Periodontitis is a chronic inflammatory disease that, if untreated, can lead to irreversible periodontal tissue degradation and tooth loss. In 2021, more than one billion people worldwide suffered from periodontitis, while in Poland, over 60% of the adult population experienced this disease. Untreated periodontitis can result in severe complications, such as Alzheimer's disease, rheumatoid arthritis, diabetes, and cardiovascular diseases. The pathogenesis of periodontitis is associated with dysbiosis of the bacterial biofilm present in dental plaque. Key pathogens involved in the disease progression include *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Filifactor alocis*, which contribute directly or indirectly to periodontitis pathogenesis. In response to infection, the host immune system exhibits limited efficacy in pathogen elimination, leading to chronic inflammation. Elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF), IL-1β, and IL-6, have been detected in the periodontal tissues of individuals affected by periodontitis. Gingival fibroblasts (GFs) constitute one of the most abundant cell populations in gingival connective tissue. Traditionally, their primary function has been considered to involve extracellular matrix synthesis and remodeling. However, recent studies suggest that under pathological conditions such as periodontitis, GFs can produce multiple inflammatory mediators, thereby modulating immune responses. Previous studies have focused on exposing cells to single factors, such as bacteria or their virulence factors, which does not fully reflect the complexity of the microenvironment in which GFs reside in the gingival tissue of periodontitis patients. Therefore, the aim of this study was to investigate how the interaction between pathogenic bacteria present in dysbiotic biofilm and the inflammatory milieu affects the biological functions of GFs.

In the first part of the study, the response of GFs to oral pathogen infection was analyzed in the presence of TNF, one of the key pro-inflammatory cytokines in periodontitis pathogenesis. It was found that infection of GFs with different *P. gingivalis* strains in the presence of TNF led to a synergistic increase in the production of pro-inflammatory cytokines, including IL-6 and IL-8, compared to cells treated with a single factor. Moreover, *P. gingivalis* virulence factors, such as *P. gingivalis* peptidylarginine deiminase (PPAD), FimA, and gingipains, played a crucial role in inflammatory activation of GFs in the presence of TNF. Transcriptomic analysis of cells infected with *P. gingivalis* in the presence of TNF revealed upregulated expression of genes involved in inflammatory processes, including *CXCL-1*, *CXCL-2*, *CXCL-3*, and *CCL-2*. Additionally, GFs infected with *F. nucleatum* or *F. alocis* in the presence of TNF also produced increased amounts of IL-6 and IL-8. Interestingly, other cells present in periodontal tissue, such as macrophages and gingival keratinocytes, did not exhibit a synergistic increase in inflammatory mediator production in response to oral pathogens in the presence of cytokines. Further studies demonstrated that in the presence of TNF, toll-like receptor-2 (TLR-2) plays a key role in the GF response to *P. gingivalis* infection, whereas both TLR-2 and TLR-4 are involved in cell response to *F. nucleatum* infection. The three-dimensional gingiva model used in this study confirmed that GFs, rather than keratinocytes, are responsible for the intense production of pro-inflammatory cytokines in the analyzed model. These findings suggest that GFs may play a significant role in the accumulation of inflammatory mediators in gingival tissue in periodontitis patients.

Osteoclasts are responsible for bone matrix degradation, and their excessive activation in periodontitis leads to alveolar bone resorption. The role of GFs in osteoclastogenesis remains unclear; therefore, the second part of the study focused on elucidating this aspect. It was found that conditioned media from GFs, regardless of prior stimulation or infection, inhibited osteoclastogenesis induced by receptor activator for nuclear factor-κB ligand (RANKL) in CD14-positive cells. A high concentration of osteoprotegerin (OPG) was observed in the supernatants produced by GFs, suggesting that these cells may regulate osteoclastogenesis by inh0ibiting RANKL-RANK interaction. A comparison of OPG concentrations produced by GFs from healthy donors and periodontitis patients revealed that cells from disease-affected individuals produced lower amounts of this cytokine. Additionally, conditioned media from GFs infected with pathogens and stimulated with TNF contained high levels of cytokines promoting osteoclastogenesis, such as IL-6. This suggests that under physiological conditions, GFs may exert a protective effect on bone tissue, whereas in periodontitis, they may be less effective in preserving the bone matrix and potentially contribute to its degradation.

In the third part of the study, the role of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE-2) in periodontitis progression was analyzed. PGE-2 is a crucial inflammatory mediator, and its levels are elevated in periodontal patients. It was demonstrated that GFs infected with oral pathogens and stimulated with TNF synergistically increased the production of COX-2 and PGE-2. Similar results were obtained following infection of GFs with *F. nucleatum* and stimulation with cytokines IFNα, IL-1α, and IL-1β. Furthermore, p38 kinase was shown to play a key role in the synergistic induction of COX-2 expression in GFs. Additional experiments revealed that PGE-2 present in conditioned media from GFs stimulated with TNF in the presence of *F. nucleatum* was responsible for inducing *IL-8* and *IL-10* expression in macrophages. This finding suggests that the synergistic induction of PGE-2 by oral pathogens in the presence of cytokines plays a significant role in GF interactions with immune cells, potentially modulating the inflammatory response in periodontitis.

In conclusion, this study describes a novel mechanism regulating GF activation: the synergistic induction of inflammatory mediator production by GFs in response to oral pathogen infection in an inflammatory environment. The impact of GFs on osteoclastogenesis was analyzed, and the functional consequences of their synergistic activation by bacteria and cytokines were demonstrated. These findings identify processes through which GFs may modulate inflammatory progression in periodontitis. The results not only expand our understanding of periodontitis pathophysiology but may also contribute to the identification of new therapeutic targets, such as p38 kinase, which could be utilized in developing host-modulating treatment strategies for periodontitis.