Periodontitis is a chronic inflammatory disease of periodontium and most recent WHO data indicates periodontitis affects 19% of the population worldwide that in its severe form. If untreated, it leads to irreversible tissue damage and eventual tooth loss. Periodontitis is caused by microbial imbalance and the anaerobic bacterium *Porphyromonas gingivalis* plays a key role in driving chronic inflammation due to its virulence and ability to evade the host immune system. The interaction between the periodontium's structural cells and oral pathogens plays an important role in disease progression. Gingival fibroblasts (GFs) are the most abundant resident cells in gingiva and if activated, they can display immune functionalities e.g. by promoting neutrophil infiltration. Recent reports show that targeting epigenetic mechanisms can revert pathological changes observed in periodontitis. However, limited data exist describing the epigenetic background of interaction between *P. gingivalis* and/or inflammatory environment and GFs.

The first aim of this study was to investigate the role of histone acetylation in the inflammatory activation of GFs. Histone acetylation is one of epigenetic processes which is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetylation leads to active transcription, whereas histone deacetylation is responsible for silencing of gene expression. However, recent studies using HDAC inhibitors (HDACi) showed that this regulation is more complex and more histone post-translational modifications are necessary to initiate transcription. In the present study, HDACi were used to test whether pharmacological modulation of the acetylation system can revert pathological changes caused by the presence of bacteria and inflammatory mediators. Application of pan-HDACi (SAHA, ITF2357) and HDAC3/6i, but not HDAC1, HADC6 nor HDAC8 inhibitors decreased inflammatory response of GFs to *P. gingivalis* and cytokines. This result suggested that HDAC3 might be responsible for GF inflammatory activation. Silencing of HDAC3 expression by transfecting cells with siRNA mostly mimicked the effects of HDAC3/6i on inflammatory gene expression in *P. gingivalis*-infected GFs. In contrast, HDAC inhibition has no effect on bacteria internalization and intracellular survival. Also, the anti-inflammatory effects of HDACi were not related to the induction of MAPK (mitogen activated protein kinase) or NFκB (nuclear factor κB) signaling pathways. Collectively, these studies allowed for the identification of HDAC3 as an important regulator of inflammatory gene expression and suggested that targeting HDAC activity may be clinically beneficial in suppressing inflammation in periodontitis.

The second part of the project was focused on studying the role of DNA methylation in GFs in the context of periodontal disease pathology. DNA methylation is another epigenetic

mechanism which leads to silencing of gene expression. It is catalyzed by DNA methyltransferases (DNMTs) which are the enzymes responsible for adding methyl groups to cytosines. The latest studies indicate that inhibiting the activity of DNMTs may have a protective effect on inflammation and bone resorption in periodontitis. Therefore, to assess the impact of DNMT inhibitors (DNMTi) on GF biological functions and inflammatory response, cells were treated with the DNMT1-specific inhibitor decitabine (5-aza-2'-deoxycitidine) for 12 days to induce DNA hypomethylation. RNA sequencing (RNA-seq) data showed that decitabine upregulated genes associated with inflammatory response, whereas genes associated with ECM and collagen fibril organization pathways were downregulated. Thus, these results showed that decitabine induced an inflammatory, periodontitis-like phenotype of GFs. Functional studies of inflammatory mediator expression confirmed the RNA-seq results. Additionally, decitabine induced ICAM1 (intercellular adhesion molecule 1) expression and increased *P. gingivalis* adherence to GFs, which may facilitate bacteria dissemination. Further studies also showed that decitabine reduced GF proliferation and had a cytotoxic effect on cells. The detrimental effects of decitabine were also confirmed in another cell type of the periodontium, namely periodontal ligament fibroblasts (PDLFs). Because decitabine is highly unstable, more studies were conducted to assess if the toxic products released during decitabine degradation may contribute to the observed effects. The results demonstrated that the products of decitabine degradation had a negligible influence on GF biological functions and inflammatory activation, indicating that the observed effects are a consequence of the hypomethylating action of the inhibitor. To sum up, this part of the PhD project revealed the limited therapeutic potential of decitabine due to its detrimental effects on GF and PDLF biological functions and stimulation of pro-inflammatory pathways. However, it is an excellent tool to study the role of DNA hypomethylation in gingival cells.

The third goal of the study was to optimize the model of extended GF *P. gingivalis* infection which would mimic the physiologic conditions observed in periodontal disease. In this model GFs were pre-infected with *P. gingivalis* for 24 h, rested for a few days after removal of bacteria, and then subjected to stimulation with TNF (tumor necrosis factor) for up to 24 h at 6^{th} day of the experiment. Bacterial challenge sensitized GFs to subsequent inflammatory stimulation and caused elevated secretion of cytokines (IL-6, IL-8), which was not proliferation-dependent. Additionally, analysis of the intracellular level of IL-6 by flow cytometry confirmed that result showing that GFs display "immune memory" of a previous infection that lasts for several days. Since epigenetic changes have been indicated as underlying mechanisms of the "innate immune

memory", the alterations in DNA methylation status were assessed. However, *P. gingivalis* infection did not cause any significant lasting changes in global or local DNA methylation levels as confirmed by reduced representation bisulfite sequencing (RRBS). However, further investigations showed an increase in p65 phosphorylation level upon TNF stimulation in pre-infected GFs. This result suggests a possible role for NFκB signaling in the observed long-term effects of infection on GF inflammatory activation. Determining the full scope and consequences of this phenomenon and confirming the exact role of the NFκB pathway requires further research.