In **Chapter 1** a general introduction on the topics addressed in this thesis, a brief summary on previous introductory studies, and an outline of this thesis are given. The general aim of this thesis is to investigate the effects of irradiation on the oral tissues, more specifically on the oral mucosa. In this respect the microstructure of the superficial cells of the oral mucosa are investigated. The irradiation-induced changes of the MPL structure in a canine model and in human subjects are described.

In **Chapter 2** a review of the literature on the microplicae (MPL) structure and its function was conducted in order to form the basis for the studies described in this thesis. The correlation between the MPL structure of oral epithelial cells and the salivary layer is hypothesized. The surface structure of the superficial cells of the oral mucosa is decorated with numerous membrane ridges, termed MPL. The MPL structure is typical of the epithelial cell surfaces that are covered with protective mucus. The interaction between MPL and the mucins has been demonstrated. However, the role of the MPL structure seen on the outer surface of the oral epithelial cells is speculative.

In **Chapter 3** a study on the defence mechanism of the superficial epithelial cells is presented. The surface of superficial cells of the oral epithelium contains ridge-like folds, the so-called MPL, which are typical of the surfaces of areas covered with protective mucus. The salivary mucus gel is part of a protective diffusion membrane against harmful substances. This membrane is built up with epithelial cells covered by a highly hydrated and viscous gel, where mucins constitute the scaffold. The salivary mucous barrier is required to protect the superficial cells. The MPL structure together with membrane anchored mucin binding protein (MBP) forms the basis for this mucous barrier.

In **Chapter 4** the results of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) on oral mucosal specimens from 32 healthy patients are described in order to classify different morphology patterns of the superficial oral mucosal cells in different locations of the oral cavity. Three main surface patterns of the oral mucosal epithelial cells could be recognised, namely parallel, branched and pitted appearance.

It is concluded from this study that the MPL structure is a determining factor for the functionality of the oral epithelium. A systemic analysis could be used to describe different disorders of the oral mucosa and to discriminate progressive stages of pathological processes.
In Chapter 5 the radiation-induced changes in the superficial cells of the oral mucosa in an experimental study in ten Beagle dogs are described. In particular, the morphological characteristics of the cell surface structure after radiotherapy (RT) are described. With radiation doses of 40 and 50 Gy, discontinuous and short MPL were the typical cell structures in irradiated oral mucosa. In the 50 Gy study group the surface structure of the epithelial cells was pitted. It is concluded from this study that irradiation disrupts superficial cells of the oral mucosa. The role of the MPL structure of the superficial cells in the development of mucositis is discussed.

In Chapter 6 the results of a light and transmission electron microscopic study on oral mucosal specimens from 6 healthy patients are presented. The ultrastructural morphology of the cell membrane of superficial cells of the oral mucosa and the membrane-associated mucins (MAMs), MUC1 and MUC4, are described. The novelty of this study is that the membrane-tethered molecules seem to occur onto the cell membrane of the superficial epithelial cells of the oral mucosa. Furthermore, the stratified squamous epithelium of the buccal mucosa produce MUC1 for the surface-saliva pellicle interface. The interaction between MPL structure, MUC1 mucin and salivary mucosal pellicle is discussed.

In Chapter 7 the effects of ionizing radiation on the cell microstructure of the human oral mucosa are described. Tissue samples from 91 patients were collected during dental implant surgery or ablative surgery. These 91 patients were divided in 4 subgroups. Group 1 consisted of 28 patients who underwent dental implant surgery after RT. Of these 28 patients, two patients developed osteoradionecrosis (ORN) postoperatively. Group 2 consisted of five patients who developed ORN after tumor surgery and postoperative radiotherapy; they did not receive dental implants. Group 3 consisted of eight oral cancer patients, who underwent ablative surgery without radiotherapy. Group 4 consisted of 50 clinically healthy, non-irradiated subjects, who served as controls. The samples were studied with SEM and quantitative pixel region analysis was performed with the ImageJ image analysis software. Radiation therapy induced breakage and destruction in the MPL morphology and decreased the density of the MPL surface structures of superficial oral epithelial cells. In some of the irradiated cells the MPL were completely vanished, especially in patients who developed ORN. In non-irradiated tissue the MPL of the superficial epithelial cells were intact in all cases (non-irradiated oral cancer patients and healthy controls).
Conclusions, considerations and directives for future research

A topic addressed in the introduction, but not investigated in this thesis, is: what is the cause of dental implant failure in irradiated cases after several years of proper function? In future studies it could possibly be hypothesized that the oral epithelial cells lose their MPL in a progressive way and consequently lose their pellicle, eventually leading to a deficient defence system. It could then be a matter of time before the peri-implant defence system gives up. When the blood supply of bone and covering soft-tissues has been damaged by former radiation, the bacterial “attack” cannot be properly managed and the infection may proceed along the transmucosal part of the dental implant. The next phase will most likely be implant loss and possibly osteomyelitis (ORN). It is unclear why some irradiated patients suffer from dental implant loss or ORN and others do not. The cell morphology of the superficial oral mucosa, specifically the absence of MPL, might be the clue to predict possible problems or safe conditions for future dental implant indications.

Another question that can be raised, is whether irradiated oral mucosal cells are capable of regeneration. Can superficial epithelial cells that are damaged or lost their MPL in time, be replaced by new (healthier) cells that do show a normal MPL structure again? This does not seem to be the case, since dental implant failures usually occur several years (4-5 years) after implant surgery. One could ask whether replacing the irradiated mandibular mucosa surrounding of (future) dental implants by keratinized mucosa from the palate (which should be far less irradiated than the mandibular mucosa) could be an advantageous surgical procedure, delivering mucosal epithelial cells with a normal surface morphology (with intact MPL). Furthermore, would a damaged surrounding mucosal bed influence negatively the transplanted healthy epithelium and make it degenerate the MPL structure as well?

An important result from the studies described in this thesis is that the superficial oral epithelial cells show deterioration of the MPL structure after RT. The degree of damage to the MPL structure depends on the irradiation dose and may define the risk of dental implant loss and the occurrence of ORN. Pre-implant diagnosis might include a mucosal biopsy for decision-making whether dental implants can be predictably placed or not.

Future research could cover the potential clinical significance of the MPL-glycocalyx/mucin complex in elucidating the pathogenesis in poorly understood oral disorders, such as dry mouth syndrome, Sjögren’s disease (mismatch of tear/salivary flow and perceived dryness), burning mouth syndrome and radiotherapy-related complications (dry mouth complaints in RT-patients that are also seen in relatively normal salivary flow), radiation caries, radiation-induced periodontitis and ORN.