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Review

THE IMPACT OF IMPLANTED BIOMATERIAL TO THE MICROBIOME

Does technology change human or we change technology?

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Abstract: Contemporary medicine is unimaginabile without biotechnological involvment in diagnostic and therapeutic purpouses, but sometimes the discovery and application of new materials go faster then the understanding and adoption of the knowledge about the consequences for our organism. Understanding of biological processes at the microscopycal and molecular level would lead to better integration of biomaterial with human body environment. The primary aim of this review was to evaluate the potential microbiological effect after biomaterial and implant insertions to oral microbiota. The secundary aim was to evaluate if such microbiological shift has any effect to surgical wound-healing, post-implant tissue reaction or rejection of implanted biomaterial. The third aim was to question previous results of similar reviews and studies regarding microbiological role in improving therapeutic responses after biomaterial implantation in dental medicine. A comprehensive systematic search via Web of Science, Scopus and PubMed databases was conducted. The data synthesis showed similar results among clinical studies and several reviews with ambiguous conclusions leaving numerous questions without straight answers. We hypothesized that relationship between host microbiome and biomaterial insertion is mutual, but within the limitations of this review, the interaction between host-oral microbiota and material inset remains uncharted territory.

Keywords: oral microbiome, biomaterial, dental implant

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Introduction

Throughout history, medicine has evolved to cure various pathological conditions and for the last several decades it has an additional role in improving quality of life while extending life expectancy. It is difficult to analyze the evolutionary path of medicine without its connection to technology. Improvement of contemporary medicine is not possible without biotechnological advances in diagnostic and therapeutic purposes. In addition, technology has changed the whole paradigm of our lives. With a scientific progress in fundamental natural science, such as physics, chemistry and biology, and subsequent discovery and development of new materials, came better understanding of their possible applications in medicine [1]. The treatment of various health issues, including healing wounds, immobilization of broken limbs, replacement of anatomical deficits requires biomaterials to shorten the treatment, and to extend and improve the quality of life [1], [2]. Today, the development of science and technology opened the door to new studies of material structure and its properties on the molecular level [2], [3]. Materials are manipulated experimentally, adding or subtracting undesired characteristic depending on the application, and by combining different materials it became possible to merge their features as well [3]. Biomaterials, no matter how biological they are, still represent a group of foreign bodies implanted in the human organism. In order for such materials to become more natural and less foreign, huge resources are being dedicated to research about their effect on human body, both on macroscopic level and increasingly on microscopic, even nanoscopic level [3]. One of the leading beneficiaries in the biomaterial research has certainly been dental medicine, which is increasingly becoming a part of aesthetic medicine, and therefore a profitable area for investment that will also enable scientific advances related to clinical applications. Through this review, we analyze the impact of biomaterials on the human microbiome.

The microbiome represents a community of microorganisms (such as fungi, bacteria and viruses) that co-exists in a particular environment. In humans, the term is often used to describe the microorganisms that live in or on a particular part of the body, such as the skin or gastrointestinal tract [4]. These groups of microorganisms are dynamic and change in response to environmental factors of the host, such as exercise, diet or medication. The concept also includes microbial genetic material, while the term microbiota describes only microorganisms. The total number of cells in our organism is approximately 10^{14}, and the number of microbiota cells is about 10^{15}, which means the ratio of our genes and microbiota is greater than 100:1 in favor of the microbiota [4]. The concept of the human microbiome was first proposed by molecular biologist Joshua Lederberg in year 2001. The term microbiome was introduced to denote the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share space in the human body [4]. The interactions between the microbiome and the human immune system are numerous, complex and, in fact-the most important, a two-way road. There are many questions, but we can assume with certainty that the discovery of the microbiome has changed our view about human body. With the ending of the human genome project, began the human microbiome project and research for this topic has become overwhelming, thankfully to the development of technology [5]. Today, almost every disease or pathological condition of unknown etiology includes some disorder in the microbiome [4], [6]. Every manipulation and intervention on humans, either diagnostic, therapeutic, restorative, or aesthetic; chemical or biological, certainly brings some change in human microbiome. However, is this change temporary and reversible or is it permanent?

Dental composites and biomaterials are routinely utilized in restorative dental procedures. These procedures are now accessible to the general public, but their longevity and their effect on the human body will probably become evident in a several decades. The integrity of the implanted material and its durability certainly depends on the metabolic activities of the oral microbiome. Therefore, many new antimicrobial dental materials have been developed in the last few decades. But is the antimicrobial trait desirable feature for a patient or a safety net for the oral surgeon? Is any biomaterial adaptation responsible for further disturbances in the balance of the microbial community (Figure 1)?

Theoretically, an ideal biomaterial should trigger a minimal possible reaction of the microbiota but is this even achievable? Such research is very demanding and complex because in vitro will never represent in vivo, and moreover, each person has its own, specific microbiome and immune system. Everyone has a distinctive microbiome same as a fingerprint [7]. Even identical twins have some differences in microbiome components [4], [8]. It implies personalized medicine and an individual approach to each patient.

Oral microbiology and the research of dental materials are faced with an additional challenge - the oral biofilm. The evaluation of implants intended for primarily sterile areas of the organism is less demanding because the anatomical niche of their placement requires absolute microbiological cleanliness. On the contrary, dental materials should be biocompatible materials that are resistant to mechanical, chemical, and biological degradation, including exposure to chewing and grinding forces, contact with eukaryotic (human, fungal) and prokaryotic (bacterial) cells organized into their own biofilm communities.

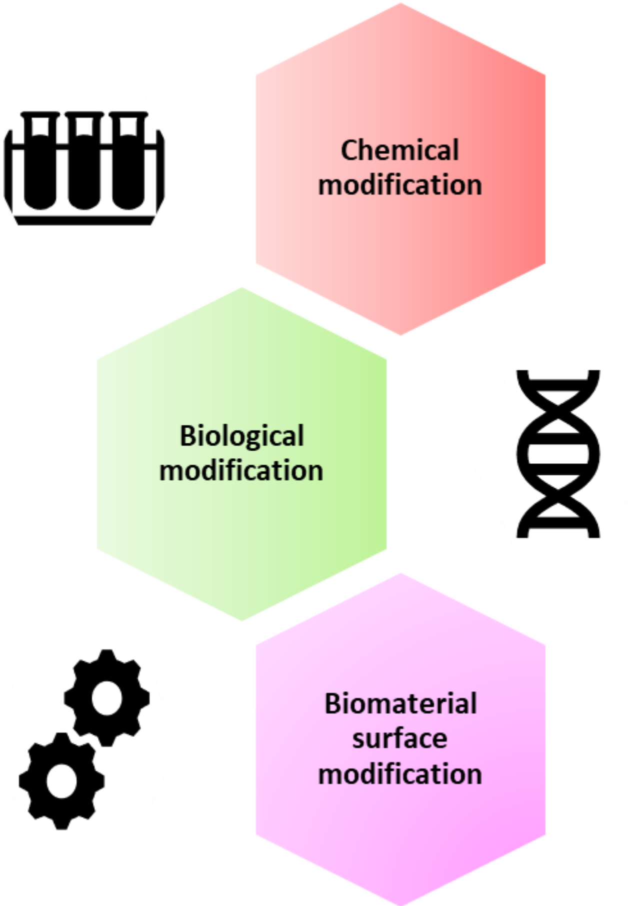

Figure 1. Do biomaterials need extensive and versatile adaptations? What is the final result of those multiple variant modifications to our organism?

This aspect is of fundamental importance to medicine development as the oral cavity has been inhabited with distinct microbial communities, which contribute to the general health but nevertheless have been associated with systematic diseases affecting the human organism as a whole. Therefore, a dysbiosis can not only affect the implant material, the following healing process and final results but also lead to complete health problems. What about biochemical properties of saliva and the food or drinks we consume on everyday basis? It is perhaps the last but most important factor that affects biomaterials in the oral cavity because they do not only have a direct impact on the material, but also determine the metabolic activity of the microbiome. The effect of the microbiome on biomaterials depends about the composition of saliva. The most abundant enzyme in saliva is alpha-amylase, which catalyzes the hydrolysis of starch [9]. The ability to directly bind amylase is conserved among streptococci, which are important early inhabitants of any oral biofilm. Many studies have shown that even the inorganic components of saliva - calcium and magnesium ions play an important role in the utilization of amylase by streptococci, while the chelation of both cations reduces the binding of streptococci to the biomaterial [10]. Currently, oral diseases are best described as problems of oral ecology, which is also called dysbiosis, meaning an ideal dental material should have immunomodulatory and bioactive properties [11], [12]. Special interest is given to molecular and genetic level of research, which should answer the question how to integrate knowledge about the evolution of genes (human, microbial) to solve the challenges that arise as a response of macro and microorganisms to the presence of biomaterials over several decades. However, a question arises whether biomaterials should have antimicrobial effect at all? Or should we strive to design a material whose composition will also benefit the microbiota, with the ultimate effect of preventing dysbiosis?

The answer is still elusive, whereby finding the ideal solution requires an interdisciplinary approach. Almost 90% of the world's population suffers from oral dysbiosis [6]. The effect of such pathological changes in the oral cavity varies from gingivitis and chronic periodontitis to the final destruction of periodontal tissue and tooth loss. The primary site of periodontal infection is the space between the root of the tooth and the gingiva - the subgingival tissue, which, as the disease progresses, can deepen into the periodontal pocket [6]. This specific anatomical niche represents the ideal conditions for the development and proliferation of small but hard-to-reach smoldering infections that not only causes local inflammation but also can be the source of focal infections with the effect somewhere else in the body. It is considered that periodontitis occurs because of disturbed equilibrium in the dental plaque [13]. It is a microbial biofilm composed of microorganisms that represent normal flora of the oral cavity [13]. Although dental plaque is the most widespread sessile community of microbes in humans, we all have it and it is impossible to avoid it, clinical signs of periodontitis occur only when chronic presence of dental plaque causes an immune response and inflammation [14]. This happens due to the sensitivity of the host or a change in the conditions in the microenvironment of the oral cavity (Figure 2) [15], [16].

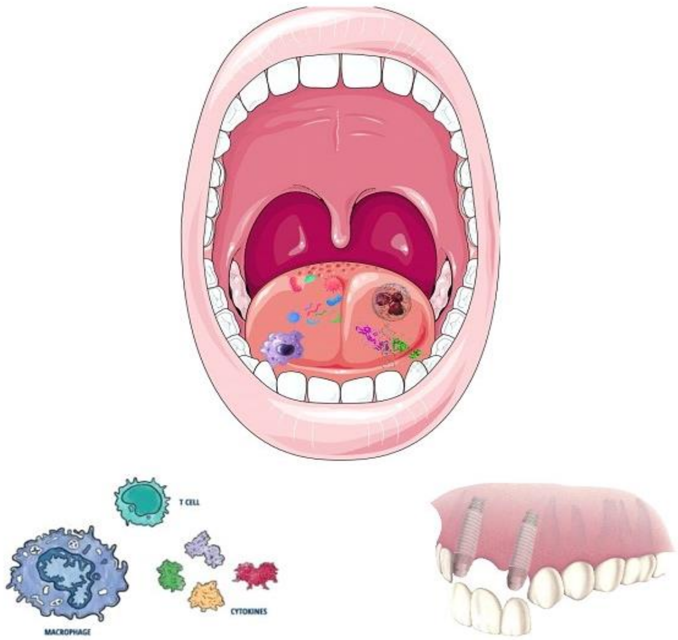

Figure 2. Individual variations of the immune system at the molecular level can significantly change the outcome of biomaterial implantation.

The sensitivity of the host is determined by genetic and environmental factors and acquired habits such as exposure to cigarette smoke [14]. Although the host's inflammatory response serves as protective mechanism, if it is excessive or insufficient, destruction of gingival and periodontal tissue can occur [14]. The expression of microorganism`s virulence factors of and the increase in pH value contribute to tissue destruction, which changes the microenvironment in the oral cavity [13], [15]. Predominant inhabitants of the early biofilm are streptococci, such as the bacteria *Streptococcus gordonii*, *Streptococcus sanguis*, *Streptococcus oralis*, *Streptococcus parasanguis* and numerous other members of the normal flora in the oral cavity [17]. But what is normal oral microflora? It is difficult to clearly define what represents a normal microbiota in the oral cavity. It is a variable community of different microorganisms, and it is almost impossible to say who is the observer and who is the cause of pathological processes, when they occur (Figure 3).

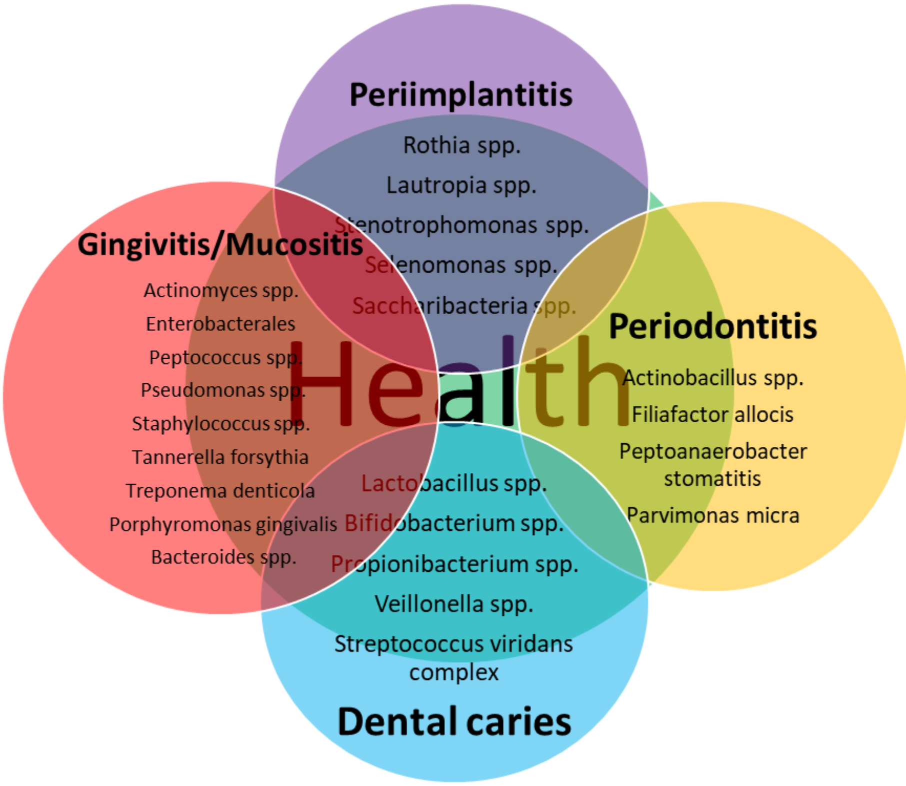

Figure 3. The same members of the microbiota can play different roles in the pathology of the oral cavity.

Ultimately, microorganisms act as a community - together, and if an infection spreads, it is always mixed and multifactorial [18]. Factors that favor streptococci as first colonizers are the expression of adhesin proteins that recognize receptors on the conditioning film, the ability to metabolize saliva components as the only sources of nutrients, and the distinct ability to coaggregate [19]. Through the process of coaggregation - intercellular recognition and adherence of genetically different bacteria, and metabolic interactions of other bacteria with streptococci, the early biofilm soon becomes a multigeneric microcommunity consisting of aerobic and aerotolerant bacterial species of the genera *Gemella, Actinomyces, Veillonella, Haemophilus* and *Neisseria* [20]. For instance, the bacterium *Fusobacterium nucleatum* is considered to be the key-mediator of the co-aggregation process between early and late colonizers in dental plaque [21]. Furthermore, it is believed that this gram-negative bacterium promotes the anaerobic microenvironment that allows strictly anaerobic, the very late colonizers to survive in an aerobic atmosphere. Those latecomers are gram-negative anaerobic bacteria, such as *Porphyromonas gingivalis*, *Tanerella forsythensis* and *Treponema denticola* [22]. The prevention of periodontitis is based on the control of oral biofilm formation, but for now, mechanical removal and chemical control have proved unsuccessful [13]. New strategies to prevent the formation of oral biofilm, such as interference of signal transduction in the biofilm, modification of the tooth surface, replacement of potentially pathogenic with genetically modified, less virulent microorganisms, and immunization, are just the beginning of efforts to control the complex microbial community [13], [23]. Is there a biomaterial resistant to plaque formation? The future will tell which of these strategies will be successful in preventing and controlling oral biofilm. Does the control-key lie in the use of probiotics? Many nutritional supplements, such as chewing gums containing probiotic bacteria, are recommended for the purpose of oral health [23], [24]. Inflammatory bowel diseases are additionally treated with probiotics, some disorders even with fecal transplantation [4], [24]. Is this experimental self-medication? How rational is it to interfere with one's own microbiome, which is subject to natural fluctuation only during the first few years of life and after that remains constant with only occasional violations of integrity due to the use of antibiotics [24]? The consumption of probiotics is still in the sphere of controversy, regardless of the indication for use, and always a good idea for clinical research, although the results are so far debatable [23], [24].

Common strategies to prevent microbial colonization of materials include surface modification of biomaterials. This includes the determination of topography by atomic force microscopy, modulation of hydrophilicity or incorporation of components that serve as protein repellents for microbial adhesion or disrupt the integrity of the microorganism cell membrane [25], [26]. The main problem seems to be the overabundance of certain microbes, not their general presence or absence [26]. It has been shown that biomaterials mainly affect biofilm composition, gene expression and protein production [27], [28]. Does this mean that future research should focus on transcriptomics and proteome analysis? So far, similar research has been documented only for *Streptococcus mutans* [28], [29]. In addition to the topography of the biomaterial surface, the composition of the biofilm is also affected by the structure of material. This was especially shown by studies in which modified acrylic dental resins were used, which contain antimicrobial substances such as silver derivatives [30]. How long would that effect last in vivo? What does the future bring us? Most microbes are incredibly adaptable, especially during chronic exposure to a particular stressor [31]. There are several reasons for this, one of them being the exchange of genetic material. It has been shown that numerous dental biofilm bacteria, especially streptococci, can develop a physiological ability to actively take up extracellular DNA [32]. This feature is especially induced in the conditions of biofilm growth and enables, among other things, the exchange of antibiotic resistance genes [33]. The question arises, can our own oral cavity become reservoir of multi-resistant microbes? Microorganism stress is a classic trigger for the horizontal exchange of genetic material, as well for mutations of the bacterial chromosome, which responds to environmental conditions with much faster adaptation (change) than any eukaryotic organism [34], [35]. Nevertheless, the speed and direction of microbial evolution will probably never be surpassed or predicted. Another strategy of microbes in the evolvement of insensitivity to antimicrobial substances is the emergence of persisters. Persisters are metabolically inactive subpopulations of microbes in the biofilm [6]. Such cells show an increased ability to survive and after (re)activation show increased expression of the *gtf* gene responsible for the production of the extrapolysaccharide matrix, the basic component of any biofilm [36]. Fortunately, persisters usually are only a small proportion of the total biofilm population, but they can be the reason for the regeneration of a depleted microbial community, with the potential emergence of new variants.

However, future looks bright judging by the results of recent research and gives hope in the fight against biomaterial infections [37]–[40]. How long will it last? The road from novel scientific discovery to professional application is far and slow. By now microorganisms have always quickly found a way to adapt against our measures and solutions. In this competition, time works against us. We rely so much on technology that it changes us, our view of human body and bring many new questions without clear answers. Does the correct approach already lie within ourselves [41]?

Conclusions

The biomaterial connection to our microbiome represents uncharted frontier. We still lack a lot of knowledge about the influence of dental materials to the ecology of any microbiome. Likewise, there is a very superficial understanding about relationship of the dental material chemical composition to the microbiome transcriptome. However, this is an important area of research that may predict the clinical efficacy of new materials. Maybe it's not the composition of the microbiota that matters at all, but gene expression or the activity of the microbial community. Ultimately, the success of inventing an ideal biomaterial, the smart bioactive materials of *the next generation*, will certainly be the result of interdisciplinary cooperation between scientists, materials experts, molecular biologists, and clinicians.

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