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BIOCOMPATIBILITY OF DENTAL CASTING ALLOYS

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ABSTRACT: Most cast dental restorations are made from alloys or commercially pure titanium (cpTi). Many orthodontic appliances are also fabricated from metallic materials. It has been documented *in vitro* and *in vivo* that metallic dental devices release metal ions, mainly due to corrosion. Those metallic components may be locally and systemically distributed and could play a role in the etiology of oral and systemic pathological conditions. The quality and quantity of the released cations depend upon the type of alloy and various corrosion parameters. No general correlation has been observed between alloy nobility and corrosion. However, it has been documented that some Ni-based alloys, such as beryllium-containing Ni alloys, exhibit increased corrosion, specifically at low pH. Further, microparticles are abraded from metallic restorations due to wear. In sufficient quantities, released metal ions—particularly Cu, Ni, Be, and abraded microparticles—can also induce inflammation of the adjacent periodontal tissues and the oral mucosa. While there is also some *in vitro* evidence that the immune response can be altered by various metal ions, the role of these ions in oral inflammatory diseases such as gingivitis and periodontitis is unknown. Allergic reactions due to metallic dental restorations have been documented. Ni has especially been identified as being highly allergenic. Interestingly, from 34% to 65.5% of the patients who are allergic to Ni are also allergic to Pd. Further, Pd allergy always occurrs with Ni sensitivity. In contrast, no study has been published which supports the hypothesis that dental metallic materials are mutagenic/genotoxic or might be a carcinogenic hazard to man. Taken together, very contradictory data have been documented regarding the local and systemic effects of dental casting alloys and metallic ions released from them. Therefore, it is of critical importance to elucidate the release of cations from metallic dental restorations in the oral environment and to determine the biological interactions of released metal components with oral and systemic tissues.

Key words. Dental alloys, metals, corrosion, biocompatibility, allergy, occupational hazards.

(I) Introduction

Casting alloys and commercially pure titanium (cpTi) are widely used in modern dentistry for temporary and permanent restorations, splints, and removable or fixed orthodontic appliances. Previous reports indicate that these dental metallic devices may cause local tissue responses, such as gingivitis/periodontitis, in the oral cavity (Wirz, 1993; Schmalz, 1999). It has been hypothesized that the severity of these alterations significantly depends upon the metal and its chemical state and concentration (Schmalz *et al*., 1997a,b; Yamamoto *et al*., 1998).

Metallic components and microparticles from cast metal restorations have been identified in adjacent plaque and gingival tissues (Rechmann, 1993, 1994; Rechmann *et al*., 1994). High-noble alloys were found to be more resistant to corrosion than noble alloys. Interestingly, most local adverse effects, such as the "burning mouth syndrome", were observed when noble and base alloys were combined (Schmalz, 1999). It has also been documented that metal ions, which are released from restorations by corrosion, can penetrate dental hard tissues (Söremark *et al*., 1968; Kratzenstein *et al*., 1986, 1988).

Cations are systemically distributed from metallic restorations shortly after insertion. It has been found that the urinary platinum concentration significantly increased immediately after the insertion of crowns, bridges, or telescopic restorations fabricated of high-noble alloys. Three months after cementation, the urinary Pt concentration was still significantly elevated. But no increased urine levels for gold or palladium were found (Begerow *et al*., 1999a; Hugger *et al*., 2000). High concentrations of Ni and Cr were released from surgical implants and systemically distributed (Traisnel *et al*., 1990).

Taken together, these findings indicate that metallic components may leach from a metallic cast restoration or appliance into the oral cavity, and may subsequently produce adverse oral or systemic effects. In particular, palladium in dental casting alloys has been the subject of major controversies and concerns about possible adverse biological reactions, such as toxic and allergenic effects (Wataha and Hanks, 1996).

Released metallic ions are critical with respect to the biological behavior of dental metals and alloys. However, other parameters, like surface structure and electrochemical features, may also contribute to local interactions. For example, it has been reported that crowns cast with cpTi may accumulate more plaque than restorations made with noble alloys (Geurtsen, 1990; Geurtsen and Marx, 1990; Yoshida *et al*., 1999a,b). This increased plaque accumulation might then cause adverse inflammatory reactions in the adjacent soft tissues. Further, most alloys were less biocompatible in the "as-cast" condition than in the polished condition when specimens were investigated by direct contact tests in cell cultures (Craig and Hanks, 1988). Slightly roughened sandblasted stainless steel was significantly less biocompatible than in the polished condition (Bataille *et al*., 1997). In addition, it was observed that substances derived from the superficial layers of cast alloys are particularly cytotoxic (Woody *et al*., 1977). It was also found that cell-surface interactions between a Co-base alloy and human peripheral blood neutrophils significantly impaired neutrophil functions (Shanbhag *et al*., 1992).

This review article will focus, first, on corrosion and the release of metal ions from dental casting alloys. This will be followed by a critical evaluation of studies which have investigated the biological effects of individual metallic cations and casting alloys.

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TABLE 1
Elemental Composition of Precious and Non-precious Alloys (Craig, 1997)

Classification of alloys for dental cast restorations and metallic appliances:
(a) High-noble (noble metal content ≥ 60 wt% + gold content ≥ 40 wt%)
(b) Noble (noble metal content ≥ 25 wt%)
(c) Predominantly base metal (noble metal content < 25 wt%)

Noble metals:
Gold (Au), platinum (Pt), palladium (Pd), iridium (Ir), ruthenium (Ru), rhodium (Rh)

Base metals:
Silver (Ag), copper (Cu), zinc (Zn), indium (In), tin (Sn), gallium (Ga), chromium (Cr), cobalt (Co), molybdenum (Mo), aluminum (Al), iron (Fe), beryllium (Be), manganese (Mn), titanium (Ti), nickel (Ni), vanadium (V), niobium (Nb), zirconium (Zr)

(II) Corrosion and Release of Cations from Dental Casting Alloys

ADA Specifications classify alloys into three groups: (a) high noble (noble metal content ≥ 60 wt% + gold content ≥ 40 wt%; (b) noble (noble metal content ≥ 25 wt%); and (c) predominantly base metal (noble metal content < 25 wt%) (Craig, 1997). Noble metals are gold (Au), platinum (Pt), palladium (Pd), iridium (Ir), ruthenium (Ru), and rhodium (Rh). Base metals are silver (Ag), copper (Cu), zinc (Zn), indium (In), *etc*. (Table 1) (Craig, 1997). It should be emphasized that silver is a noble element in metallurgy. But based upon the relatively high reactivity in the oral cavity, Ag is considered by the ADA to be a base metal (Anusavice, 1996).

Due to the dramatic increase in the price of gold over the past 20 years, the use of both gold-reduced noble alloys and base metal alloys has increased significantly. Castable cpTi has also received increasing interest and importance during the past decade for the fabrication of fixed and removable restorations (Berstein *et al*., 1992; Geurtsen, 1994).

Adverse effects due to dental metallic materials are chiefly caused by corrosion, which results in release of metal ions and subsequent metal-protein or metal-cell interactions. Generally, corrosion is characterized by electrochemical phase boundary reactions which cause liberation of metal ions (Geis-Gerstorfer, 1996).

Many scientists have investigated the release of metal ions due to corrosion from different dental alloys *in vitro*. The amount and nature of released cations varied depending upon the type of alloy and other parameters, *e.g*., type of corrosion (corrosion due to concentration of cells; crevice, galvanic, stress, and pitting corrosion, *etc*.). Composition and chemical characteristics of the corrosive solution—such as pH and ionic composition, artificial saliva, cell culture medium, serum, *etc*.—were also of great significance (Covington *et al*., 1985; Nelson *et al*., 1999a,b). Ni-based alloys, in particular, revealed a significantly increased corrosion and release of Ni ions after storage at pH 1 or 4. However, high-noble and noble alloys were not significantly affected by low pH (Schwickerath, 1988; Johansson *et al*., 1995; Leyhausen *et al*., 1998; Wataha *et al*., 1998). Non-beryllium-containing Ni-Cr alloys were more resistant to corrosion than Be-containing alloys, but base metal alloys with a high Ni content released Ni ions in the order of magnitude which was found from food and drink intake (Brune, 1986; Bumgardner and Lucas, 1994). In particular, base metal alloys containing both Be and Ni revealed a very high Be leakage, which may pose a health risk (Covington *et al*., 1985; Geis-Gerstorfer and Passler, 1993). Noble alloys also release metal ions. Interestingly, it was found that Zn and Ag leach from most noble alloys into artificial saliva. This observation corroborates the findings of other investigators that Ag may be classified as base metal under oral conditions (Lappalainen and Yli-Urpo, 1987). Further, there is evidence that the alloy surface composition is of decisive importance for the release of cations. It was found that higher amounts of metal ions were released when the superficial atomic ratio of noble to non-noble elements was less than 1 (Wataha and Malcolm, 1996).

Khan *et al*. (1999a) compared the *in vitro* corrosion of three Ti alloys in various protein solutions with phosphate-buffered saline (PBS = control) at different pHs. Corrosion in PBS was higher at low pH. The addition of proteins to PBS reduced the influence of the pH. On the other hand, protein solutions in comparison with pure PBS further reduced surface hardness of the metallic specimens, indicating that proteins may interfere with the re-passivation of the alloy surfaces. These *in vitro* observations point to the complex interactions that can simultaneously produce protective and detrimental effects in an *in vivo* environment.

The nature and amount of metal ions released from 10 alloys into cell culture medium, over a three-day period, were correlated with their composition and microstructure. In contrast to previous studies, with simple experimental ternary alloys, cation release could not be predicted based upon nominal composition of the commercial alloys, unless multiple-phase formation occurred. Au, In, and Pd were not identified in the medium extracts. But Ag, Cd, Cu, Ga, Ni, and Zn were often found (Wataha *et al*., 1991a). Elevated element medium-alloy (EMA) ratios, however, were observed for multi-phase alloys. This indicates that multi-phase alloys may release metal ions according to their nominal composition (Wataha *et al*., 1991a). These short-term results were confirmed by subsequent long-term investigations. The release of cations into cell culture medium at pH 7.2 from 8 commercial casting alloys (high noble, noble, base metal) was investigated over a clinically relevant period of 10 months. Metal ions were constantly released during this entire period. Interestingly, cation release was not generally correlated with the concentration of an individual metal in an alloy or the nobility of the alloy (Wataha and Lockwood, 1998).

It must be emphasized that the *in vitro* data do not necessarily reflect the *in vivo* clinical conditions. In vivo, numerous biological factors, including organic acids and enzymes which are produced by oral micro-organisms or are contained in food, may contribute to alloy corrosion. Interactions between various restorations and specific factors of individual persons, such as consumption of acidic food and beverages, composition of saliva, *etc*., can significantly influence intra-oral corrosion (Bumgardner and Johansson, 1998). Moreover, corrosion may be accelerated by phagocytotic cells, such as human neutrophils (Yang *et al*., 1992). This is corroborated by clinical observations with removable titanium prostheses. Ti denture-base plates used by patients for six months corroded more than Ti plates which had not been inserted (controls) (Canay *et al*., 1998). In contrast, it was found that several casting and implant alloys had similar

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polarization resistances and corrosion currents in artificial saliva (*in vitro*) or in the oral cavities of baboons (*in vivo*). However, the nature and quantity of the released metal ions were not identified (Gettleman *et al*., 1980). Thus, this study does not basically disprove the poor correlation between *in vitro* and *in vivo* corrosion data which was observed by other researchers (Bumgardner and Johansson, 1998; Canay *et al*., 1998).

Wear is another important factor which can accelerate corrosive processes *in vivo*, especially due to a local breakdown of the passive layer (Khan *et al*., 1999b). Thus, conjoint action of corrosion and wear may occur in the oral environment. In addition, it must be noted that the heat treatment of the alloys for establishment of a chemical bond between veneering ceramic and metal surfaces, and the quality of the casting process itself may significantly influence the solubility and the corrosion resistance of a cast dental restoration (Bumgardner and Johansson, 1998; Schmalz *et al*., 1998a).

Few *in vivo* studies have been published on the release of metal ions from metallic dental materials. The solubility of 7 Ni-containing base metal alloys (removable partial dentures) in saliva was studied in a clinical trial. Polished as well as passivated specimens were investigated. Saliva samples were taken prior to insertion (baseline) as well as 20 min and 3 hrs later. It was found that the release of Ni from the alloys *in vivo* was similar to that reported in previous *in vitro* experiments: Salivary concentration of Ni significantly increased 20 min after insertion. Three hours after insertion, however, no increased Ni concentration was found (Schwickerath, 1988).

Saliva samples from 47 individuals with different types of fixed orthodontic appliances were analyzed for Ni and Cr concentrations prior to insertion and up to one month after insertion. There was no significant elevation of the salivary concentration of these two elements (Kerosuo *et al*., 1997). Thus, these *in vivo* data contradict the *in vitro* observations published by Wataha and Lockwood (1998) and Rose *et al*. (1998).

The release of metal components from base metal alloys into adjacent soft tissues was investigated in a rabbit model. Specimens were implanted intramuscularly for periods of up to 12 weeks. High tissue concentrations of Ni and Cr were found close to the implants, and these concentrations decreased toward the periphery. Corrosion behavior of the implanted alloy specimens was not dependent upon the implantation period. Interestingly, corrosion characteristics of Ni-Cr alloys with almost identical elemental composition were significantly different (Reuling *et al*., 1990). This confirms that metal ion release from alloys is not generally proportional to nominal alloy composition (Wataha *et al*., 1991a; Wataha and Lockwood, 1998).

Altogether, these discongruent findings clearly bring into focus the problems of comparing various *in vitro* and *in vivo* studies. Further, it may be concluded that the release of metal ions cannot be predicted from the nobility or the overall composition of a casting alloy. Each product must be evaluated individually for its corrosion behavior and the release of its components in various corrosive environments.

(III) Cytotoxicity of Metals and Dental Casting Alloys

Various types of primary and immortalized cell lines (fibroblasts or fibroblast-like cells and bone cells) derived from humans or animals have been used for the assessment of cytotoxic effects due to dental metallic materials in culture systems (Naji and Harmand, 1990; Berstein *et al*., 1992; Leyhausen and Geurtsen, 1992; Wataha *et al*., 1994a,b; Schmalz *et al*., 1997a,b). Alloy extracts and isolated salt solutions (metal cations) were also studied for cytotoxic effects (Schmalz *et al*., 1997a). A great many different parameters and bioassays have been used to identify the mechanisms of the cytotoxic alterations: cell proliferation and alteration of cellular morphology, release of glycosaminoglycans, expression of transforming growth factor β, succinic metabolic activity, influence on various enzyme systems, *etc*. (Berstein *et al*., 1992; Wataha *et al*., 1994a; Locci *et al*., 1996; Sauvant *et al*., 1997; Leyhausen *et al*., 1998).

Cytotoxicity of salt solutions and biological effects of metal ions

Single-salt solutions were investigated for the determination of cytotoxic alterations due to an individual metal. Further, combinations of these salts were used for the detection of synergistic, antagonistic, or additive effects caused by different mixtures of metal ions (Wataha *et al*., 1992; Schmalz *et al*., 1998a; Yamamoto *et al*., 1998). A comprehensive screening of the toxic behavior of 43 metal salts was undertaken with fibroblasts and osteoblast-like cells. The study revealed that the IC_{50} (inhibitive concentration causing a 50% reduction of cell growth) of the most toxic salt ($CdCl_2$: 1.36×10^{-6} mol L^{-1}) differed by four orders of magnitude from the least toxic salt (Yamamoto *et al*., 1998). The authors observed that the toxic response was dependent upon the type of metal as well as on the valence and the concentration. Very high toxicity was observed with K^+, Cd^{2+}, V^{2+}, Ag^+, Hg^{2+}, Sb^{3+}, Be^{2+}, and In^{3+}, whereas Sn^{4+}, Zr^{4+}, Nb^{5+}, and Mo^{5+} caused a significantly lower cytotoxic reaction in both cell types. Inconsistent results were found by Schmalz *et al*. (1998a), who compared the cytotoxic potency of medium alloy extracts and identical salt solutions. Medium extracts were constantly less cytotoxic than the salt solutions. This may be due to antagonistic or protective effects caused by various metal combinations, which have been previously described by Wataha *et al*. (1992). But these scientists also found that some combinations of two metal ions caused synergistic (increased) cytotoxic effects.

The influence of cell type and cell and serum concentration on the cytotoxicity of 17 metal ions was investigated by Schmalz *et al*. (1997b). Neither cell type nor cell concentration significantly influenced the ranking order of the toxic reactions. Serum concentration altered the cytotoxicity of Nb^{5+}, Ni^{2+}, and Sn^{2+} only, whereas toxic reaction to all other cations did not vary, regardless of the serum concentration. The most toxic ions were Ag^+, Zn^{2+}, and Cd^{2+}, whereas Hg^{2+}, Nb^{5+}, Mo^{5+}, Ga^{3+}, In^{3+}, and Sn^{2+} caused less-cytotoxic reactions (Table 2).

In contrast, other authors have reported that cellular parameters may significantly influence the toxic ranking of cations. Fourteen metal ions which leach from dental casting alloys were screened for cytotoxicity by the use of 4 different cell lines. It was found that the cell lines responded differently to most of the metallic elements studied. In addition, passage number of the cells also influenced toxic ranking. The authors concluded from these data that clinically relevant cell lines should be used when more sophisticated, pre-clinical information "beyond simple toxic ranking" is necessary (Wataha *et al*., 1994a).

Various biological interactions between metal ions and metabolic pathways or cell structures have been documented. Several cellular parameters (cell viability, DNA-/RNA-/protein-synthesis, membrane integrity, *etc*.) were examined after the incubation of human gingival fibroblasts with several metal salts. Cr^{6+} and Be^{2+} were the most toxic cations, Ni^{2+} was mod-

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TABLE 2
Cytotoxic Ranking of Various Metal Ions Evaluated in L-929[a] or 3T3[b] Mouse Fibroblast Cultures

Wataha *et al.*, 1991[bb]*	Wataha *et al.*, 1994[aa]	Schmalz *et al.*, 1997[ba]	Sauvant *et al.*, 1997[a]*	Yamamoto *et al.*, 1998[a]		Kappert *et al.*, 1998[a]
Cd^{2+}	Cd^{2+}	Ag^{+}	**Cu^{2+}**, V^{5+}	K^{2+} (most toxic)	**Zn^{2+}**	In^{3+}
Ag^{+}	Ag^{+}	**Zn^{2+}**	Hg^{2+}, **Zn^{2+}**	Cd^{2+}	Y^{3+}	Ga^{3+}
Zn^{2+}	**Zn^{2+}**	Cd^{2+}	Co^{2+}	V^{3+}	$W6^{+}$	**Zn^{2+}**
Cu^{2+}	V^{3+}	Hg^{2+}	Cd^{2+}	Ag^{+}	Fe^{3+}	**Cu^{2+}**
Ga^{3+}	Au^{3+}	Au^{3+}	Ti^{4+}	Hg^{2+}	Pd^{2+}	Au^{3+}
Au^{4+}	**Cu^{2+}**	Pt^{4+}	Nb^{5+}	Sb^{3+}	Fe^{2+}	Co^{2+}
Ni^{2+}	Co^{2+}	Co^{2+}	Fe^{3+}	Be^{2+}	Ti^{4+}	Ni^{2+}
Pd^{2+}	Pd^{2+}	**Cu^{2+}**	Sb^{3+}	In^{3+}	Hf^{4+}	Pd^{2+}
In^{3+}	Ti^{4+}	Ni^{2+}	Sn^{4+}	Cr^{3+}	Ru^{3+}	Sn^{2+}
	Be^{2+}	Pd^{2+}	Mn^{3+}, Ge^{4+}	Hg^{+}	Sr^{2+}	Mo^{5+}
	Ga^{3+}	Mn^{2+}	Cr^{3+}	**Cu^{2+}**	Sn^{4+}	La^{3+}
	Ni^{2+}	Nb^{5+}	Pb^{2+}	Rh^{3+}	Ba^{2+}	
	Ti^{4+}	Ga^{3+}	Ba^{2+}	Ti^{3+}	Cs^{+}	
	Cr^{3+}	Cr^{3+}		Sn^{2+}	Nb^{5+}	
	Al^{3+}	In^{3+}		Ga^{3+}	Ta^{5+}	
		Sn^{2+}		Pb^{2+}	Zr^{4+}	
				Cu^{+}	Al^{3+}	
				Mn^{2+}	Mo^{5+}	
				Tl^{+}	Rb^{+}	
				Ni^{2+}	Li^{+} (least toxic)	

* The rank of toxic potency based on the most sensitive assay out of several investigated parameters. Bold-face elements were investigated in all studies.

erately toxic, and Cr^{3+} and Mo^{6+} were the least toxic ions (Messer and Lucas, 1999). A different toxic ranking based on 6 bioassays in L929 was reported by Sauvant *et al*. (1997), with V^{5+} and Cu^{2+} being the most detrimental cations (Table 2).

Heavy metals released by corrosion, like Ni^{2+} and Co^{2+}, can enter the circulatory system and be distributed systemically by proteins, such as albumin (Traisnel *et al*., 1990). These ions may then induce gene activation in endothelial cells, similar to pro-inflammatory mediators like interleukin 6 (IL-6) and interleukin 8 (IL-8) (Wagner *et al*., 1998). Very low concentrations of Zn^{2+}, Ni^{2+}, and Co^{2+} also stimulated leukotriene B4-release, *in vitro*, due to activation of polymorphonuclear neutrophil granulocytes (PMNs) (Klein *et al*., 1994a). There is also indication that Ni and Zn can activate T- and B-cells, whereas Cd and Cu were inhibitory (Smith and Lawrence, 1988; Warner and Lawrence, 1988). Consequently, cations released by corrosion can provoke inflammatory reactions and may modulate the immune response by activation or inhibition of T- and B-cells.

In addition to affecting adjacent oral soft tissues, ions released from cast metal restorations may also have an adverse effect on nearby alveolar bone. This aspect has been addressed by various scientists, who performed short-term (48-hr) studies to determine acute toxic effects, whereas long-term incubation periods (up to 4 wks) were applied to monitor sublethal "chronic" alterations. Osteogenic bone marrow cells were significantly damaged by Cr^{6+} after an incubation period of 48 hrs, whereas Ti^{4+}, Al^{3+}, V^{5+}, and Mn^{2+} were only slightly cytotoxic. Moderate toxic potency in osteogenic cells was observed with Co^{2+}, Mo^{6+}, and Ni^{2+} (Puleo and Huh, 1995). In contrast, V^{5+} was significantly cytotoxic in long-term experiments for up to 4 wks, whereas the other metal ions caused only slight or no cytotoxic effects (Thompson and Puleo, 1995).

Osteoblastic differentiation of human bone marrow cells was influenced, *in vitro*, by Fe^{3+}, Cr^{3+}, and Ni^{+}, depending upon the state of differentiation of the cultured cells (Morais *et al*., 1998). Ni^{2+}, Co^{2+}, Ti^{4+}, and V^{3+} affected DNA synthesis, alkaline phosphatase activity (ALP), and *in vitro* calcification processes (osteopontin and osteocalcin expression) of cultured osteoblast-like cells, even at subtoxic concentrations. However, Al^{3+} influenced only ALP expression and calcification (Sun *et al*., 1997).

Thus, cations released from dental alloys may cause significant biological alterations at non-cytotoxic concentrations. Since most casting alloys release several metal ions into the biological environment simultaneously, it would be of great interest to investigate the interactive effects of various combinations of metal ions on metabolic processes.

The question that arises at this point is whether experiments with metal salt solutions rightly represent cytotoxic reactions due to alloy extracts. There is evidence, in fact, that medium extracts of cast specimens of various types of alloys are slightly less cytotoxic than corresponding salt solutions. This might be partly due to limitations of the sensitivity of the chemical analysis of the specimen extracts. However, the interactive effects between and among various cations must also be taken into account (Wataha *et al*., 1992; Schmalz *et al*., 1998a). Additionally, it has been reported that corrosive extracts of surgical stainless steel and the corresponding salt solutions elicited significantly different effects on biomineralization of cultured osteoblast-like cells. The corrosion products caused only slight effects, whereas the salt solutions induced more pronounced alterations (Morais *et al*., 1998). Investigators should keep these discrepancies in mind when interpreting cytotoxicity data which have been reported with isolated salt solutions.

CYTOTOXICITY OF SOLID ALLOY SPECIMENS AND ALLOY EXTRACTS

Most corrosion studies are performed at low pH, *e.g*., pH 1, 2.3, or 4.2 (Pfeiffer and Schwickerath, 1989, 1991; Johansson *et al*., 1995; Leyhausen *et al*., 1998; Wataha *et al*., 1998). The resulting

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acidic extracts, however, are not compatible with the physiological conditions necessary for cell culture studies. For those investigations, therefore, alloy or metal specimens are most frequently extracted with medium at neutral pH. Thereafter, the effects of the medium extracts on cells are tested. In these assays, only effects caused by released ions are determined. Further, direct contact experiments with solid alloy specimens placed together with the cells in the culture wells at the same time have been performed. In these studies, effects due to released metal ions and to surface parameters as well can be investigated (Geurtsen and Leyhausen, 1995). Mono- and co-cultures were applied to monitor cytotoxic effects. Screening tests of pure metals and experimental and commercial alloys revealed that cytotoxicity does not correspond to the overall composition of the alloy or % value of any single metal (Craig and Hanks, 1990; Schade *et al*., 1997; Leyhausen *et al*., 1998; Schmalz *et al*., 1998a; Wataha *et al*., 1999c). But castable cpTi was invariably highly biocompatible (Berstein *et al*., 1992; Wang and Li, 1998). Further, it was observed that corrosion resistance of a noble or base metal alloy does not permit one to draw conclusions on its biocompatibility (Schade *et al*., 1997). It was also found that solid specimens of gold-based solders combined with a substrate alloy were very often less cytotoxic than the solders alone. Only three solder-substrate alloy combinations revealed more pronounced toxic reactions than the single solders (Wataha *et al*., 1999b). Various studies documented that Cu was the most toxic element, whereas Au, Pd, and Ti were the least cytotoxic metals. Generally, high-copper alloys were severely toxic (Bumgardner *et al*., 1989; Berstein *et al*., 1992; Grimsdottir and Hensten-Pettersen, 1993; Wataha *et al*., 1995a). Interestingly, high Pd concentration improved biocompatibility of high-copper alloys more effectively than high Au or Ag concentrations (Craig and Hanks, 1990). Be, when released in high quantities, reduced cell growth significantly (Bumgardner and Lucas, 1995; Wataha *et al*., 1995a, 1999e; Schmalz *et al*., 1997b). But contradictory cytotoxicity results were reported for Ni and Ag. Ni was highly cytotoxic in primary human gingival fibroblasts (Bumgardner and Lucas, 1995) but was only moderately toxic in immortal fibroblast cultures (Wataha *et al*., 1991b, 1994a; Schmalz *et al*., 1997b). Solid Ag specimens provoked moderate cytotoxic reactions in immortal 3T3-fibroblast cultures, whereas Ni specimens were severely cytotoxic (Craig and Hanks, 1990). Ag-salt solutions, however, were highly cytotoxic in various studies (Wataha *et al*., 1991b, 1994a; Schmalz *et al*., 1997b).

Several cobalt-chromium alloys were investigated in direct contact tests with human gingival fibroblasts. The degrees of cellular alterations varied between "no cell injury" and "severe cellular damage". Although there was no general correlation between overall alloy composition and cytocompatibility, severely cytotoxic alloys contained higher amounts of Ni than biocompatible products (Arvidson *et al*., 1987). These data were corroborated by a later study, with Ni-based casting alloys, which showed that energy metabolism of human gingival fibroblasts decreased, in particular, when higher quantities of Ni and Be ions leached from the tested alloys (Bumgardner *et al*., 1995).

A significant shortcoming of conventional cell-culture studies is that they do not accurately reflect the *in vivo* long-term behavior of dental casting alloys. Therefore, attempts have been made to develop adequate cell-culture assays. For this, alloys were tested immediately after being polished as well as 10 mos after being conditioned in a biologic medium. Alloys which were cytocompatible at baseline were not cytotoxic after 10 mos, and highly cytotoxic alloys were significantly less cytotoxic after 10 mos. The authors, therefore, concluded that short-term *in vitro* tests do not sufficiently determine long-term behavior of dental casting alloys (Wataha *et al*., 1999e).

The influence of the surface treatment and topography upon cytocompatibility of various alloys was investigated in direct contact tests with "as-cast" and polished specimens. Crown and bridge casting alloys and some base metal alloys revealed a better biocompatibility in the polished condition than in the "as-cast" condition. The authors stated that this approach might simulate interactive effects between different alloy surfaces and the gingival tissue. Interestingly, two alloys with high Cu content (50-60 wt%) were severely cytotoxic independently of the surface topography (Craig and Hanks, 1988). Similar results were reported for a Co-Cr alloy with various surface states (Naji and Harmand, 1990).

Powders of cpTi, Ti alloys, and a Co-Cr-Mo alloy were used for the investigation of direct interactions between alloy surfaces and cells. Fine particles of these metallic materials reduced cell viability when in direct contact with the cells. It was speculated that small particles with a diameter of less than 5-10 μm will cause cellular injuries, even when the materials are well-tolerated in bulk form. Fine particles of cpTi (diameter < 3 μm) also decreased biosynthesis of collagen types I and III in human osteoblast-like cells. But the nature of those chemical/physical/biological interactions remained unclear (Evans, 1994; Yao *et al*., 1997). In contrast, fine Ti-particles (diameter 1-3 μm) stimulated secretion of IL-1 and prostaglandin E2 from peritoneal macrophages and significantly enhanced bone-resorbing activity in the medium from those stimulated cells. These observations strongly indicate that Ti particle wear debris may cause bone resorption in adjacent areas (Glant *et al*., 1993). The significance of particle size and particle surface area as well was corroborated by Shanbhag *et al*. (1994). Small Ti particles increased the secretion of IL-1 from macrophages, whereas larger particles with higher surface areas inhibited DNA synthesis of macrophages, which is indicative of cell damage and cell death.

It may be hypothesized, though, that the topography of a metallic substratum can be of decisive importance for the orientation of cells growing on or adjacent to a cast metallic restoration. For example, it has been observed that human gingival fibroblasts exhibited a random orientation on a smooth titanium surface, but were oriented along grooves on a V-shaped grooved titanium substratum. In addition, surface topography significantly influenced the expression of metalloproteinase-2 (Chou *et al*., 1998). Taken together, observations made by several investigators indicate that an irregular topography ("as-cast" condition, irregular fine particles, *etc*.) can interfere with tissue remodeling, which eventually might result in tissue inflammation (Craig and Hanks, 1988; Naji and Harmand, 1990; Evans, 1994; Yao *et al*., 1997).

Phase formation is another parameter which may play a considerable role in determining the biocompatibility of a casting alloy. Several scientists investigated the correlation between cytocompatibility and phase formation, on the one hand, and overall elemental composition, on the other hand. There was no correlation between cytotoxicity and Cu content in a study with "directly tested" Ag-Pd-Cu casting alloys. Phase formation, however, significantly influenced biocompatibility. Multi-phase Ag-Pd-Cu alloys were all cytotoxic, whereas single-phase products were cytocompatible (Niemi and Hensten-

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Pettersen, 1985; Niemi *et al*., 1985). Subsequently, it was reported that phase formation might, in fact, significantly influence biocompatibility. Contrary to previously reported data, it was also found that single-phase alloys with only moderately high Cu concentration were severely cytotoxic, as were multi-phase alloys (Craig and Hanks, 1990).

Although there is no uniform pattern of cytotoxic effects due to a single metal, many studies indicate a high toxic potency of Cu, Ni, and Be. However, no correlation exists between the nobility of alloys and cytotoxicity. In addition to the nature and quantity of released cations, surface topography of a cast metallic structure may be of decisive importance for its biological compatibility. Thus, released metal ions as well as surface topography may contribute to local adverse effects in the gingiva and periodontium adjacent to cast restorations. In addition, there is evidence that small metal or alloy particles generated by wear may cause tissue inflammation. But it must also be emphasized that the currently used *in vitro* assays obviously do not accurately measure the long-term biological effects of dental metallic materials.

IN VITRO AND *IN VIVO* EFFECTS OF METALS AND CASTING ALLOYS ON IMMUNOLOGICAL PARAMETERS

There is great concern that metal ions may impair the local and systemic immune responses of patients with cast metallic restorations. Therefore, interactive effects of metal ions with immunological parameters have been investigated *in vitro* and *in vivo*. The production of various immune mediators after treatment with metallic materials has been studied in lymphocyte cell lines. The expression of the inflammatory mediator interleukin 2 (IL-2) and the immune effector, IgG, by T- and B-cells was determined after incubation with three copper-based casting alloys. The production of both immune mediators was altered by released cations (Bumgardner *et al*., 1993). Lipopolysaccharide (LPS)-stimulated osteoblast-like cells revealed an increased production of IL-1α and tumor necrosis factor-α (TNF-α) after incubation with Ti, Co, and Cr. In addition, synthesis of type I collagen was reduced (Wang *et al*., 1997).

Polymorphonuclear leukocytes (PMNs) are primarily involved in the host defense against bacteria, *e.g*., by the production of reactive oxygen species (ROS). Thus, the expression of ROS was used as parameter for the determination of interactive effects between several metal ions and PMNs. While Mo, Al, and V enhanced ROS production, no such effect was observed with Cr, Co, Ni, and Ti (Ciapetti *et al*., 1998). Release of several cytokines from unstimulated and stimulated peripheral blood mononuclear cells (PBMCs) as well as cell viability and DNA synthesis were investigated after incubation with Cr ions. This cation caused a dose-dependent reduction of cell viability, DNA synthesis, secretion of IL-6, and expression of soluble interleukin-2 receptor (sIL-2r). These findings suggest that chromium has the potential to suppress the immune system (Granchi *et al*., 1998).

Ti wear particles did not significantly alter several investigated immunological parameters such as peripheral blood lymphocyte activation and IL-2 secretion (Kohilas *et al*., 1999). Similar data have been reported with murine macrophages which had been exposed to wear debris of a Co-Cr alloy (Prabhu *et al*., 1998). But IL-1 secretion from macrophages and bone-resorbing activity were significantly stimulated by fine Ti particles. In contrast, the respiratory burst and function of human PMNs were significantly inhibited by Co-base alloy particles of non-phagocytosable size (Shanbhag *et al*., 1992).

The pro-inflammatory effects (IL-6 production) of numerous metals and one noble alloy were investigated in a three-dimensional co-culture model with human fibroblasts and keratinocytes. Increased IL-6 expression, suggestive of a pro-inflammatory potency, was detected with Cu, Zn, Co, Ni, and Pd at low or non-toxic concentrations (Schmalz *et al*., 1998b).

Several metal ions (Ag, Au, Cu, Hg, Ni, Pd, Pt, Zn) altered the secretion of proteins from macrophages (Wataha *et al*., 1995b). Sub-toxic concentrations of a Ni solution also induced an increase of IL-1α and TNF-α secretion from unstimulated and LPS-stimulated THP-1 monocytes (macrophages), whereas Ag and Cu enhanced IL-1α expression only from LPS-stimulated cells (Wataha *et al*., 1996).

Cell adhesion molecules (CAM) like E-selectin, intercellular adhesion molecule 1 (ICAM-1), *etc*., are important for mediating cell-cell interactions of platelets and leukocytes with endothelial cells. Thus, it is of great interest to determine if metal ions can alter the production of CAM by endothelial cells. Very low, sub-toxic concentrations of Zn, Ni, and Co increased the expression of E-selectin, ICAM-1, and other CAM similar to that observed with inflammatory mediators. The authors concluded that clinically relevant very low concentrations of these metal ions, which may be released by corrosion, might participate in the pathogenesis of tissue inflammation (Klein *et al*., 1994b). Controversial observations were reported by Wataha *et al*. (1997). Contrary to previous experiments, ICAM-1 expression of LPS-stimulated endothelial cells was reduced by sub-toxic Ni concentrations, but no effect was determined in the absence of LPS. At clinically relevant, cytotoxic concentrations, Ni stimulated ICAM-1 production. These results show that Ni might exhibit a dual action regarding ICAM-1 expression, which is modulated by microbial factors, like LPS. Solid specimens of a Ni-containing alloy caused a significant increase of IL-1α secretion from THP-1 monocytes, confirming the pronounced inflammatory potency of Ni ions (Wataha *et al*., 1999a).

The significance of bacterial LPS stimulation on the inflammatory responses of various cell types following incubation with several metal ions was corroborated by Wang *et al*. (1996). After LPS stimulation, human monocytes/macrophages expressed significantly more cytokines following incubation with sub-toxic concentrations of three metal ions: IL-1α (Ti, Cr, Co), TNF-α (Ti, Cr), and IL-6 (Ti). In addition, all cations that were used reduced the production of tumor growth factor-α1 (TGF-α1). In the absence of LPS, however, no stimulation of monocytic cytokine expression was observed.

The effects of noble alloys and Ni-containing base metal alloys on the levels of lymphocyte subpopulations after implantation were investigated *in vivo* in a murine model (Zalkind *et al*., 1998). No alteration in the proportion of the lymphocyte subpopulations was observed.

The concentrations of circulating T-lymphocytes and other parameters—such as hematocrit, total and differential leukocyte count, serum and salivary nickel concentrations, *etc*.—were determined over a six-month period after patients received fixed partial prostheses which had been made with a Ni-Cr alloy. No changes in salivary and serum Ni levels were found. Though the T-lymphocyte and monocyte populations did not change, the number of circulating eosinophils decreased and the neutrophil and basophil populations increased. Since basophils participate in hypersensitivity reac-

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tions, it was concluded that hypersensitivity caused by metal ions which are released from the Ni base alloy cannot be ruled out (Arikan, 1992). On the other hand, T-lymphocyte proportions increased from 56% to 77% of all lymphocytes after removal of a Ni base alloy crown in a 20-year-old patient. This case report indicates that Ni may adversely influence the number of circulating T-lymphocytes (Eggleston, 1984). Taken together, the contradictory studies published by Arikan (1992) and Eggleston (1984) do not allow for definite conclusions about the effect of Ni on circulating T-lymphocytes.

There is evidence that metallic components derived from dental cast restorations can modulate the expression of various immunological factors and, therefore, may participate in the etiology of various intra-oral (and systemic?) pathological conditions. The increased expression of cytokines after LPS stimulation of various cell types indicates that bacterial toxins and material-related factors, *e.g.*, cation release due to corrosion, may produce synergistic or additive inflammatory effects in the pathogenesis of oral diseases, like oral mucositis, gingivitis/periodontitis, and alveolar bone resorption.

(IV) Genotoxicity, Mutagenicity, and Carcinogenicity of Metallic Materials

Individual metal ions have been thoroughly assessed for genotoxic and mutagenic effects in prokaryotic and eukaryotic test systems. Various platinum salts [*e.g.*, carboplatin, cisplatin (II), and $PtCl_4$(IV)] were clearly genotoxic in the *Escherichia coli* PQ37 genotoxicity assay (SOS chromotest) and the eukaryotic micronucleus test (MNT). Other platinum salts, like $PtCl_2$(II) and K_2PtCl_6(IV), and all investigated palladium salts were not genotoxic (Gebel *et al*., 1997). The two Rh and two Cr salts also exhibited genotoxic potency in the bacterial SOS chromotest with *E. coli* PQ37 (Lantzsch and Gebel, 1997). Cd^{2+} induced DNA single-strand breaks in various cell types, which is indicative of a genotoxic potency of this cation (Latinwo *et al*., 1997).

An oxidative DNA damage or an interaction with DNA repair primarily correlates with the carcinogenic potency of metallic cations. Mammalian cells are used to detect carcinogenic effects. Interestingly, in most instances, carcinogenic metal ions are not mutagenic in bacterial test assays. Alteration of DNA repair processes and carcinogenic effects have been observed with Cd^{2+}, Ni^{2+}, and Co^{2+} (Hartwig, 1995). Ni revealed a weak to moderate mutagenic activity. However, it was reported that Ni can enhance the genotoxic effects of physical parameters (*e.g.*, UV light) and cytostatic compounds like cisplatin (Hartwig *et al*., 1994). It has been observed that various Ni compounds may contribute to the development of nasal and lung cancers (Norseth, 1980; Hayes, 1997).

Genotoxic effects have been associated with Be and Ga salts (Kuroda *et al*., 1991). Analysis of experimental and occupational data also indicates that Be may increase the risk of lung cancer and other tumors in man (Aller, 1990; Ashby *et al*., 1990; Ward *et al*., 1992; Hayes, 1997). Further, Ti is a suspected carcinogen (Hadfield *et al*., 1998). Tumors were found in rodents after the application of Ga, Be, In, Rh, or Pd (Lyon *et al*., 1966; Schroeder and Mitchener, 1971; Groth *et al*., 1980). But there is no conclusive evidence that Sn, Zn, and V have mutagenic or carcinogenic potency (Wataha and Hanks, 1996; Rojas *et al*., 1999) (Table 3).

There are very few data on the genotoxic effects of dental alloys. Various orthodontic appliances (wires, brackets, extension screws) fabricated with different base metal alloys and cpTi were screened for genotoxic alterations. Different assays—like the Comet assay, Salmonella reverse-mutation test, the chromosomal aberration test, and the electron microscopy *in situ* end-labeling assay—have been used, and no genotoxic effects were observed (Assad *et al*., 1998; Wever *et al*., 1997; Tomakidi *et al*., 2000). Similar data were found with several Ti-containing alloys, cpTi, and one Ni-Cr-based alloy in the Ames test (Wang and Li, 1998).

TABLE 3
Mutagenicity/Genotoxicity and Carcinogenicity of Metal Ions

Metal	Mutagenic/Genotoxic	Carcinogenic
Cd	+	+
Co	+	+
Cr	+	+
Ni	+	+
Sn	?*	-
V	-	-
Zn	?	?
Be	+	+
Ga	+	+
Ti	-	?
Pd	?	+
Pt	+	?
Rh	+	+
In	?	+

Sources: Aller, 1990; Fishbein, 1984; Hadfield *et al*., 1998; Hartwig, 1995; Hayes, 1997; Kanematsu *et al*., 1990; Kuroda *et al*., 1991; Leonhard and Lauwerys, 1987; Lyon *et al*., 1966; Rojas *et al*., 1999; Schroeder and Mitchener, 1971; Wataha and Hanks, 1996.

* ? = inconclusive, controversial, or unclear data.

These reports show that various metals are genotoxic and/or carcinogenic. There is strong evidence that Ni, Co, Cr, and Be, in particular, increase the risk of cancer in humans. However, no studies have been published which support the hypothesis that dental casting alloys are genotoxic or might be a carcinogenic hazard to man.

(V) Interactions between Casting Alloys and Micro-organisms

In contrast to amalgam, there is scant information on the microbial effects of dental metals and casting alloys (Dummer and Harrison, 1982; Morrier *et al*., 1998). Microbial growth promotion or inhibition as well as bacterial adhesion and plaque formation on metallic surfaces have been studied. The influence of a Pd-Ag alloy upon the growth of the intra-oral aerobic and micro-aerophilic flora from healthy persons was investigated *in vitro*. A significant inhibition of the growth of aerobic bacteria was observed which might favor proliferation of periodontopathogenic anaerobic bacteria (Stipetic *et al*., 1998).

Antibacterial activity of Ti granules was compared with the microbial effects of amalgam, Cu, and Sn. In general, Ti only slightly reduced the viability of micro-organisms such as *S. sanguis*, *S. mitis*, *A. naeslundii*, Fusobacterium spp., and *P. intermedia*. But amalgam and Cu clearly decreased viable counts of all types of bacteria studied (Leonhardt and Dahlén, 1995). Joshi and Eley (1988) reported similar observations. Interestingly, it was observed that serum significantly reduced the antibacterial activity of amalgam and Cu (Leonhardt and Dahlén, 1995).

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Adhesion of *S. oralis*, *A. viscosus*, and *C. albicans* to a Co-Cr alloy was significantly correlated with the surface structures of the alloy specimens. Polished surfaces revealed fewer bacteria than did sandblasted samples (Taylor *et al*., 1998). Early plaque formation on different types of dental materials, including amalgam, casting alloy, and Ti, was studied *in vivo*. The test dental materials were attached to the buccal and oral aspects of the upper first molars. There was no difference in plaque formation on the various materials. But the location of the specimens was clearly important. While only scarce amounts of bacteria were identified orally, abundant micro-organisms were found on all specimens which were attached to the buccal aspects. The author concluded that the type of restorative material is significantly less important for intra-oral plaque formation than the location of the restoration (Hannig, 1999).

In contrast, Steinberg *et al*. (1998) reported that various types of oral bacteria might have different adhesion patterns to cpTi and Ti alloys. *A. viscosus*, *A. actinomycetemcomitans*, and *P. gingivalis* adhered less to the cpTi than to the Ti alloy. In addition to location, other intra-oral parameters can significantly influence the adhesion of micro-organisms to metallic surfaces. There was less adhesion of *A. viscosus*, *A. actinomycetemcomitans*, and *P. gingivalis* to the Ti alloy and cpTi when the specimens were coated with saliva or albumin (Steinberg *et al*., 1998).

Freshly isolated strains of *S. mutans* from two persons showed different levels of adhesion to dental gold alloy specimens which were covered with pellicles from the same test subjects. Attachment of *S. mutans* was better on pellicles of homologous origin, *i.e.*, from the same test person. Therefore, it was concluded that individual biological factors significantly influence the adhesion of micro-organisms to dental materials (Ørstavik *et al*., 1982). There is also evidence that physicochemical parameters of the surface, like the hydrophobicity and the zeta-potential of a restorative alloy, are important for the adhesion of certain bacteria, such as *S. sanguis* and *S. mutans* (Satou *et al*., 1988).

The attachment of bacterial LPS (from *E. coli* and *P. gingivalis*) varied depending upon the alloy studied, whereas surface finish did not significantly influence LPS adhesion (Knoernschild *et al*., 1994, 1995, 1997; Nelson *et al*., 1997).

But whether bacterial adhesion and attachment of microbial components, like cytotoxic LPS, are predominantly correlated to alloy composition or the surface structure of a cast restoration is still a controversial subject. Further research is necessary to clarify this important and clinically relevant aspect.

(VI) Histocompatibility of Metallic Dental Materials

Many authors have investigated the tissue reactions to orthopedic or implant alloys, but few studies have addressed the histocompatibility of dental casting alloys. This may be due to the minor significance of those studies for the biological characterization of casting alloys, since these materials are not implanted into oral tissues. Thus, implantation tests furnish only an indication of the non-specific toxic behavior of cast alloys. Histological changes due to dental alloys were investigated in rabbits, rats, guinea pigs, and hamsters (Piliero *et al*., 1979; Bessing and Kallus, 1987; Reuling *et al*., 1991; Kansu and Aydin, 1996a). Specimens of high noble, noble, and base metal alloys were implanted subcutaneously into rats. Sections were evaluated, at different time periods, for up to 60 days after implantation. Ni-Cr alloys caused severe histologic alterations. A high noble and two Ag-Pd alloys caused only slight irritations. The investigated noble alloys and Co-Cr base metal alloys induced moderate tissue irritation (Kansu and Aydin, 1996a). In another study, in contrast, a Ag-Pd alloy demonstrated "extreme" reaction after subcutaneous implantation into guinea pigs. However, the second Ag-Pd alloy, which was included in this investigation, revealed the best compatibility among the 5 alloys investigated (Bessing and Kallus, 1987). Various noble and base metal alloys caused no tissue irritation after implantation into the cheek pouches of hamsters. In addition, no differences among the various alloys were observed (Piliero *et al*., 1979).

The Cu-Pd-rich component of a Ag-Pd-Cu-Au casting alloy resulted in an acute and permanent inflammation after subcutaneous implantation into guinea pigs, whereas the Pd-rich alloy component and the alloy itself induced no significant tissue alteration (Niemi *et al*., 1985).

Reuling *et al*. (1991) and Reuling (1992) investigated the biocompatibility and corrosion behavior of 12 dental casting alloys (high noble, noble, base metal) in rats, guinea pigs, and rabbits. None of the metallic materials caused acute systemic toxic or lethal effects when the animals were fed powdered alloys (1000 mg *per* day) over a period of seven days. But depending upon the composition of the alloys, significant changes in organs, such as the lungs, liver, kidneys, *etc*., were found. The most severe alterations, such as tissue necrosis, ulceration, and chronic inflammatory reactions, were observed with alloys containing high quantities of Cu, In, or Be. The pronounced toxic effects of alloys containing these elements were confirmed when they were implanted subcutaneously or intramuscularly. A subsequent analysis of the peri-implant concentrations of Ni, Co, and Cr after intramuscular implantation of 5 base metal alloys revealed that these metals were evenly released into the surrounding tissue and in amounts that correlated with alloy composition. In particular, very high Ni and Cr levels were identified in the tissue close to Ni-Cr alloy implants.

As with *in vitro* studies, various results have also been observed *in vivo*. Generally, *in vivo* investigations have demonstrated that dental casting alloys may cause severe local and systemic effects which cannot be predicted from the nobility of the material or the overall composition of the alloys. Analysis of the available data clearly indicates that materials containing higher amounts of Cu, In, or Be are likely to cause tissue alterations, such as inflammation and necrosis, in particular. It may also be concluded that Ni-Cr alloys show increased corrosion in a biological environment.

(VII) Allergenic Effects of Metals and Dental Casting Alloys

The allergenic behavior of metals and alloys has been investigated *in vitro* and *in vivo*. Experimental assays, such as the lymphocyte transformation test, along with dermal, intradermal, and intra-oral *in vivo* tests have been used for detection of the allergenic potency of metals or casting alloys (Veien and Kaaber, 1979; Axéll *et al*., 1986; Nordlind, 1986; Van Loon *et al*., 1986; Grimsdottir *et al*., 1994).

Metal ions, which are released from dental restorations, can provoke systemic and local allergic reactions. A case has been reported of an individual who suffered from an IgA nephropathy after placement of Ni alloy base crowns (Strauss and Eggleston, 1985). An analysis of 139 published cases of allergic reactions to dental metallic restorations showed that those patients

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suffered from local irritations predominantly in the form of gingivitis and stomatitis (Hildebrand *et al*., 1989). Only 33 out of 139 persons revealed general reactions. Analysis of these data demonstrates that local allergic reactions to metals may often be misdiagnosed as inflammatory reactions. The higher incidence of oral side-effects in comparison with systemic reactions was corroborated by Hensten-Pettersen (1992). Most frequently, lichenoid reactions and swelling and pain of oral soft tissues and lips were observed. In several patients, Au ions caused an allergic contact gingivo-stomatitis which was similar in appearance to an erosive lichen planus (Izumi, 1982; Laeijendecker and Van Joost, 1994). Interestingly, many patients had a history of intolerance to gold jewelry. Lichenoid reactions of the oral mucosa have also been found when Ni was used in an intra-oral patch test (Axéll *et al*., 1986). A double-blind clinical study including a patch-test screening of 708 patients showed 12 positive results (1.7%): Ni (five), Cr (six), and Co (one) (Morris, 1987). But it should be noted that positive skin reactions to metals such as Ni are not necessarily associated with intra-oral allergic reactions. For instance, it was observed that patients with a previous history of positive skin reactions to Ni did not develop local or systemic adverse effects in response to a Ni-containing alloy during an observation period of up to 15 yrs (Spiechowicz *et al*., 1984, 1999). Similar observations were published by Yontchev *et al*. (1986). No association between patients' orofacial complaints and patch test results was found. This clearly indicates that routine patch-testing of patients with subjective intra-oral symptoms should not be performed. It is also noteworthy that patch tests may even cause sensitization in some cases.

Various "porcelain fused" casting alloys contain iridium (Ir) and indium (In). Positive patch-test reactions to these metals were observed in five out of 205 patients investigated (Marcusson *et al*., 1998).

A study with 60 persons documented that Ni has the highest allergenic potency, followed by K, Co, Ag, Cu, Pd, Pt, and Au (Kansu and Aydin, 1996b). Schmalz examined 86 patients who suffered from adverse intra-oral effects due to cast metallic restorations (Schmalz, 1999). Twenty-three percent of these persons exhibited gingivitis which did not disappear after thorough plaque control. Alterations of the tongue were observed in 16% of the patients, and five out of 86 persons demonstrated oral lichenoid lesions.

Allergic reactions to 49 selected casting alloys, cpTi, and amalgam were investigated in an epidemiological study population of 763 patients. Most frequently, Ni, Co, and Ag caused allergic irritations. In addition, a surprisingly high incidence of positive dermal reactions to Ti was observed. Analysis of these data indicates that Ti should also be considered in the diagnosis of allergic alterations due to metallic dental restorations (Mau *et al*., 1998). Contact sensivity to a Ti device (pacemaker) has also been reported by Abdallah *et al*. (1994).

Metals of the platinum group exhibit a considerable allergenic potency (Renner, 1984). In particular, allergic reactions to palladium—such as contact dermatitis, intra-oral contact mucositis, and lichenoid reactions—have been reported (Estler, 1992). Interestingly, from 34% to 64.5% of patients who are allergic to Ni are also allergic to Pd ions. Further, Pd sensitivity always occurs with Ni allergy. But only a few of these patients will also react to the metal form of Pd (Ketel and Niebber, 1981; Van Loon *et al*., 1986; Augthun *et al*., 1990; Rebandel and Rudzki, 1990; Van Wataha and Hanks, 1996; Schaffran *et al*., 1999; Gawkrodger *et al*., 2000).

A high sensitization rate to Pd (8.3%) was found in randomly selected eczema patients. Therefore, the authors questioned the "clinical future" of Pd-Ag alloys (Aberer *et al*., 1993). In contrast, Wataha and Hanks (1996) stated, in a later review article, that Pd, as a component of dental casting alloys, does not pose an increased risk to the health of patients, since the dissolution rate of Pd ions from these alloys is very low.

Gold restorations significantly increase the prevalence of gold sensitivity. One hundred thirty-six asymptomatic patients were patch-tested for gold sensitivity. Of these, 33.8% of persons with gold restorations had positive skin reactions, whereas only 10.8% of the patients without gold restorations revealed positive reactions (Schaffran *et al*., 1999).

A test battery has been created for the detection of allergic reactions to metal ions released from dental restorations (Van Loon *et al*., 1986). The most important cations concerning corrosion and related allergy were used for this test battery and included Au^{3+}, Pd^{2+}, Zn^{2+}, Mo^{6+}, Sn^{2+}, Ga^{3+}, Co^{2+}, Cr^{3+}, Cr^{6+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, and Si^{4+}. Subsequently, the relevance of those cations for the detection of allergic reactions to dental metallic restorations was investigated in patients with a history of contact stomatitis, contact dermatitis, and healthy control persons. Patch tests revealed a significantly higher percentage of positive reactions in allergy patients in comparison with healthy control persons. The most relevant cations were Pd and Ni, whereas Ga, Sn, and Zn caused no adverse reactions.

Although allergies to dental castings are not very frequent, a suitable alloy should be selected only after careful evaluation of its allergenic or immunological consequences. This is corroborated by Namikoshi *et al*. (1990), who investigated the prevalence of sensitivity to amalgam and casting alloys in 95 randomly selected persons. Six individuals developed positive reactions in epicutaneous patch tests to constituents of cast alloys, such as Cu, Ni, Co, Au, and Zn. In addition, medical devices (pacemakers), jewelry, and dental metallic restorations may cause metal allergy in patients. Ni, in particular, has been shown to be highly allergenic. Therefore, Ni-containing alloys should be avoided in persons with a history of Ni allergy. But there is a lack of agreement if the application of Pd alloys significantly increases the risk of allergic reactions to this ion. It must be emphasized, however, that many patients (from 34% to 65.5%) who are allergic to Ni are also allergic to Pd (Schaffran *et al*., 1999; Gawkrodger *et al*., 2000). This aspect should be taken into serious consideration before the use of Pd-containing alloys in patients who are allergic to Ni.

(VIII) Occupational Hazards for Dental Personnel

Dental technicians seem to have an increased risk for occupational diseases during the fabrication of dental metallic restorations. It has been found, for instance, that dental technicians excrete significantly more Pt, Pd, and Au than do road construction workers or high school graduates. This would suggest that laboratory technicians have an elevated occupational metal exposure (Begerow *et al*., 1999b). Inhalation and/or aspiration of dust and toxic vapors, which can be generated during the processing of low-fusing alloys, may irritate the respiratory and gastro-intestinal system (Material safety data sheet, 1992; Wee *et al*., 1998).

Pneumoconiosis is the most important fibrotic lung disease which can result from chronic exposure to inorganic dust. It was observed that the manufacturing of base metal restorations signifi-

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cantly increases the incidence of pneumoconiosis in dental technicians working in laboratories with no or insufficient local exhaust ventilation (Selden *et al*., 1995). A recent clinical screening among Cretan dental technicians revealed a 9.8% prevalence of pneumoconiosis (Froudarakis *et al*., 1999). Previous epidemiological studies have documented a prevalence of this lung disease in *ca*. 15% of technicians (Choudat, 1994). Exposure to dust from Co-Cr-, Co-Cr-Mo-, and Be-containing alloys was identified as a possible reason for dental technicians' pneumoconiosis. Particles from abrasives like silica and silicon carbide, which are also generated during the finishing of base metal frameworks, additionally increase individuals' risk of developing occupational lung diseases (Carles *et al*., 1978; Rom *et al*., 1984; De Vuyst *et al*., 1986; Selden *et al*., 1995, 1996; Nayebzadeh *et al*., 1999). The significance of abraded particles other than dust from alloys was verified experimentally. It was found that hard metal dust consisting of Co and tungsten carbide was more toxic than pure Co dust (Roesems *et al*., 1997).

Kulak and Arikan (1997) evaluated the risk of allergic reactions to Ni and Be in dental technicians who processed base metal alloys. No increased IgE concentration or alteration of various other blood parameters indicative of allergic reactions was found. These observations confirmed previous clinical observations that the processing of base metal alloys containing Ni, Cr, or Co does not increase the risk of allergy to these metals (Arikan and Kulak, 1992).

There are no reports that metallic restorations threaten the health of dental personnel other than technicians. The installation of sufficient dust exhaust ventilation in dental laboratories is therefore recommended.

(IX) Conclusions

Numerous *in vitro* studies have documented that each metallic dental restoration releases cations due to corrosion. These ions may be distributed in the oral cavity and systemically. Ni-based alloys in particular exhibit an increased corrosion at low pH. Beryllium-containing Ni-based alloys were significantly more susceptible to corrosion than were Be-free alloys. Generally, however, cation release cannot be predicted on the basis of the overall composition or the nobility of an alloy. But little is known about whether these *in vitro* data represent *in vivo* conditions accurately.

The biocompatibility of cast metallic restorations is primarily determined by the amount and nature of released cations. The biological effects of these metal ions are significantly different. Although contradictory data have been documented, many investigators have reported that Cu, Ni, and Be have pronounced cytotoxic potency. There is also evidence from *in vitro* investigations that various metallic elements, like Ni, Co, and Cr, can modulate the immune response. The *in vivo* effects of these metals on the immune response are as yet unknown.

Local and systemic allergic reactions to many metals have been observed, with Ni being the most frequent allergenic element. Additionally, various other factors could contribute to biological interactions of metallic restorations, such as physicochemical surface parameters (atomic ratio of noble to non-noble metals, *etc*.), phase formation, wear, and the quality of the manufacturing process itself. The importance of these factors still remains unclear, since very scarce information from *in vivo* studies is available.

There is no evidence to suggest that metallic dental restorations increase the mutagenic and carcinogenic risk in humans. Except for laboratory technicians, there is also no indication that metallic dental materials are an occupational hazard for dental personnel. These laboratory technicians have a higher risk for fibrotic lung diseases due to dust from metals and abrasives, but only if there is insufficient exhaust ventilation in dental laboratories.

Taken together, the knowledge about the mechanisms of biological interactions between metallic dental restorations and oral or systemic tissues is still very fragmentary. One might speculate that metal ions induce local allergic reactions which may be misdiagnosed as inflammatory reactions. Surprisingly, little information is available about the effects of casting alloys and individual metallic ions on micro-organisms.

Therefore, a high priority should be given to clarification of the following issues:

- determination of released metal ions from metallic dental restorations *in vivo*;
- development of *in vitro* tests, in particular "long-term assays", which better simulate the biological interactions of metallic components *in vivo*;
- determination of subcellular effects of individual metal ions and clinically relevant combinations of these cations which are decisive for the biocompatibility of metallic dental restorations; and
- clarification of the effects of casting alloys and individual metallic elements on micro-organisms.

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