Summary

INNATE IMMUNE REACTIVITY TO DENTAL ALLOYS
Oral exposure to metals and metal alloys is frequently associated with local and systemic adverse reactions. The studies presented in this thesis shed a new light on the mechanisms by which dentally applied metals can cause irritation, inflammation or allergy. In addition the question is addressed why some metals, such as nickel, are known as strong sensitizers, while others seldom cause allergy. Overall, this thesis contributes to the understanding why metal-sensitivity occurs so frequently and it helps the researcher, dentist and patient to become more aware of the (potential) health effects of dental alloys. This data might also be useful for dentists to select alloys which have minimal immune stimulatory capacity. In addition, the results can contribute to a rational basis for future guidelines on the use of metal alloys in the oral cavity. Below, a summary of the chapters is given and the most relevant findings are highlighted.

In chapter 1 six major questions on the relation between metal exposure and inflammation or allergy are selected and introduced by evaluating the present knowledge on the immune events that can lead to allergy. It is explained how the activation of innate immune cells by metals, in casu dendritic cells (DC), can induce inflammation and eventually adaptive immune responses to these metals. To become activated, DC receive ‘danger signals’, that are normally given by micro-organisms but can be mimicked by metals, such as nickel. Important receptors for such ‘danger signals’ are the so called toll like receptors (TLR), that are expressed on DC and other innate immune cells..

The results of chapter 2 show that, like nickel, also cobalt and palladium strongly activate DC in vitro, as shown by the production of IL-8, an important inflammatory mediator. Copper and zinc, but not iron and chromium, induced low but significant innate activation. As shown in experiments with different TLR transfected cell lines next to nickel, cobalt and palladium could trigger the cells via TLR4 ligation, the receptor for bacterial endotoxin.

In chapter 3 also the high molecular weight transition metals gold and mercury were tested for their innate stimulatory capacity. Gold and, to a lesser extent, mercury caused distinct DC activation and maturation. Both metal salts induced IL-8 production by DC as well as by the cell line THP-1, although to a lower extent than we had found for nickel, cobalt and palladium. Importantly, when studying gold induced responsiveness in the TLR transfected cell lines, TLR3 rather than TLR4 ligation was shown to be involved. TLR3 is the (intracellular) receptor for dsRNA associated with viral infection. The nature of the low-level innate response to mercury remains to be clarified.

Chapter 4 describes the direct stimulatory effects of gold, mercury, copper and nickel salts on keratinocytes (KC). First we observed that human KC as well as skin or gingiva derived KC cell lines express functional TLR3, but not TLR4, 5, 7/8 or 9. Indeed, gold induced robust IL-8 production by KC. Of note, also mercury, copper and nickel did activate KC. Whereas
these findings confirm our hypothesis that gold triggers TLR3, the mechanism(s) by which mercury, copper and nickel trigger KC still remain to be elucidated.

In chapter 5 we tested dental cast alloys as solid specimens, reflecting the actual situation in the oral cavity, in DC and THP-1 cell cultures. The results fit with our earlier findings that most metals used for dental alloys show innate stimulatory activity. Importantly, the latter finding indicates that such stimulatory activity can be observed at low metal concentrations such as released from alloys in regular media. Of note, strongest and consistent IL-8 release was found with gold and palladium-copper containing alloys. In the presence of bacterial endotoxin, the exposure to these alloys, as well as to the 24 hrs supernatants of them, resulted in even stronger innate stimulation, suggesting a synergy between the metal exposure and endotoxin. The use of actual dental cast alloys in in vitro studies, instead of metal salt solutions, provides an effective strategy to study potential immune stimulatory effects of orally applied metals.

In chapter 6 we investigated whether metal exposure can activate brain microglia, thereby providing a potential clue to the development of neuro-degenerative disease. The results confirmed that microglia can be triggered by metal salts, although at concentrations which may not readily be seen in situ. Nevertheless, also microglial cells reveal a strong synergy between exposure to metals, in particular to copper and zinc, and microbial endotoxin, indicating that such unfortunate coinciding activities may contribute to or augment chronic inflammation and neurotoxicity in humans.

Chapter 7 focused on clinical and serological parameters for autoimmune disease (AID) in relation to oral metal exposure in non-allergic and metal allergic individuals, as well as in patients with oral lesions attributed to dental restorations. Metal exposure, anamnesis and immune responsiveness to metals were evaluated extensively. The results of this study support the view that oral exposure to palladium, gold and mercury does not facilitate the development of AID. Surprisingly, in this limited group, we found a correlation between oral exposure to nickel and the presence of clinical autoimmune disease. Therefore, further investigations into a possible role of transition metals, in particular nickel, in the development of autoimmune disease are warranted.

In chapter 8 the six major questions on the relation between oral metal exposure and inflammation, allergy, neurotoxicity and autoimmune disease have been answered and discussed in the light of the results of this thesis.

In short, the use of metal alloys in dentistry as well as in numerous other applications will never be avoided. Metal alloys cannot always be replaced by other materials such as methacrylates since their characteristics are essential for distinct clinical requirements.
Results from this study indicate that dental metal alloys may initiate local and systemic immune reactivities. Still, we should emphasize that careful production of dental metal alloys and appropriate clinical monitoring all contribute to their safe use in oral applications.