THE ADVANCES IN HUMAN ORAL BIOLOGY AND BIOTECHNOLOGY

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Abstract

The oral cavity is a very specific and complicated structure. It plays the role of a speech apparatus but is also a gate and a preliminary processing plant for fuel nourishing the body. Analyzing the embryological development of the oral cavity, we can distinguish here the stages of growth, migration and fusion. This processes mainly involves the formation of branched arches that appear between the fourth and fifth weeks of fetal development. In recent years, interest in researching its various features has been still growing. After a thorough description and anatomical examination, researchers began to think about its other possibilities than just crushing, throwing, swallowing, bringing out the sounds and articulating words. It turns out that the whole range of its potential is not limited to just a few functions mentioned above. The following review article covers specific studies that have emerged most frequently over the past year. You will find here information about the microbiome of the oral cavity which plays an significant role in maintaining a proper physiological environment of the oral cavity, oral tumors and how the SCC or squamous cell carcinoma is histologically the most common and its treatment, even in the context of the Covid-19 pandemic, as well as the least discovered and still fascinating for scientists wound healing processes and unknown exosomes that can transport biological molecules in their original, intact and active form, including nucleic acids and proteins with their signaling pathways.

Running title: The advances in human oral biology and biotechnology

Keywords: oral cavity, oral mucosa cells, biotechnology, oral cancer, wound healing

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Introduction

Over time, we learn about newer and newer possibilities of the human body. We deal with very detailed aspects of it, often forgetting the bigger picture that is taking into account matters that may seem obvious to scientists. Let us see then, how the recent oral research - taking into account its widely reaching biology and biotechnology aspects - has been taking place over recent years.

The specificity of the oral cavity is quite unique considering the close relationship with the external environment it represents. It is also worth mentioning that no part of the human body represents the amount of diverse functional anatomical units and does not contain such a plentiful bacterial flora as we can find in the mouth. However, a structure with so many different functions is exposed to just as many factors. Eating, drinking and many common stimulants usually have a negative effect on the appearance and condition of the mouth. The rich bacterial flora is an indispensable part of it, supporting many processes in the oral cavity. However, if the tissues inside are damaged, bacteria that have previously had a positive role can infect the proximal or distal areas of the mouth. In such a situation, the constantly moist environment and saliva, which prevents the hard closing of wounds, i.e. scabs, are also not helpful. The early signs of many deficiencies in the body, degenerative diseases and metabolic diseases are first visible inside the mouth before we can physically see them at the destination. That is why lips are often called body mirror, which perfectly reflects their important and broad role in further scientific research [1].

A short prologue - anatomy of oral cavity

The oral cavity is an extremely complex structure. It is a three-dimensional organ comprising teeth, muscles, sensory receptors and secretory glands. It is a gateway to the gastrointestinal tract and plays very important roles such as biting, chewing, swallowing, but also breathing and speaking. Analyzing the embryological development of the oral cavity, we can distinguish here the stages of growth, migration and fusion. This processes mainly involves the formation of branched arches that appear between the fourth and fifth weeks of fetal development. These arches are separated by clefts from the ectoderm, while in themselves come from mesodermal tissue. They are also separated by internal pharyngeal sacs which originate in endoderm [2,3]. The main support for the moving structures in the mouth is the mylohyoid muscle. It is located just above the anterior belly of the digastric, having a flat, triangular shape. This muscle helps raise the hyoid bone when talking and swallowing. Together with the geniohyoid and the anterior belly of the digastric muscle, they form the muscular floor of the mouth. Cheeks and lips surrounding the alve-

olar arches are mainly built by the mimetic muscle. The submucosal connective tissue of the lips and cheeks contains numerous salivary glands that discharge continuously into the atrium of the mouth. CN VII known as facial nerve provides motor innervation of mimetic muscles including orbicularis oris and buccinator, which forms the muscular structure of the cheek [4–6]. The mouth and nose are separated by a hard palate that goes from the teeth to the back of the cleansed cavity. The soft palate is located on the back of the hard palate and is formed as a result of the specific arrangement of the palatoglossus and palatopharyngeus muscles. These muscles mainly have two functions: they pull the back of the tongue up to separate the mouth from the throat and stretch and lift the soft palate when swallowing to prevent food from entering the nasal area [2,4]. The muscle structure that covers most of the mouth and rests on its bottom is the tongue, which consists of intrinsic and extrinsic muscles. They facilitate precise language movements when chewing, swallowing or articulating spoken sounds and words. The papillae covering the mucous membrane of the tongue distinguish its anterior part from the posterior one. They are largely responsible for the perception of taste and are divided into 4 types: fungiform, filiform, vallate and foliate [1,7]. In the oral cavity there are four muscles of mastication: the temporalis, masseter, medial and lateral pterygoid. They move the jaw during the processes of biting, pounding and crushing food, as well as during speech. Teeth play an equally important role here. We divide the maxilla and mandible into two parts, of which every half in an adult has a set of eight permanent teeth: two incisors, one canine, two premolars and three molars. Molars grind, incisors cut and chop food [4,8].

Oral microbiome possibilities

As we know well, what is invisible to the naked eye is equally important. The microbiome plays an important role in maintaining a proper physiological environment of the oral cavity. Although we often talk about its positive effect, it should also be remembered that the same microbiome can play a role in the development of oral diseases and even cancer and other chronic diseases, through general inflammatory effects and direct metabolism of chemical carcinogens [9–11]. Stimulants, in particular smoking, diseases such as diabetes, chronic stress and obesity are among the many factors that can disturb the symbiosis of bacterial flora. Dysbiotic oral microflora disrupts the host's defense mechanisms, causing chronic periodontal disease or even oral cancer [12–14].

Just a few months ago, a study on the body's immunity related to the dependence of malnutrition and the bacterial flora of the mouth and intestines was published. Malnutrition is saying to be associ-

ated with increased intestinal barrier permeability, microbial dysbiosis and weakened responses to oral vaccination [15].

Host-microbe interactions in the intestine are largely mediated by immunoglobulin A (IgA). For example, IgA helps maintain a diverse and resilient community of commensal microbes, thus promoting reciprocity between the host and microbiome. IgA also protects against infection by limiting both the growth and virulence of enteropathogenic bacteria [16]. To investigate how nutrient-induced changes in bacterial flora affect the body's response to oral vaccines, gnotobiotic mice was colonized with faecal microbiota of Bangladesh children with SAM - severe acute malnutrition in the past. Research was carried out by Di Luccia et al. His team's results provide important preclinical evidence that the intestinal microflora of malnourished children is causally related to the response to the oral mucosal vaccine and that these phenotypes can be reversed by the introduction of additional bacteria. [17]. Several years ago, there were also reports showing dependence microbiome in the oral cavity in relation to autoimmune diseases. It has been shown that problems with the integrity of oral microflora can be directly related to systemic lupus erythematosus (SLE), Sjorgen's Syndrome (SS), rheumatoid arthritis (RA) and Crohn's disease (CD). Such results unbelievably broaden the diagnostic possibilities and ways of earlier detection of these or related autoimmune diseases [18].

Over the past 20 years, scientists have become interested in the possible effects of probiotics on healthy oral care. In vitro studies have shown that probiotics can effectively inhibit the growth of *bees* and *Candida albicans* that inhabit the oral cavity. Many strains have the ability to eliminate harmful bacteria found in saliva and dental plaque. So far, eight random researches have been carried out and in as many as 75% of the treatment with probiotics (mainly *Bifidobacterium* spp. and *Lactobacillus* spp.) proved effective in the fight against caries and in improving the general condition of the oral cavity [19–22].

There is also a connection between the stimulants used and the oral microbiome. Smoking changes the microbiological properties of the mouth by reducing the population of commensal microbes and increasing the number of pathogenic microorganisms. In 2019, the differences between the salivary microbiome of smokers and non-smokers were examined. Six types of bacteria - *Streptococcus*, *Vellionella*, *Neisseria*, *Prevotella*, *Rothia* and *Haemophilus* - definitely dominated (to varying degrees) the salivary microflora of all samples, which scientists perceive as being able to restore the microbiome in saliva after quitting smoking. Three types - *Prevotella*, *Streptococcus* and *Veillonella* - showed significantly elevated levels among smokers at the expense of *Neisseria* in non-smokers.

Thus, it has been shown that smoking has a definite impact on the salivary microflora changes in smokers. It can therefore be used to distinguish between smokers and non-smokers, which can be useful in many fields of science, in particular in criminology [23–25].

Without focusing only on one factor, scientists began to consider the issue of mouth microbiome globally. The teeth of different individuals show different susceptibility to caries, gingivitis and periodontitis. Knowing that dental diseases arise mainly as a result of changes in the structure, composition or function of the colonies of microorganisms inhabiting the oral cavity, studies of these aspects emphasize the usefulness of considering the impact of the relative effects of geographical, environmental (e.g. size, tooth shape, morphology) and host-specific (demographic, genetic, behavioral, socio-economic) on the structure and function of oral microorganisms. Understanding certain patterns not only of the composition of the microbiome of the oral cavity but also its correlation with other factors, will allow a global view on the problem of oral cavity problems and will significantly facilitate both the diagnosis and treatment of individual diseases or even prevent them in time [26–28].

When examining the oral microbiome, one should not forget about the necessary tool which is the Human Oral Microbes Database (HOMD). Its main purpose is to provide key information on the dominant bacterial species inhabiting the oral cavity. This widely developed database combines information on phylogenetics, phenotype, clinical research and bibliography regarding this microbiome. Thanks to the available BLAST tools and technologies using 16S rDNA sequences, it is possible to quickly determine and identify the composition of the microbiome in a given sample, which makes this technique common and suitable for rapid development of the database and research in which it is used [29,30].

Cancer of oral cavity

In developing countries, oral cancer has been very common over many years. Due to the frequency and variety, it is not easy to treat and patients struggle with its effects for a long time. SCC or squamous cell carcinoma is histologically the most common and is usually caused by excessive alcohol consumption or the use of stimulants, mainly smoking tobacco [31,32]. Despite the relatively easy diagnosis at an early stage, we often observe extremely advanced cases of this cancer. Standard treatment is resection of the oral cavity and adjuvant treatment. By combining several treatment techniques (e.g. resection with chemotherapy or radiotherapy), recovery statistics have improved significantly, as has the quality of life of people with cancer. Squamous cell carcinoma (SCC) accounts for over 90% of all oral cancer cases. Other malignancies may arise from

epithelium, connective tissue, small salivary glands, lymphatic tissue and melanocytes or metastases from a distant tumor. SCC accounts for over 90% of registered cases of oral cancer. The remaining 10% are other cancers arising from connective tissue, epithelium, lymphoid tissue, small salivary glands and even melanocytes or metastases from a tumor not in close proximity to the mouth [33,34].

As mentioned above, oral self-monitoring is relatively simple, but despite this, patients sometimes present highly advanced cancer. That is why doctors pay special attention to neck and head examinations in situations where the patient has suspected oral cancer. Known techniques such as visual inspection, palpation allow you to characterize the tumor in terms of its location, extent to some extent, the presence of bone invasion or visible skin breakdown. There are currently many known methods for dealing with oral cancer, but most often these are surgical removal, followed by chemotherapy and radiation therapy. The choice of techniques depends on the attending physician who assesses how the tumor is located, what tissues it is located near, what structure it has etc. and how to remove it safely and most effectively. In patients with a high risk of local-regional relapses, adjuvant treatment is highly recommended. These patients most often had large primary tumors (pT3 or pT4), bulky nodal disease (pN2 or pN3) or tumor invasion around the lymphatic vessels and nerves [35–37].

Patients with SCCOC (SCC of the oral cavity) have a high risk of recurrence and the development of new primary cancers. When using comprehensive, annual head, neck and mouth examinations, the risk of developing recurrences is about 4-7%. Unfortunately, we are not yet in possession of effective chemoprevention and the only effective method of prevention is to control your lifestyle and frequent examination, not only superficial but also blood tests, hormone levels and the work of secretory glands located in the vicinity of the mouth [38–40].

At this time, it is impossible not to mention the COVID-19 pandemic. Despite the very unfavorable situation that has prevailed in many countries, we unexpectedly managed to get something valuable out of it. In countries affected by the pandemic, there have been drastic decreases in the performance of previously planned treatments for operations of patients infected with a virus in need of immediate care. A report was sent from Wuhan, China, that among 34 asymptomatic patients with scheduled operations, the ICU admission rate was 44% and the mortality rate was 20% [41]. There are many disputes about what steps to take for patients with mouth, head or neck cancers. One source claims that if the COVID test result is positive, surgery should be postponed due to its specificity - high-level exposure to aerosols from the patient. The situation is different when the test result is negative and the

operation would be a life-saving measure - then postponing it could definitely reduce the chances of the patient surviving from high to very low. However, there are also voices that unanimously call for not delaying the planned treatments, regardless of the result of the test for the presence of the virus [42,43]. An attempt was made to give guidance by Deo et al. to help oncological surgeons make critical surgical decisions. Currently, these guidelines are only considered in India. They suggest neoadjuvant chemotherapy, oral metronome therapy for locally advanced oral cancer or, ultimately, postponing surgery until the tumor progresses [44]. Metronomic therapy seems to be a great solution because it meets all the criteria: it prevents tumor progression, influences its regression and “buys time” ensuring patients’ operability awaiting surgery. It is also minimally toxic, home based and cost-effective. It inhibits tumor angiogenesis, stimulates the immune response of a weakened patient and induces tumor specific dormancy [45]. Therapy consists of oral methotrexate once weekly and celecoxib twice daily. The patient is assessed after 4 and 8 weeks of treatment along with comprehensive tests. Both preparations are easily available and affordable. Currently, 23 patients with locally advanced T4a tumors have sent therapy. After 8 weeks, complete response was observed in 2 patients - one with cT4aN0M0 lip cancer and the other with cT4aN0M0 central arc cancer. According to RECIST 1.1 (criteria for assessing response in solid tumors), stabilization was found in 7 patients, partial stabilization in 17 patients, and lack of response and disease progression in 3 patients. After treatment, deferred surgery was offered to 20 out of 23 patients, which is a high result [46]. Thanks to the method used, it has been proved that the use of metronome therapy allows to postpone the operation to a large extent without side effects, which in the future can not only contribute to not worsening the patient’s condition, but also to protect healthcare workers from the risk of infection.

Wound healing processes and using of exosomes

Undoubtedly, the topic that needs to be addressed is the excellent ability of the mouth to heal itself. The wound healing process results in the formation of scars. Wounds of mucous membranes usually heal much faster and more efficiently than wounds on the skin, additionally leaving minimal or zero scars. Healing stages are similar for skin and mucous membrane wounds. It is said, however, that wound healing in the mucosa is more like healing of fetal tissues due to rapid remodeling and minimal scarring. These wounds also show a lower inflammatory response with lower infiltration of macrophages, neutrophils and T lymphocytes, as well as reduced expression of the TGF- β 1 proinflammatory

matrix. It is an isoform of transforming growth factor b (TGF-b) that is activated in the wound healing process and promotes the recruitment of inflammatory cells. It is considered an important factor in the formation of scars due to the fact that it recruits fibroblasts that produce the collagen necessary for scarring. In turn, the TGF-b3 isoform with anti-fibrotic properties has increased expression in the wounds of the oral mucosa, if compared to that in skin wounds [47–50]. In a study by Erik William Evans, it was a surprising discovery that mucosal wounds showed a significant difference in the number of myofibroblasts compared to skin wounds, where there were significantly fewer. It is interesting that their presence usually contributes to contracture, and thus a more visible scar [51]. In this paragraph, exosomes also deserve attention, as their promising source may be tissues with high regenerative potential. Such tissue is undoubtedly the oral mucosa. This region is constantly exposed to numerous injuries, however it regenerates very efficiently and quickly. It could be assumed that this is due to the lack of immune mediators (such as osteoprotegerin and haptoglobin), fewer blood vessels, and the presence of profibrogenic mediators. Due to these and many other features of the oral cavity, one should look at the exosomes coming from it. These are vesicles excreted by many cell types, both into extracellular matrix and biological fluids. Exosomes belong to the group of extracellular vesicles (EV) and are separated from the surrounding environment by a lipid bilayer, just like the microbubbles in this group. Exosomes are heterogeneous sizes (from 40 to 120 nm), released by many types of cells, both into the extracellular space and biological fluids. About 30 years ago, immediately after their discovery, it was believed that their main role was the selective removal of unwanted components from cells. Current knowledge says that they can transport biological molecules in their original, intact and active form, including nucleic acids and proteins. This discovery makes it possible to claim that they are load-bearing elements that are part of the intercellular signaling pathways that are not fully understood [52–60]. Moreover, not only exosomes isolated from cells have extraordinary properties. Salivary exosomes have been identified as highly interesting nanoparticles. The mechanism and signaling pathway by which tumor-derived biomarkers can be transmitted through the systemic circulation to the oral cavity have been demonstrated. Hence, by analyzing the oral exosomes, we can learn about the occurrence of a tumor in the body that we did not know before. This forms the basis for research into the interaction of saliva, oral mucosa and systemic circulation, which, as it may turn out, have a close relationship and connecting pathways.

Conclusions

The above article indicates the aspects most widely described in the latest scientific publications of the last year. Each of them covers a different aspect of oral research, but seen globally, they create a comprehensive picture of biology and biotechnology concepts that are currently being explored. It is impossible to talk about all new research, because more and more of them are growing every day. Thanks to this, we can get to know the properties of the processes associated with the oral cavity better and on the basis of it, develop new scientific theories, design research, invent new therapies. We know more about human body than yesterday, but less than tomorrow.

Ethical approval

The conducted research is not related to either human or animal use.

Acknowledgements

Not applicable.

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Conflict of interest statement

The authors declare they have no conflict of interest.

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