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Cytotoxicity of Dental Alloy Extracts and Corresponding Metal Salt Solutions

G. Schmalz*, H. Langer, and H. Schweikl

Department of Operative Dentistry and Periodontology, University of Regensburg, D-93042 Regensburg, Germany; *corresponding author

Abstract. Adverse tissue reactions of the gingiva and the periodontium close to dental cast alloys may be caused by the effects of released metal elements. Tissue reactions depend upon the amounts of elements available which are a function of corrosion rates. Since pH values of standard corrosion solutions are as low as 2.3, such extracts are *a priori* not biocompatible. In the present study, elements released from dental cast alloys into cell-culture media were determined, and the cytotoxicity of these medium extracts was compared with the effectiveness of metal salt solutions prepared according to the metal elements found in extracts. The elements Ag, Cu, Ni, and Zn were detected in extracts of dental alloys and copper (positive control) by inductively coupled plasma atomic emission spectrometry (ICP-AES). Medium extracts of dental alloys were non-toxic in mouse fibroblasts (L929 cells). However, the amounts of elements found in these extracts were weakly cytotoxic when tested as salt solutions prepared from chloride (Cu^{2+}, Zn^{2+}, Ni^{2+}) or sulfate (Ag^{1+}) salts. When the test specimens were heat-treated before extraction, extracts were clearly cytotoxic in a dose-related manner. Again, the amounts of elements that caused 50% cell death (TC_{50}) were slightly lower in corresponding salt solutions than in extracts. In general, cytotoxicity of medium extracts consistently proved to be slightly less than that of the corresponding salt solutions, probably due to the limitations of the chemical analysis of extracts. This should be taken into account if salt solutions are used instead of the medium extract.

Key words: dental cast alloys, metal ions, corrosion, cytotoxicity.

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Introduction

Clinical observations of local adverse tissue reactions such as enhanced gingivitis and periodontitis in the direct vicinity of dental cast alloys may be related to the effects of metal cations released into the surrounding tissue (Wirz *et al.*, 1993; Wirz, 1993). This hypothesis is supported by animal (Reuling *et al.*, 1990) and *in vitro* (Wataha *et al.*, 1995) studies, which show that certain dental alloys may elicit adverse tissue and cell reactions. Since dental alloys are medical devices, the possibility of tissue damage has to be analyzed through pre-clinical risk assessment (International Organization for Standardization, 1995). Therefore, relevant pre-clinical biological tests are highly desirable as a basis for such a risk assessment.

Biological reactions to dental alloys depend upon the dose of available metal ions released from the materials—thus, upon corrosion rates (Brune, 1986). The corrosion rate is a function of the corrosion conditions—*e.g.*, the pH (Klötzer, 1985; Pfeiffer and Schwickerath, 1991). Reduced pH values may occur in the oral cavity because of acidic food, metabolites from oral bacteria in dental plaque (Pfeiffer and Schwickerath, 1991), and crevice corrosion (Wirz *et al.*, 1987). Standard corrosion tests, therefore, have been proposed to be performed at pH values down to 2.3 (Geis-Gerstorfer and Weber, 1987; International Organization for Standardization, 1993, 1996), resulting in an extract which contains metal ions. However, biological testing of such an extract presents a problem, since the low pH of the extract is not compatible with physiologic conditions of the biological test system.

A possible solution to this problem is to separate the extraction (corrosion) process from the biological testing procedure. In a first step, a medium extract containing metal elements derived from corrosion testing is chemically analyzed. In a second step, a test solution is prepared by the addition of metal ion salts to a biocompatible physiologic liquid (*e.g.*, cell-culture medium) based on the results of the

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chemical analysis of the medium extract. This test solution can then be used for biological evaluation. However, this approach then may present other problems, since it is dependent upon variables such as the limitations of the chemical analysis—*e.g.*, its sensitivity: Some metal ions may not be detected, but they may contribute to toxicity or they may reduce toxicity (Wataha *et al.*, 1992). Furthermore, the oxidation state of the metal ions cannot be determined by current chemical methods such as atomic absorption spectroscopy (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES).

To determine the possible role of these limitations and thus the suitability of the above-suggested approach, we compared the biological activity of the medium extract obtained from corrosion tests with that of a test solution prepared from metal salts. Data are reported from standard cytotoxicity tests.

Table 1. Composition of dental cast alloys

Alloy[a]	Composition, wt%
Degulor M	Au 70.0; Ag 13.5; Cu 8.8; Pt 4.4; Pd 2.0; Zn 1.2; Ir 0.1
Degulor C	Au 74.0; Ag 13.5; Cu 7.0; Pt 2.4; Pd 2.0; Zn 1.0; Ir 0.1
DG 92	Au 80.7; Pt 17.0; Zn 1.8; Ir 0.3; Ta 0.2
APO 16	Au 67.7; Ag 21.0; Pt 1.5; Pd 5.8; Zn 3.4; Sn 0.5; Ir 0.1
Palliag NF IV	Ag 52.0; Pd 39.9; Zn 4.0; Sn 2.0; In 2.0; Ir 0.1
Stabilor 7404	Au 58.0; Ag 25.0; Pd 12.95; Zn 4.0; Ir 0.05
Rexillium III	Ni 76.0; Cr 13.0; Mo 3.0; Ti 2.0; Be 1.5; Ga 1.0; Si 1.0; Co 0.5
Copper	99.98

[a] Dental alloys were manufactured by Degussa (Hanau, Germany), and copper was obtained from Aldrich (Steinheim, Germany).

Materials and methods

Test materials and extraction procedures

Specifications of the dental alloys used in this study are presented in Table 1. Pure copper was included as a reference material. Various dental alloys were cast by the manufacturer in specimen sizes of 35 mm x 11 mm x 1.1 mm. After being sandblasted, the surfaces of the test specimens were polished with abrasive paper (wet paper, type 1200). Selected specimens of alloys Degulor M, Degulor C, Stabilor 7404, APO 16, and Palliag NF IV were heat-treated at 800°C for 10 min; specimens of DG 92 and Rexillium III were heated to 950°C.

The test specimens were cleaned twice in 10 mL of deionized water in an ultrasonic bath for 10 min and then dried. Six specimens of each dental alloy were sterilized individually by being autoclaved in closed 10-mL glass vials (Duran; Schott, Wetzlar, Germany) at 121°C, 1.1 atm for 20 min. Then, a 7-mL quantity of cell-culture medium (Eagle's basal medium supplemented with 5% newborn calf serum) was added to each test tube at room temperature, and the test specimens were extracted for 3 days and 7 days at 37°C in an air atmosphere containing 5% CO_2 according to ISO 10993-5 (International Organization for Standardization, 1992). Extracts of 6 test samples were pooled and used as stock solutions for further chemical and biological analyses (see below). One of the 6 specimens which were extracted was heat-treated. For alloys Stabilor 7404 and APO 16, the same regimen was followed, but extracts were prepared from 6 specimens which were heat-treated at 800°C for 10 min. These extracts are referred to as extracts of heat-treated samples.

Analysis of element release and preparation of salt solutions

The medium extracts of dental cast alloys were analyzed for traces of Ag, Al, Au, Be, Co, Cr, Cu, Ga, In, Ir, Ni, Sn, Ta, and Zn by inductively coupled plasma atomic emission spectrometry, ICP-AES (Spectroflame-ICP D, Spectro Analytical Instruments, Kleve, Germany). The detection limit was 0.01 ppm except for copper and zinc (0.05 ppm). Either one or two elements were detected in the medium extracts, as presented in "Results". Accordingly, salt solutions were prepared which contained either one cation or various combinations of two metal cations. Sterile salt solutions (stocks) were made from $CuCl_2$, $ZnCl_2$, Ag_2SO_4, and $NiCl_2$ (Sigma, Deisenhofen, Germany) and were diluted in cell-culture media at concentration ranges encompassing the concentrations of elements found in the extracts. The cytotoxicity of these ionic salt solutions was determined as described below.

Cytotoxicity testing

L929 mouse fibroblasts were routinely cultivated in Eagle's basal medium (Sigma, Deisenhofen, Germany) supplemented with 5% newborn calf serum (Gibco BRL, Life Technologies, Eggenstein, Germany) at 37°C in an air atmosphere containing 5% CO_2. For cytotoxicity testing, 1×10^4 cells/well were seeded in 96-well plates (Greiner, Frickenhausen, Germany) and incubated for 24 hrs. Then, the cells were exposed to 200 µL of the various serial dilutions of extracts of dental cast alloys or salt solutions for 24 hrs. Exposure of L929 cells was stopped by the discarding of the exposure medium, and cell viabilities were recorded immediately by means of the MTT assay. Briefly, MTT assay solution (1 mg/mL) was added to each well. After a three-hour incubation period (37°C, 5% CO_2), the supernatant was removed, and the intracellularly stored MTT formazan was solubilized in 200 µL dimethyl sulfoxide for 1 hr at room temperature. Optical densities were then measured at 570 nm in a multiwell spectro-photometer (EL311, Biotek Instruments, Burlington, VT, USA).

Statistical analysis

All cell cultures were performed in 8 replicates *per* dose. Optical densities read from treated cell cultures were related to untreated cell cultures (= 100%) and expressed in percent. Dose-response curves between cell survival rates and serial dilutions of medium extracts of dental cast alloys were fitted to the data (Table Curve TM 3.10, Jandel Scientific, San Rafael, CA, USA). TC_{50} values (concentration corresponding to 50% relative optical density) were calculated together with the corresponding 95% confidence limits, and cytotoxic effects were considered different if the 95% confidence intervals were distinct.

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Table 2. Elements released from various dental cast alloys into cell-culture medium as analyzed by ICP-AES

Dental Alloy	Extraction Period (days)	Element	µg/mL (mean ± SD)	µmol/L (mean)	Element	µg/mL (mean ± SD)	µmol/L (mean)
Degulor M	3	Zn	0.51 ± 0.01[a]	7.8	Cu	0.89 ± 0.00	14.0
	7	Zn	0.51 ± 0.01	7.8	Cu	1.99 ± 0.01	31.3
Degulor C	3	Zn	0.68 ± 0.01	10.4	Cu	0.83 ± 0.01	13.1
	7	Zn	0.53 ± 0.01	8.1	Cu	1.26 ± 0.02	19.8
DG 92	3	Zn	0.77 ± 0.02	11.8			
	7	Zn	0.85 ± 0.02	13.0			
Stabilor 7404	3	Zn	0.93 ± 0.01	14.2			
	7	Zn	0.99 ± 0.03	15.1			
Stabilor 7404, ox.[b]	3	Zn	3.72 ± 0.08	56.9			
	7	Zn	3.39 ± 0.02	51.9	Ag	0.14 ± 0.01	1.3
Palliag NF IV	3	Zn	0.51 ± 0.02	7.8	Ag	0.19 ± 0.00	2.9
	7	Zn	0.47 ± 0.01	7.2	Ag	0.41 ± 0.00	3.8
APO 16	3	Zn	1.59 ± 0.06	24.3			
	7	Zn	1.37 ± 0.02	21.0			
APO 16, ox.	3	Zn	3.28 ± 0.04	50.2			
	7	Zn	3.37 ± 0.02	51.5			
Rexillium III	3				Ni	0.39 ± 0.00	6.6
	7				Ni	0.65 ± 0.00	11.1
Copper	3				Cu	57.2 ± 0.50	900
	7				Cu	103.1 ± 0.46	1622

[a] The concentrations given were obtained from pooled samples as described in "Materials and methods"; measurements were carried out in triplicate.
[b] ox. = All samples were heat-treated before extraction.

Results

Chemical analysis of the medium extract

Specimens of high-noble dental cast alloys, a Ni-Cr alloy, and copper, which was used as a positive control material, were extracted into cell-culture media for 3 and 7 days, and the alloy element release was chemically analyzed. The elements Ag, Cu, Ni, and Zn were detected in the cell-culture medium analyzed by ICP-AES (Table 2). Zinc, which was present in all high-noble alloys tested, was also detected in all cell-culture extracts obtained from high-gold alloys and a Ag-Pd-based alloy. The quantities of Zn extracted from test samples were almost identical after a three-day and a seven-day extraction period. However, the amounts of Zn released into the cell-culture medium were about 2 to 4 times higher in pooled extracts of heat-treated test samples (Stabilor 7404, APO 16) compared with extracts of test samples which contained only one heat-treated specimen (Table 2). Cu was extracted from 2 copper-containing high-gold alloys in similar quantities. Ag was not detected in extracts of these alloys, but Zn was present in both extracts in almost equal amounts. Interestingly, Ag, Ni, and Cu were consistently released at higher rates when the extraction period was increased from 3 to 7 days. The amounts of Ag extracted from an Ag-Pd-based alloy (Palliag) slightly increased with extraction time. The quantities of Ni released from a Ni-Cr-based alloy and of Cu released from copper test samples were doubled with increased extraction times.

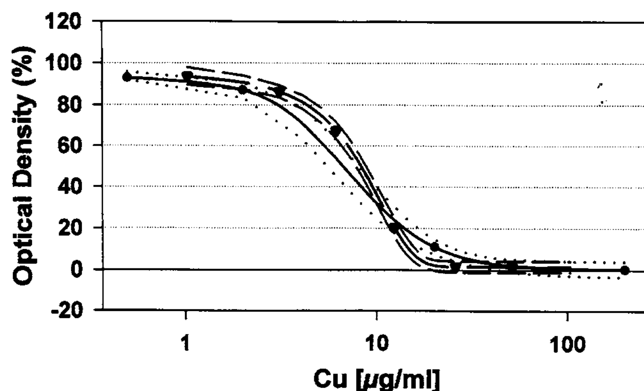

Figure 1. Cytotoxicity of copper and copper (2^+) chloride. Copper content was analyzed in a medium extract of copper test specimens (▼) after a seven-day extraction period, and a copper chloride salt solution (●) was prepared accordingly. Cell survival rates are expressed as normalized optical density readings; typical dose-response curves, including 95% confidence limits (dotted line for copper and dashed line for copper chloride), were fitted from original data.

Cytotoxicity of the medium extracts

Extracts of copper specimens were cytotoxic in L929 mouse fibroblast cells, and Fig. 1 shows an example of the cell reaction in a typical dose-response curve fitted from normalized optical density readings. The dilutions of the

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Table 3. TC_{50} values of cytotoxic extracts of dental cast alloys

Dental Alloy	Extraction Period (days)	TC_{50}	95% Confidence Limits
Stabilor			
7404, ox.[a]	3	45.0 (Zn)[b,c]	43.3 - 46.1
	7	42.6 (Zn)	41.0 - 43.6
		1.1 (Ag)	1.07- 1.14
APO 16, ox.	3	39.9 (Zn)	35.3 - 45.0
	7	43.9 (Zn)	42.2 - 45.0
Copper	3	123.6 (Cu)	110.8 - 135.5
	7	147.3 (Cu)	139.9 - 158.3

[a] Test specimens were heat-treated and then extracted in cell-culture medium as described in "Materials and methods".
[b] The metal elements found in medium extracts are given in parentheses.
[c] The numbers are mean values from 8 replicate cultures to indicate concentrations (µmol/L) of elements determined in extracts that caused 50% cell death (TC_{50}) and 95% confidence limits.

Table 4. TC_{50} values of metal salt solutions

Salt Solution[a]	Cytotoxicity[b] TC_{50}	95% confidence limit
Copper chloride	107.0	88.1 - 125.9
Zinc chloride	25.5	22.0 - 28.9
Silver sulfate	4.8	3.6 - 6.0
Nickel chloride	332	295 - 365
Copper chloride/zinc chloride	88.0 (Cu)	75.8 - 102.4
(Degulor M-based)	21.0 (Zn)	18.1 - 24.4
Zinc chloride/silver sulfate	21.3 (Zn)	17.4 - 25.8
(Palliag NF IV-based)	10.4 (Ag)	8.5 - 12.6
Zinc chloride/silver sulfate	29.0 (Zn)	23.6 - 34.9
(Stabilor-based)	0.8 (Ag)	0.7 - 1.0

[a] The salt solutions were prepared according to the levels of various metal ions detected in medium extracts of dental cast alloys and copper specimens given in Table 2.
[b] The numbers are mean values from 8 replicate cultures to indicate concentrations (µmol/L) which caused 50% cell death (TC_{50}), and 95% confidence limits were also included.

original copper extracts which caused a 50% reduction in cell viability contained Cu at concentrations of 123.6 µmol/L and 147.3 µmol/L after extraction periods of 3 days and 7 days, respectively (Table 3). These concentrations were calculated on the basis of ICP-AES analyses of pooled cell-culture media extracts. Serially diluted extracts of selected dental cast alloys were also cytotoxic when all samples were heat-treated before extraction. The extracts of Stabilor 7404 which caused 50% cell death (TC_{50}) contained 45 µmol/L Zn (three-day extract) and 42.6 µmol/L Zn and 1.1 µmol/L Ag (seven-day extracts). Likewise, extracts of heat-treated APO 16 test samples reduced cell numbers to 50% at Zn concentrations of 39.9 µmol/L (three-day extract) and 43.9 µmol/L (seven-day extract) (Table 3). Extracts obtained from dental alloys with only one heat-treated sample and from a Ni-Cr-based alloy were non-toxic in L929 cells (see below in Table 5).

Cytotoxicity of ionic salt solutions

The elements Cu, Zn, and Ag were detected in extracts of various high-noble dental cast alloys and of copper test specimens. Therefore, we also tested corresponding salt solutions to compare the cytotoxicity of medium extracts of dental cast alloys and metal ion salt solutions. Table 4 summarizes the cytotoxicity data of various ionic salt solutions. TC_{50} values for Cu, Zn, and Ag were 107 µmol/L, 25.5 µmol/L, and 4.8 µmol/L, respectively. Ni, which was present in non-toxic extracts of a Ni-Cr-based alloy, reduced cell survival rates to 50% at concentrations between 295 µmol/L and 365 µmol/L (Table 4). Copper and zinc were combined as chloride salts in a test solution based on the Cu and Zn levels analyzed in non-toxic extracts of a copper-containing high-gold alloy (Degulor M, seven-day extract). The test solution which reduced the viability of L929 cells to 50% of control cultures contained Cu and Zn levels of 88 µmol/L (Cu) and 21 µmol/L (Zn). Silver sulfate and zinc chloride salts were combined in two different ratios based on the cytotoxic extracts of an Ag-Pd alloy (Palliag NF IV) and heat-treated Stabilor 7404 samples (seven-day extracts). The test solution based on an extract of Palliag NF IV specimens exhibited a TC_{50} value of 10.4 µmol/L Ag and 21.3 µmol/L Zn. The TC_{50} value of the Stabilor-based combination of the elements was 0.8 µmol/L Ag and 29 µmol/L Zn (Table 4).

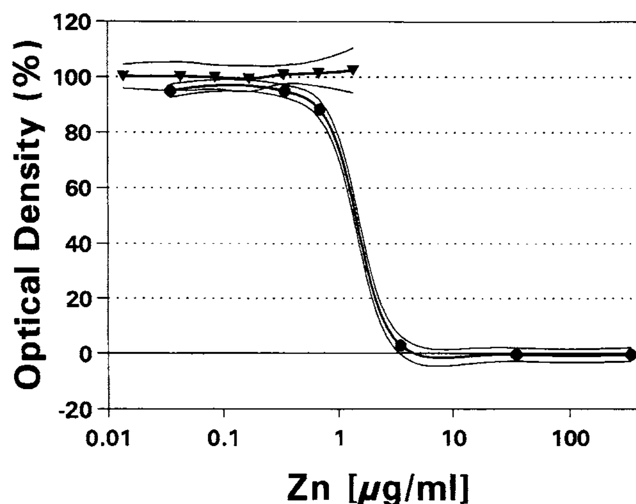

Figure 2. Cytotoxicity of a dental cast alloy (APO 16) expressed on the basis of zinc levels detected by chemical analysis in medium extracts of non-heat-treated test specimens (▼). In comparison with these data, survival rates of mouse fibroblasts are shown after exposure to amounts of zinc present in a test solution prepared from zinc chloride (●). Cell survival rates are expressed as normalized optical density readings; typical dose-response curves, including 95% confidence limits, were fitted from original data.

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Table 5. Comparison of the cytotoxic potencies of medium extracts of dental alloys and ion salt solutions

		Cytotoxicity of Medium Extracts[a]		Cytotoxicity of Ion Salt Solutions[b]	
Dental Alloy	Extraction Period	Cell Survival[c]	95% Confidence Limit	Cell Survival	95% Confidence Limit
Degulor M	7 (days)	102.1	94.8 - 109.2	79.3	76.2 - 82.2
DG 92	3 (days)	115.6	107.9 - 123.3	86.2	82.6 - 90.0
DG 92	7 (days)	117.1	111.6 - 122.6	83.3	79.4 - 87.6
Stabilor 7404	3 (days)	93.9	86.6 - 101.3	80.7	76.1 - 85.3
Stabilor 7404	7 (days)	119.3	112.7 - 125.9	78.4	73.3 - 83.2
Palliag NF IV	7 (days)	93.8	84.9 - 102.8	76.0	72.6 - 79.7
APO 16	3 (days)	107.6	104.4 - 110.8	52.6	41.8 - 62.1
APO 16	7 (days)	102.4	93.6 - 111.3	61.6	53.7 - 69.8
Rexillium	3 (days)	102.0	96.2 - 107.9	94.2	90.0 - 97.6
Rexillium	7 (days)	107.0	98.3 - 115.7	83.1	79.6 - 86.7

[a] Cytotoxicity is expressed as cell survival of exposed cell cultures related to untreated cell cultures.
[b] Cytotoxicity of ion salt solutions means survival rates of cell cultures which correspond to metal element levels found in medium extracts (as given in Table 2).
[c] The numbers are mean values from 8 replicate cultures to indicate concentrations (µmol/L) which caused 50% cell death (TC_{50}), and 95% confidence limits were also included.

Comparison of cytotoxicity of medium extracts and the corresponding salt solution

The survival rates of cell cultures after exposure to extracts of APO 16 test specimens extracted for 7 days in cell-culture medium serve as an example for the observations of different cytotoxic effects of identical amounts of Zn, depending on the source of the element applied to cell cultures (Fig. 2). Serial dilutions of the medium extract were non-toxic, and the Zn levels indicated were calculated from the Zn content of the medium extract. A dose-response relationship between increasing concentrations of Zn in a zinc chloride salt solution and cell survival is shown in the second graph. A Zn concentration that corresponds to the Zn levels found in the medium extract decreased cell survival in cell cultures exposed to the zinc chloride solution to almost 60%. Confidence limits (95%) that described cell survival by both graphs overlapped at non-toxic Zn concentrations but were different at Zn levels present in the undiluted medium extract (Table 5).

Zinc was also detected in non-toxic extracts of DG 92, Stabilor 7404, and APO 16 test specimens. Interestingly, these amounts of Zn were cytotoxic in L929 cells when tested as a zinc chloride salt solution. Cell survival rates were reduced to 86% and 83% relative optical density by a test solution based on Zn levels found in DG92 extracts. Cell survival was even lower with Zn levels found in extracts of Stabilor 7404 (81% and 78%) and in extracts of APO 16 (53% and 62%) test specimens (Table 5). Confidence limits of these cell survival rates were different from those observed after the medium extracts were tested. Also, extracts of a Ni-Cr-based alloy were non-toxic, but the amounts of Ni present in extracts reduced cell survival rates to 94% and 83%, respectively, when tested in a $NiCl_2$ test solution (Table 5).

The two elements copper and zinc were present in non-toxic extracts of Degulor M after 7 days of incubation. A metal ion salt test solution, however, based on exactly these copper and zinc levels, reduced cell viability to approximately 79% of untreated control cultures. Likewise, extracts of Palliag NF IV which contained zinc and silver after 7 days of incubation were non-toxic, but a test solution which contained these levels of zinc and silver reduced cell viability to approximately 76%. Again, the confidence limits of the survival rates were, after exposure to salt solutions, different from those of the medium extracts (Table 5).

Similar to the more toxic effects of ionic salt solutions containing the amounts of metal elements found in non-toxic medium extracts by chemical analysis, TC_{50} values were lower in salt solutions than in extracts. Confidence limits (95%) of TC_{50} values of cytotoxic extracts and ionic salt solutions were distinct except for the cytotoxic effects of copper levels extracted after a three-day extraction period and the copper chloride solution (Tables 3, 4).

Discussion

The alloys tested in the present study on element release and cytotoxicity represent currently available materials used in dental practice. Selected test specimens were heat-treated before being tested. Heat treatment of dental alloys is necessary for establishing a chemical bond between ceramic and metal surface, *e.g.*, through an oxide layer. Heat treatment changes the surface of the metal to a depth of 30 µm, and elements like zinc can diffuse to the surface of the alloy (Kappert, 1992). This phenomenon has also been demonstrated for nickel-chromium alloys (Schwickerath, 1990). The altered surface promotes enhanced solubility and release of metal elements (mainly zinc) (Kappert, 1992), which may be the reason for enhanced toxicity. Therefore, the manufacturers of dental alloys recommend removing this surface layer mechanically or—more recently—chemically. One out of a total of 6 test specimens was heat-treated in the present study to simulate the incomplete removal of this surface layer which may occur in a clinical

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situation. A set of experiments was performed with heat-treated samples only to demonstrate the biologic effect of this procedure. The ratio of the volume of the extraction medium to sample surface was kept at 0.8 mL/cm^2 surface and was within the range recommended by standard protocols (International Organization for Standardization, 1992).

Metal element levels in extracts of the dental alloys were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES). This method had been used in previous studies (Weber *et al.*, 1986; Reuling, 1992); other investigators frequently use atomic absorption spectroscopy (AAS) (Pfeiffer and Schwickerath, 1990; Bumgardner and Lucas, 1995; Wataha *et al.*, 1995). Although the detection limits of the ICP-AES depend on the type of element analyzed and are in most cases higher than those of AAS (Schedle *et al.*, 1995), ICP-AES analysis is preferably used if concentration ranges are varying widely and a large range of linearity of the standard curve is necessary. Furthermore, another advantage is that multiple analyses of metal elements can be run by ICP-AES (Schrader, 1982).

Only serially diluted extracts prepared from heat-treated test samples were cytotoxic in the present investigation. This is in accordance with the results from the chemical analysis and can be attributed to the enhanced solubility of unnoble metals, like copper and zinc. The extraction of copper and zinc from copper-containing high-noble alloys in the present study is in accordance with data presented in a recent investigation (Wataha *et al.*, 1995). Ag was also detected in cytotoxic extracts, but these concentrations were obviously below those necessary to reduce cell survival. A similar correlation of element release from dental alloys and toxicity was observed previously. Noble and Ag-based alloys released greater amounts of metal elements than high-noble alloys, and Cu and Ag were identified as candidates that cause cytotoxicity with these materials (Wataha *et al.*, 1995). A high-noble alloy (Degulor M) was severely cytotoxic under test conditions different from those in the present investigation. This effect was probably caused by copper released from the dental alloy (Berstein *et al.*, 1992). Ni-Cr-based dental alloys, on the other hand, did not reduce cell viability or change cell morphology but decreased cell proliferation which was associated with the release of beryllium (Bumgardner and Lucas, 1995).

The TC_{50} value of copper chloride found here is in accordance with data described in the literature. For the TC_{50} for copper chloride, we found 107 µmol/L in the present investigation and 137 µmol/L in a recent study (Schmalz *et al.*, 1997). Wataha *et al.* (1991) reported 240 µmol/L, and Schedle *et al.* (1995) found 97 µmol/L. The range for zinc chloride was from 22 to 29 µmol/L, and for silver sulfate, from 3.6 to 6.0 µmol/L. These findings are identical to those reported by Wataha *et al.* (1991). Zinc chloride toxicity differed considerably from the data presented by Schedle *et al.* (1995). There is strong evidence that the cytotoxic effects of the metal ion salt solutions were caused by cations alone. Anions like chloride or nitrate were not effective at concentrations as low as tested here (Hulme *et al.*, 1987; Wataha *et al.*, 1991; Schmalz *et al.*, 1997).

A simple extrapolation of the present data to the clinical situation is not possible—*e.g.*, because of the neutral pH during the extraction process. However, these conditions were used so that we could compare the original extract with a test extract prepared from ion salts, taking into consideration that only extracts with neutral pH can be applied to cells in culture without damaging the cells simply by the pH and not by the metal ion. Maybe even fewer amounts of metal ions are released from a dental alloy into a corrosion solution of low pH compared with solutions at neutral pH value, because of passivation of the surfaces of alloy specimens by hydrogen molecules.

Corrosion of dental cast alloys is routinely tested by means of a lactic acid/NaCl solution in static immersion assays. Cell-culture medium had to be used here as an extraction medium instead of a corrosion solution for various reasons. Cell-culture medium is appropriate when cells are exposed to a test substance for at least 24 hrs. Therefore, the cytotoxicity of metal ions is usually tested in cell-culture medium (Schedle *et al.*, 1995; Wataha *et al.*, 1995; Schmalz *et al.*, 1997). Also, the biological activity of a standard corrosion solution cannot be analyzed *in vitro* because of incompatible low pH values, lactic acid, and sodium chloride concentrations. Adjustment of the low pH values of a corrosion solution to neutral is possible; however, this procedure would add alkali metal ions, and, most importantly, any dilution of the neutralized solution would change the equilibrium between monomeric and oligomeric lactic acid. Furthermore, any dilution step would change two experimental parameters—the concentrations of lactic acid and sodium chloride—simultaneously. The influence of only one of these parameters on cell survival rates cannot be distinguished.

It was consistently observed that the metal salt solution showed slightly higher toxicity values than the medium extract for a range of different dental alloys. The levels of metal elements detected in the extracts, however, were high enough to produce cytotoxic effects when used as salt solutions. Reduced toxicity of extracts may be due to the specific extraction conditions or to a protecting effect by other metal ions which could not be detected with the analytical method used in this study. Such a protecting effect has been described for a number of metal combinations (Wataha *et al.*, 1992). Another explanation for this unusual effect is that the valencies of metal elements released from dental cast alloys are different from those of the corresponding cations in chloride or sulfate salt solutions. Therefore, the bioavailability of metal elements released from cast alloys might be lower compared with that of metal cations, resulting in lower toxic potencies.

In summary, salt solutions containing the species and the amounts of metal ions detected in cell-culture extracts of dental alloys are slightly more toxic than the medium extract which is regarded as reflecting the impact of the limitations of the chemical analysis. If salt solutions are used as a substitute for the medium extract, this should be taken into account.

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