Aspiration pneumonia is a lung disease caused by an uncontrolled aspiration of the stomach or oral cavity contents. It usually has a bacterial etiology, with the contribution of both aerobic and anaerobic bacteria. The aspiration pneumonia affects children and elderly individuals with dysphagia, or patients subjected to frequent inhalations or intubations during hospital treatment. These conditions favor the contact of bacteria with the lung tissue and lead to the infection. The excessive colonization of oral cavity with bacteria, viruses, or fungi, resulting from insufficient hygiene or oral diseases, including periodontal disease, can be a source of unwanted colonizers in the airways.

The fast development of diagnostic methods in recent years allowed to identify microorganisms that appear in lungs during various infectious diseases, including the aspiration pneumonia. Among numerous bacterial species identified in these infections, an anaerobic bacterium *Porphyromonas gingivalis* has been occasionally found – an inhabitant of periodontal tissue and the major causative agent of periodontal disease, often associated with other serious disorders such as the rheumatoid arthritis, various heart diseases and, perhaps, as has been recently suggested, the Alzheimer disease. Those findings have inspired the recent increasing interest in the presence and role of *P. gingivalis* in lung infections. Other colonizers of the oral cavity that have also been detected in lungs during the aspiration pneumonia are yeast-like fungi of the *Candida* genus. They can exist in various forms, commensal or pathogenic, depending on environmental conditions in the host, predominantly on the relative strength of the immune system. During the aspiration pneumonia, *Candida* yeasts have been occasionally detected in the invasive form, and their frequent presence in lung specimens has been usually considered to be an artifact of the sample withdrawal procedure. However, recent studies strongly support a hypothesis that candidal pathogenic potential can play an essential role in the infections caused by aspiration.

Recent studies indicate that many microbial infections are mixed multi-species infections, based on the formation of biofilms, that are a form of microbial co-existence, cooperative or competitive, at the invaded niche in the host. What is most important, within such mixed biofilm a so-called matrix is formed, composed of the microbial cell wall constituents or even cytosol-derived proteins that acquire here some additional, protective functions. Surrounded by the matrix, microorganisms generate favorable microenvironment, as has recently been demonstrated for *Candida albicans* that in the mixed biofilm can generate favorable conditions for anaerobic bacteria by decreasing the oxygen concentration to the level, low enough for the viability of *P. gingivalis* cells.

Notably, this form of microbial self-protection limits the effectiveness of antibacterial or antimycotic therapies, thus making the treatment of different infectious diseases, such as the aspiration pneumonia, more difficult. Standard antimicrobial therapies were usually developed against single-species infections or mixed-species but purely bacterial. The formation of protective biofilms by various microbial species implicates searching for novel therapeutic approaches based on the knowledge of protective and cooperative mechanisms as potential targets for future therapies. Hence, this research project aims at exploring how the components of biofilm matrix affect the host organism and whether the matrix composition is modified under the direct contact with the host as a way for the adaptation to the host environment. After defining these mechanisms, the influence of antibiotics and antimycotics on these interactions will be verified for the determination if such type of selective therapy can be successful or, in the opposite, will favor the excessive generation of the therapy-resistant pathogen.

For these purposes, in this project, it is planned to develop a tridimensional cellular model, with a similarity to the lung tissue in terms of intercellular contacts, signaling pathways, and gene expressions. This model will be used to characterize the development of mixed infection with biofilm formation, and to test the effect of antimicrobial therapy. The most interesting observations will be confirmed in a mouse model of aspiration pneumonia. Among the biofilm matrix constituents, special attention will be paid to yeast glucans and chitin, that can undergo changes under the influence of bacterial factors or enzymatic actions from the host side. Some protective role can also be exerted by extracellular bacterial DNA, while the importance of yeast non-coding DNA is not recognized at all, especially in terms of its influence on the host cells.

Moreover, the biofilm contains extracellular vesicles that bear several virulence factors – a significance of this type yeast-derived vesicles in the mixed infections also needs to be explored. The role of all above-specified matrix components will be verified in a context of the application of antibiotic- and antimycotic therapies and the promising combinations of selected drugs will be searched for. A measurable effect of the planned studies will be the recognition of the mechanisms of co-infections with the contribution of *Candida* yeasts in the aspiration pneumonia, and the indication of the mixed biofilm elements as potential targets for treatments. The proposed recognition of the effects of combined antibiotic therapy will enable to verify the current medical procedures used for treatments of the aspiration pneumonia and similar diseases.