Chapter 7:

General Abstract
*P. gingivalis* is a Gram-negative, anaerobic bacterium associated with peri-implantitis. *P. gingivalis* induces inflammatory and immunological responses in host cells which are aimed at eliminating the infection and restoring tissue homeostasis. In susceptible hosts, interaction of *P. gingivalis* with host cells may result in aberrant inflammatory responses which lead to inflammation and tissue destruction around the dental implant. Titanium dental implants under occlusal stresses can undergo corrosion and wear. As a result, titanium wear particles (TiO$_2$) are present in the peri-implant soft tissues. However, influence of TiO$_2$-particles to host-pathogen interaction in peri-implantitis is largely unknown. Fibroblasts are important host cells, as they not only provide structural support to the implant but also take part in inflammatory host reactions to external stimuli. In the current thesis, we aimed to study the role of interaction between fibroblasts, viable *P. gingivalis* and titanium particles in the pathogenesis of peri-implantitis. In addition, effects of non-surgical peri-implantitis treatment on the clinical and microbiological parameters were studied.

We found that viable *P. gingivalis* can invade gingival fibroblasts *in vitro* and the non-encapsulated *P. gingivalis* is more efficient in invading gingival fibroblasts. It has been suggested that the predominant mode of encapsulated *P. gingivalis* strains to evade host defenses is blunting the host immune responses; the non-encapsulated strains may evade the host defenses by invading host cells. Interestingly, even high concentrations of antibiotics were not able to completely eradicate the *P. gingivalis* internalized into gingival fibroblasts. This may imply that internalization of *P. gingivalis* into fibroblasts protects it from antibiotics to which it is otherwise susceptible. We speculate that, internalized *P. gingivalis* may potentially act as a reservoir for future re-infection of peri-implantitis and periodontitis sites. Therefore, internalization of *P.
*P. gingivalis* into gingival fibroblasts may contribute significantly to the pathogenesis of peri-implantitis.

We also studied gene expression and protein production of selected pro-inflammatory cytokines and matrix-metalloproteinases in *P. gingivalis*–fibroblast interaction. Peri-implant granulation tissue fibroblasts from peri-implantitis patients (PIGFs) were challenged with viable *P. gingivalis* and compared with gingival fibroblasts from periodontitis patients (PGFs) and periodontally healthy controls (HGFs). Our results indicate that before the *P. gingivalis* challenge, interleukin (IL)-1β, IL-8 and monocyte chemotactic protein (MCP)-1 are expressed at higher levels in PIGFs and PGFs compared to HGFs. After the *P. gingivalis* challenge, significant up-regulation was observed in the gene expression of IL-1β, IL-6, IL-8, MCP-1 and MMP-1 in PIGFs and PGFs but not in HGFs. Interestingly, the *P. gingivalis* challenge down-regulated MMP-8 expression in PIGFs. In addition, PIGFs sustained higher induction of IL-1β, MCP-1 and MMP-1 compared to PGFs, after the removal of *P. gingivalis*. Our results indicate that non-challenged fibroblasts from peri-implantitis and periodontitis lesions are in a pro-inflammatory state and give higher pro-inflammatory responses when challenged with *P. gingivalis*. In addition, PIGFs may play a role in the pathogenesis of peri-implantitis by sustaining inflammation in the peri-implant tissues.

We studied the effects of TiO₂-particles alone and in combination with *P. gingivalis* on the inflammatory reactions in PIGFs. Despite the biocompatibility properties of titanium, our results suggest that TiO₂-particles can induce pro-inflammatory reactions in PIGFs. A challenge with *P. gingivalis* alone elicited pro-inflammatory reactions in PIGFs. Interestingly, a combined challenge with TiO₂-particles and *P. gingivalis* caused a stronger increase in the gene and protein expression of TNF-α and MCP-1 when compared to a challenge with *P. gingivalis* alone. Our
findings may implicate that *P. gingivalis* infection in combination with the presence of titanium wear particles has the potential to enhance inflammation associated with peri-implantitis.

Peri-implantitis is treated non-surgically as well as surgically in contemporary dental practice. Non-surgical treatment of peri-implantitis mainly consists of mechanical debridement of the implant surface with/without the use of local and/or systemic antibacterial agents. However, the effects of non-surgical treatment of peri-implantitis on the clinical outcome have not been thoroughly studied. We retrospectively studied the effects of non-surgical peri-implantitis treatment with or without the use of systemic antibiotics, on the clinical and microbiological parameters of peri-implantitis. Regardless of systemic antibiotics use, non-surgical peri-implantitis treatment was effective in improving clinical parameters such as bleeding on probing (BoP), mucosal recession (MR) and peri-implant pocket probing depth (PPD), at three months evaluation when compared to baseline. Use of systemic antibiotics showed additional value in improving BoP and MR around the implants. However, use of systemic antibiotics did not significantly affect the presence or proportions of target bacteria in submucosal plaque. 47.5% implants did not need surgery three months after non-surgical peri-implantitis treatment, regardless of antibiotic use. These findings indicate that non-surgical peri-implantitis treatment is effective in reducing peri-implant inflammation.

In conclusion, research presented in the current thesis indicates that non-encapsulated *P. gingivalis* can evade host defenses by internalization into gingival fibroblasts and that internalized *P. gingivalis* can survive antibiotic treatment *in vitro*. Further, *P. gingivalis* may enhance and sustain inflammation and facilitate matrix breakdown by means of interaction with fibroblasts from peri-implantitis and periodontitis lesions. Moreover, peri-implantitis fibroblasts react to titanium wear particles by releasing pro-inflammatory cytokines and this effect is
increased in presence of *P. gingivalis*. Therefore, *P. gingivalis*-fibroblast-titanium interaction may significantly contribute to the pathogenesis of peri-implantitis. Lastly, non-surgical treatment of peri-implantitis is effective in reducing inflammation in peri-implantitis.