HIV is an infection that attacks the immune system of the patient and causes a big drop in CD4+ T cells. The World Health Organization said that by the end of 2020, more than 37.7 million people throughout the world had HIV, and around 73% of them were on Antiretroviral Treatment (ART). The sickness (HIV) and the treatment (ART) have changed the composition and diversity of the oral microbiome. Changes in the components of saliva that are secreted, the body's natural and adaptive immune responses, and the way saliva works in the body may all lead to dysbiosis of the oral microbiome in people with HIV.[11,12]

Mouth Microbiome Transplantation (OMT) is similar to fecal microbiome transplantation in that it involves transferring mouth microorganisms from a healthy donor to a patient with dental caries or periodontitis. Recently, OMT therapy has been successfully used on dogs with naturally occurring periodontitis. The procedure involved putting healthy donor plaque samples into a sterile saline solution, putting the mixture on a nylon swab, and swabbing and irrigating the tooth surface of the transplant recipient. There have been many suggestions on how to treat individuals with this condition, but none of them have been tried on people yet, and the best and most effective technique is still unknown. OMT tries to restore microbial balance by moving helpful microbes from a healthy person into the mouth of a sick person. Using rinses, gels, or swabs, the donor's saliva or dental plaque is carefully collected, processed, and sent to the recipient. Early studies suggest that OMT may reduce inflammation, stop toxic biofilms, and bring back a healthy microbial population. Even though OMT has a lot of promise, it is hard to put into practice since it is hard to find the right donors, make sure that it is microbiologically safe, standardize methods, and deal with ethical difficulties. Researchers are looking at the long-term effects of OMT. As research goes on, OMT may become a very useful tool in both preventive and therapeutic dentistry. Possible future advancements include personalized oral probiotics, ways to keep the microbiome healthy, and using probiotics to treat systemic illnesses. OMT might change the way we think about oral and systemic health thanks to additional clinical research and technological progress.[13,14]

Conventional treatment of oral diseases

Causal therapy is one of the traditional ways to treat dental cavities and periodontal disease. To treat dental caries, you have to cut off the demineralized tissue and replace it with restorative material. The main treatment for periodontal disease is to get rid of plaque biofilm mechanically and deal with the risk factors that come with it. For example, those with diabetes should work on controlling their blood sugar better, and people who smoke should quit smoking. Current methods do a good job of treating tooth decay and periodontal disease, but they don't do a good job of preventing these problems before they start, which means that they only work for a short time and the disease is likely to come back.[15,16] Dental restorative materials have a significant failure rate, with 8% of them failing each year because to secondary caries and bulk fracture.[17] Earlier treatments, including putting resin-based sealants on permanent molars, only lower the risk of cavities by 11% to 51% for a short time, up to 48 months.[18,19] Almost all patients with periodontal disease who are getting periodontal maintenance therapy show signs of continuous clinical attachment loss. One to two-thirds of these patients lose at least one tooth throughout a lengthy period of maintenance care. Finding ways to improve the prevention and treatment of periodontitis and dental caries is very important. This is especially important since older individuals are losing teeth more often and young children are getting serious cavities more often.[20-22]

mouth Microbiota Therapy (OMT) may be a viable approach for addressing prevalent mouth illnesses.

Developing oral microbiome transplantation therapy in Australia

Mouth Microbiome Transplantation (OMT) is similar to Fecal Microbiome Transplantation (FMT) in that it involves transferring mouth microbes from a healthy donor to a sick patient. Recently, OMT therapy has been successfully used on dogs with naturally occurring periodontitis. The successful procedure involved putting healthy donor plaque samples into a sterile saline solution, putting the mixture on a nylon swab, and swabbing and irrigating the tooth surface of the transplant recipient. There have been many suggestions on how to use this therapy on people, but it hasn't been tried on people yet, and the best and most effective way to do it hasn't been found yet.[23-25]

Finding healthy donors is a very important part of our study. The microbiome of a "healthy" person varies a lot from person to person, making it unlikely that there is one "healthy" state that applies to everyone. This makes it harder to choose a donor. The term "super donor" comes from fecal microbiome transplantation and refers to fecal donor material that works much better than other donors' stools. For example, the super-donor effect has been seen in several clinical trials for treating inflammatory bowel disease. The microbiota in the stomach of super donors is mostly made up of keystone species or high diversity traits that don't necessarily lead to health improvements in the mouth (for example, keystone species like P. gingivalis may be seen as oral pathogens, and increased diversity).[26-28]

The oral microbiome is associated with diseases, including periodontal disease.[29] Although they were unable to determine what exactly constitutes a "healthy donor," the authors of the research on canine OMT did find that the oral microbiota of healthy dogs was different from that of periodontitis and edentulous patients. Therefore, we propose that the following characteristics be present in the microbiota of an OMT super donor: display low numbers of sophisticated "red" microbes. Streptococcus mutans and other known cariogenic organisms do not possess any infectious or transmissible viruses, such as herpes or HIV. They show an increased ratio of viable to non-viable bacterial cells in their effective *in vitro* proliferation. The ability of these species to colonize a host by altering their microbiome to resemble a donor has demonstrated effectiveness in rodent models of oral diseases, including inflammation reduction, with few side effects, such as increased risk of oral infections or disease markers. 'Super donors' who have been successful need to be studied in humans further. It is our expectation that these characteristics of donor microbiota will improve the chances of OMT success in human research. However, before OMT therapy is used on humans, further safety, ethical, and practical considerations need to be evaluated. This study will provide the necessary information to determine the feasibility and technique of OMT clinical trials on humans.[3-6]

METHODOLOGY

Stage 1: Identifying the healthy donor and Collection of the Biofilm

"Super donors" are donors who have perfect microbial profiles, meaning they have a lot of different microbes but very few harmful ones. Six locations with the deepest probing in each sextant were used to extract supra- and subgingival plaque. Every periodontal pocket and gingival sulcus had one sterile paper point inserted into the very center. Using a sterile cotton applicator, biofilm was collected from the oral mucosae by swabbing the epithelial surfaces of the lip, buccal mucosae (both sides), palate, and tongue's dorsum. Preserved at -80°C, samples were used for DNA contamination analysis.[30]

Stage 2: Sample Preparation and Cultivation

Microbial DNA was extracted from cells by physical and chemical disruption using zirconia/silica beads and phenol-chloroform extraction in a FastPrep-24 bead beater. Prokaryotic 16S rRNA genes were amplified using universal primers (27 F and 1392R) with the GemTaq kit from MGQuest (Cat# EP012). The PCR protocol included a pre-amplification phase of 10 cycles at an annealing temperature of 56°C, followed by 20 amplification cycles at an annealing temperature of 58°C.[31] During each cycle, the elongation duration was 1 min and 10 sec at 72°C. The PCR was completed with an extended elongation period of 5 min. PCR products were purified with DNA Clean and Concentrator columns (Zymo Research, USA) and quantified with the Nano Drop (Agilent, USA). For some participants, subgingival and mucosal microbiotas were consolidated into a single vial due to technological constraints, but for other subjects, these microbiotas were preserved separately. To examine the whole microbial community, equal amounts of PCR products from separately stored supra- and subgingival microbiota samples, generated from swab and paper point samples, were combined for each patient. No paper point samples were available for edentulous patients. Each purified PCR product, at 500 ng, was tagged with a Multiplex Identifier (MID) during the Roche Rapid Library preparation phase. Four MID-tagged sequences, corresponding to each condition, were amalgamated at equimolar quantities and underwent emPCR and DNA sequencing procedures as per the manufacturer's guidelines for the Roche 454 Jr. equipment.[32]

Step 3: Develop a delivery system for OMT therapy

Hydrogels including dopamine and oxidized sodium alginate will be synthesized using a multi-step procedure for use as an OMT delivery vehicle. Sodium alginate will first undergo oxidation by sodium periodate in a 1:1 v/v ethanol-water combination. Dopamine (DA) will thereafter be conjugated to the oxidized sodium alginate using a recognized bioconjugation technique. EDC (1-(3-(Diethylamino) propyl)-3-ethylcarbodiimide hydrochloride) and NHS (N-hydroxy succinimide) will serve as coupling agents to selectively conjugate the amine group of dopamine with the carboxylic acid group of oxidized sodium alginate. The product will be used for hydrogel manufacture after extensive dialysis for purification. The hydrogel synthesis will commence using Acrylamide (AM) and dopamine-grafted oxidized sodium alginate. Ammonium Persulfate (APS), N, N0-methylenebisacrylamide (BIS), and N, N, N0, N0-tetramethylethylenediamine will thereafter be introduced in an inert nitrogen environment. An inert atmosphere is necessary to prevent the over-oxidation of dopamine.[33,34]

A clear hydrogel will develop after 10 hr. The hydrogel's composition may be modified by adjusting the quantity of dopamine conjugated to oxidized sodium alginate in the final solution. A hydrogel comprising Polydopamine (PDA) and Polyacrylamide (PAM) will be synthesized as an alternative. Dopamine will first undergo polymerization in the presence of sodium hydroxide, followed by the sequential incorporation of AM, APS, and BIS while mechanically stirring in an ice bath. A PDA-PAM single network hydrogel will be synthesized after 10 min. The DA/AM ratio will be adjusted to enhance the characteristics of the hydrogels. The mechanical stability of hydrogels will be evaluated by measuring their storage modulus (G') and the loss tangent (the ratio of loss modulus G" to storage modulus G'). The hydrogel's chemical characterisation will be evaluated using FT-IR spectroscopy, XPS, and thermogravimetric analysis. The hydrogel's morphological characterisation will be conducted using Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM).[35]

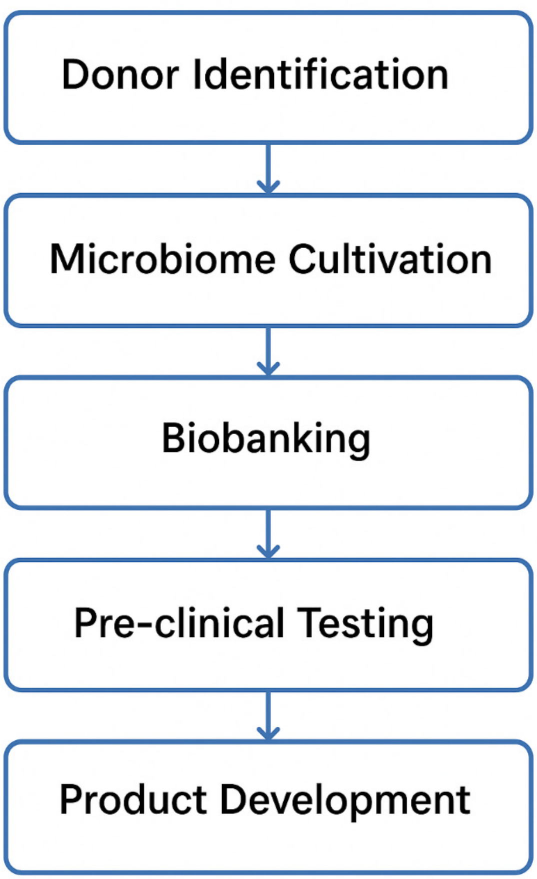

Figure 1: Summary Chart of OMT Development Process.

Once the bacteria have been properly characterized, they will be added to the hydrogel and their mortality will be checked using microscope and sequenced (as mentioned above) every 12 hr for three days. This is similar to the time needed for OMT therapy for shipping and application. This method of application still needs to be tested on murine models to see how well it works. If we run into problems with the hydrogel method, we will look into other options, like using plaque microbiota in buffers (like the canine OMT study did), varnishes, or mouth washes.[36] A summary chart of the Oral Microbiome Transplantation (OMT) development process is presented in Figure 1.

DISCUSSION

Maintaining good dental and overall health depends on the oral microbiome. Oral ailments like periodontitis and dental caries, as well as systemic issues like diabetes, cardiovascular disease, and maybe cancer, can arise from an imbalance in this microbial community, which is called oral dysbiosis. According to the theory of by introducing beneficial microbes from a healthy donor into a patient's Oral cavity, Microbiome Transplantation (OMT) aims to restore microbial balance.

The current study explored potential of OMT as an innovative approach to managing oral dysbiosis. Unlike conventional treatments, which focus on causative therapy such as plaque removal and dental restoration, OMT targets the root cause by reintroducing a balanced microbial community. Traditional methods, though effective to an extent, often result in high failure rates and disease recurrence, primarily due to their inability to address the microbial imbalance directly. OMT, therefore, offers a promising therapeutic alternative by focusing on restoring a healthy oral microbiome rather than merely managing the symptoms.

One of the key challenges in developing OMT is identifying suitable donors, as oral microbiomes exhibit significant interpersonal variability. The term "super donor" originates from fecal microbiome transplantation, whereby certain donor characteristics provide superior therapeutic results. In the context of OMT, a super donor would ideally possess a microbiome with high diversity, low pathogenic species, and the capacity to establish a stable and beneficial microbial community in the recipient. In this study, we developed a hydrogel-based delivery system for OMT, utilizing dopamine-grafted oxidized sodium alginate to create a stable and biocompatible medium for microbial transfer. The hydrogel's physicochemical properties, including mechanical stability and microbial viability, were thoroughly characterized. This innovative approach addresses some of the logistical challenges associated with microbial transplantation, such as maintaining microbial viability during transport and application.

Preliminary data from animal studies, particularly those involving canine models, indicate that OMT may significantly reduce inflammation and inhibit the formation of pathogenic biofilms. Nonetheless, translating these findings to humans requires rigorous testing and optimization. Safety concerns, such as the potential transmission of infectious agents, must be thoroughly addressed through stringent donor screening and microbial safety protocols. Moreover, longitudinal studies are necessary to assess the durability of the transplanted microbiome and its therapeutic effectiveness in the management of oral illnesses. The capacity to use OMT as both a therapeutic intervention and a preventative strategy might transform dental treatment, especially for those susceptible to recurrent oral infections.

Applications of OMT

Treatment of Periodontitis

OMT has shown promise in treating periodontitis by introducing healthy oral microbial communities that can outcompete pathogenic bacteria, reduce inflammation, and restore oral health. Studies have demonstrated significant reductions in periodontitis-associated pathogens following OMT.

Management of Dental Caries

By transplanting health-associated oral microbiota, OMT aims to reverse dysbiosis linked to dental caries, potentially reducing the recurrence and severity of cavities.

Adjunct to Standard Periodontal Therapy

OMT can be used alongside conventional treatments (like scaling and root planning) to help re-establish a stable, health-promoting oral microbiome, improving long-term outcomes for patients with chronic periodontal disease.

Prevention and Early Intervention

OMT may be applied during key life stages, such as the eruption of permanent teeth in children, to establish a healthy oral microbiome early and prevent future disease. It could also benefit aging populations and those receiving dental prosthetics, who are at higher risk for oral dysbiosis.

Potential for Other Oral Diseases

Research suggests OMT could be beneficial for a range of oral conditions associated with microbiome imbalance, including gingivitis, halitosis (bad breath), oral mucositis, oral cancers, and xerostomia (dry mouth).

Systemic Health Benefits

Since periodontal disease is linked to systemic conditions such as diabetes, cardiovascular disease, chronic kidney disease, osteoporosis, and Alzheimer's disease, OMT might indirectly contribute to improvements in these areas by reducing oral inflammation and pathogenic load.

CONCLUSION

Oral Microbiome Transplantation (OMT) signifies a transformative approach in the treatment of oral and systemic disorders linked to microbial dysbiosis. By restoring the natural microbial ecosystem, OMT offers a more holistic approach compared to conventional treatments. The development of a hydrogel-based delivery system demonstrates a significant advancement in the practical application of OMT, potentially overcoming challenges related to microbial viability and stability. While promising, the clinical implementation of OMT in humans requires further research, particularly to establish safe and standardized protocols for donor selection and microbiome transfer. The identification of super donors, the optimization of microbial delivery systems, and the long-term monitoring of transplant success are crucial for advancing this therapeutic innovation. If proven successful, OMT could significantly improve patient outcomes by addressing the root causes of oral dysbiosis and related systemic diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HOMD: Human Oral Microbiome Database; **OMT:** Oral Microbiome Transplantation; **WHO:** World Health Organization; **HIV:** Human Immuno Virus; **FMT:** Fecal Microbiome Transplantation.

SUMMARY

Oral Microbiome Transplantation (OMT) Presents A Novel and Promising Strategy for Managing Oral and Systemic Diseases Associated with Microbial Imbalance. It Aims to Restore the Natural Oral Microbiota, Offering A Holistic Alternative to Traditional Therapies. The Use of Hydrogel-Based Delivery Systems Enhances the Feasibility and Effectiveness of OMT. However, Further Research Is Necessary to Ensure Clinical Safety, Identify Optimal Donors, And Standardize Treatment Protocols. With Continued Advancements, OMT has the Potential to Significantly Improve Long-Term Oral Health Outcomes.

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