Pulmonary fibrosis is an increasingly frequent pathology in the aging global population. Unfortunately, these disorders are characterized by a bad prognosis: currently, no effective treatment is known and the survival is dramatically low (3-5 years). Pulmonary fibrosis occurs in a number of respiratory diseases; unfortunately, the etiology of the majority is unknown. The exception is hypersensitivity pneumonitis (HP). The HP is caused by chronic exposure to organic dust (particles of plant, animal, or microbial origin) or inorganic (some metals). More than 200 antigens responsible for HP have already been identified and they can be found in the workplace, at home, and in recreational places. Consequently, HP is one of the most common causes of pulmonary fibrosis in the world. Repeated injuries of respiratory epithelium caused by the chronic exposure to antigens in susceptible subjects provoke a hypersensitivity immune reaction and to a deficiency of tissue repair, which includes the phenomenon of epithelial-mesenchymal transition (EMT - a process involving the transformation of epithelial cells into migratory cells with a mesenchymal phenotype). Recent studies have shown that in the case of pulmonary fibrosis the EMT and inflammation drive each other until the fibrotic process reaches the point where fibrosis cannot be attenuated by silencing the inflammation. As the main mechanism of pulmonary fibrosis in the course of HP is a pathology of repair of damaged epithelium due to chronic inflammation, we assume that effective therapeutic options should combine immune balance restoration with EMT inhibition. Based on literature data the biologically active form of vitamin D3 (calcitriol) seems to meet the indicated criteria perfectly, because 1) affects cells involved in lung immune responses to environmental antigens, and thus may regulate the development of pulmonary fibrosis in the course of HP; 2) modulates the expression of cathelicidin (immune peptide), which importance for pulmonary fibrosis was revealed by our research group; 3) inhibits EMT via induction of expression of genes responsible for the acquisition and maintenance of the epithelial phenotype. Furthermore, calcitriol regulates the expression of key EMT transcription factors.

The aim of the project is to assess the usefulness of vitamin D3 in the prevention and treatment of pulmonary fibrosis. In order to achieve the mentioned goal, the influence of vitamin D3 on lung tissue in normal and pathological conditions as well as the molecular mechanism of its action will be investigated in mice model of HP. Studies will be conducted using both 1,25(OH)$_2$-vitamin D3 (biologically active form) and 25(OH)-vitamin D3 (calcitriol precursor) delivered directly to the lungs via nebulization. Furthermore, pulmonary fibrosis development will be monitored in mice on a diet with the standard and reduced content of vitamin D3 (a reference to vitamin D3 deficiency, which is a common phenomenon in society).

The studies will be conducted in the mice model of HP, in which pulmonary fibrosis is induced in prone to fibrosis mice strain C57BL/6J by chronic exposure to *Pantoea agglomerans* extract (one of the most important etiological factors of HP). *P. agglomerans* antigen and vitamin D3 metabolites will be provided into the mice airways using an inhalation tower. The research groups were designed to bring the answers to the following questions: What is the impact of vitamin D3 content in the diet on lung fibrosis development in mice model of HP? What is the influence of inhaled vitamin D3 metabolites on lung tissue in physiological and pathological conditions? Do inhaled vitamin D3 metabolites prevent or inhibit the development of pulmonary fibrosis in mice model of HP? Answers for the abovementioned questions will reveal the results of mice respiratory parameters analysis as well as results of the assessment of collected blood and lung tissue samples. Lung tissue samples will be subjected to the following analysis: ● histological examination regarding the presence of inflammatory and fibrotic lesions; ● measurement of fibrosis markers; ● analysis of lung cells subpopulations involved in the immune response to an antigen; ● measurement of the levels of cathelicidin, cytokines and grow factors taking part in fibrosis development; ● analysis of expression of both genes and proteins involved in EMT. Furthermore, collected blood samples will be used to determine the endogenous level of 1,25(OH)$_2$-vitamin D3, which is necessary for the proper interpretation of the remaining test results.

Due to unique properties, vitamin D3 and its derivatives have become a target of new therapeutic approaches including the use of chronic lung diseases, especially as an immunomodulating and antimicrobial agent. However, according to our best knowledge, the presented project is the first approach to use vitamin D3 in the prevention and treatment of pulmonary fibrosis in the course of HP. To minimize the possibility of systemic toxicity, vitamin D3 will be delivered directly to the lungs via nebulization, which is also a novel approach as well as the predicted mode of its delivery in the human pulmonary fibrosis treatment. The final effects of the project will be: confirm or exclude the possibility of vitamin D3 using in pulmonary fibrosis prevention and/or treatment; determination of the effect of vitamin D3 (present in food as well as its metabolites delivered by nebulization) on lung tissue under physiological and pathological conditions; enable increased the knowledge and understanding the molecular mechanism of vitamin D3 action during HP development with particular emphasis on its impact on EMT phenomena, immune responses, and endogenous cathelicidin level. Positive results may become an excellent starting point for clinical trials and perhaps a milestone in the development of an effective and safe strategy of pulmonary fibrosis prevention and/or treatment.