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RESEARCH AND EDUCATION

Immunostimulatory capacity of dental casting alloys on endotoxin responsiveness

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ABSTRACT

Statement of problem. Oral metal exposure has been associated with systemic and local adverse reactions, probably due to elemental release from the alloys. Although supraphysiological concentrations of salts from dentally applied metals can activate innate cells through TLR4 (Ni, Co, Pd) and TLR3 (Au), whether direct exposure to solid alloys can also trigger innate immune reactivity is still unknown.

Purpose. The purpose of this in vitro study was to determine whether dental cast alloy specimens can activate innate cells and influence their responsiveness to bacterial endotoxin.

Material and methods. Human monocyte-derived dendritic cells (MoDC) and THP-1 cells were cultured on top of different alloy specimens (Ni-Cr, Co-Cr, Pd-Cu, Pd-Ag, Ti-6Al-4V, amalgam, gold, and stainless steel) or in alloy-exposed culture medium with or without endotoxin (lipopolysaccharide [LPS]; *Escherichia coli* 055:B5). Interleukin-8 (IL-8) production was used as the parameter for innate stimulation and evaluated by enzyme-linked immunosorbent assay after 24 hours of culture. The statistical significance of the effects of various casting alloys on the secretion of IL-8 was analyzed by using the nonparametric Wilcoxon rank sum test (α=.05).

Results. Dental cast alloys induced IL-8 production in MoDC and THP-1 cells, with Au and Pd-Cu providing the strongest stimulation. The alloy-exposed culture media tested contained sufficient stimulatory metal ions to induce detectable IL-8 production in THP-1 cells, except for the Ni-Cr and stainless steel exposed media. Au and Pd-Cu alloys were also most effective in potentiating LPS responsiveness as measured by IL-8 production.

Conclusions. Using an in vitro culture system to expose MoDC and THP-1 cells to different alloy specimens this study showed that contact with the solid alloys, in particular when they contain Pd or Au, can trigger innate immune responses and augment responsiveness to bacterial endotoxin. (J Prosthet Dent 2017;117:677-684)

The oral cavity is a port of entry for early infections. Many Gram-positive and -negative bacteria contribute to dental plaque around the teeth and colonize the mucosal surface. Balancing the inflammatory reactions effectively to fight pathogenic invaders without excessive damage is of major importance. How far the presence of dental cast alloys in the oral cavity can affect innate responsiveness against bacteria is unclear.

Previous studies have concentrated on the immune stimulatory capacity of nickel (Ni), cobalt (Co), and other transition metals. In those studies Ni, Co, and palladium (Pd) were shown to activate monocyte-derived dendritic cells (MoDC) by binding to TLR4, gold (Au) predominantly to TLR3, and copper (Cu) and mercury (Hg) activated innate cells by still unknown mechanisms.[1,2] Low, near-physiological metal ion concentrations, like those found in the blood, did not activate innate cells but could potentiate the endotoxin responsiveness of THP-1 cells, a cell line used as a model

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Clinical Implications

Dental alloys, particularly those containing Pd or Au, may cause irritation by triggering innate immune reactivity. In addition, these alloys may increase the patient's antimicrobial immune responsiveness.

for innate immune reactivity.[3] The local concentrations of metal ions in the oral cavity, particularly in the immediate vicinity of metal restorations, may, however, exceed these physiological plasma levels.[5] Conditions in the oral cavity created by temperature, microbiota, pH, saliva, exogenous agents like tobacco and alcohol, ongoing immune reactions,[7-12] the combination of different metals, and the shape and surface treatment of the dental alloys may increase corrosion and subsequent metal ion release.[6,13]

All of the previously cited studies were performed with metal salt solutions. Also, the potential allergenicity of metals is commonly evaluated by testing metal salts.[1-3,14] The use of metal salt solutions was thus considered most relevant by most investigators,[6,13] and as a consequence, the direct effects of the different dentally applied alloys on innate immune cells have so far received little attention.

In the present study, dental cast alloys were tested in vitro as solid specimens, reflecting the actual situation in the oral cavity. To study the effects of dental alloys on innate cells in vitro, the alloys most frequently used were selected (Table 1). For innate responder cells ex vivo, human MoDC and the THP-1 cell line were selected, as a cell line would be more convenient for future large scale screening studies. The release rates of metals from dental alloys are generally in the range of nanograms to micrograms per square centimeter of exposed surface area as reported in various studies (Table 2).

Because the pathogenic role of gram-negative bacteria in the oral cavity, such as in subgingival plaque[15] and periodontitis,[16-18] is of major importance, the endotoxin lipopolysaccharide (LPS) was used to represent bacterial innate stimulation. LPS is a powerful innate stimulant acting through ligation of TLR4[19] and represents the major PAMP molecule produced by Gram-negative bacteria. Interleukin-8 (IL-8) release was taken as a sensitive read-out for innate immune signaling involving NF-κB activation. It is a prominent proinflammatory chemokine that attracts various immune cells to inflammatory sites, thus facilitating both innate and adaptive immune responses.[20-23]

The present study questioned how far different dental cast alloys can trigger innate responsiveness in MoDC and THP-1 cells as a model and whether these cast alloys or the low levels of metal ions released from them can potentiate or interfere with their endotoxin responsiveness.

Table 1. Alloys selected

Number	Material	Manufacturer	Composition	Usage
1	Acrylate (methyl methacrylate)	GC Europe NV	• Copolymer of methyl methacrylate and diethyl phthalate, poly (methyl methacrylate) (powder) • Methyl methacrylate and *N,N*-dimethyl-p-toluidine (liquid)	Interim crown and fixed prosthesis resin
2	Ni-Cr alloy	Noritake Dental Supply	Ni 62%, Cr 19.1%, Mo 7.1%, Ga 2%	Metal-ceramic restorations
3	Co-Cr alloy	Brident Intl	Co 63%, Cr 27%, Mo 6%, Other 4%	Partial denture alloy
4	Pd-Cu alloy (Orion Vesta)	Elephant Dental BV	Au 2%, Pd 78.9%, Cu 10%, Ga 9%, Ir <1%	Metal-ceramic restorations
5	Pd-Ag alloy (Orion Argos)	Elephant Dental BV	Au 0.1%, Pd 53.3%, Ag 36.3%, Sn 7%, In 2%, Zn <1%	Metal-ceramic restorations
6	Ti (Ti-6Al-4V)	Straumann AG	Al 6% and V 4%	Implants, crowns, fixed prostheses
7	Amalgam (Cavex non-gamma-2)	Cavex Holland BV	Hg 50%, Ag 22-32%, Sn14%, Cu 8%, and other trace metals	Direct restorations
8	Au alloy (Degudent G) (Pd free)	Elephant Dental BV	Au 86%, Pt 11.5%, Zn 1.6% In, Rh, Fe each <1%	Metal-ceramic restorations
9	Stainless steel	Dentaurum	Fe 69.35%, Ni 10%, Cr 17%, Mo 2-4%, Si 1%	Pediatric crowns; orthodontic brackets and wires

MATERIAL AND METHODS

MoDC culture

MoDC were generated from freshly prepared peripheral blood mononuclear cell as previously described.[24] Briefly, adherent monocytes were cultured for 6 to 7 days in a humidified incubator in 10 mL of Iscove's modified Dulbecco's medium (BioWhittaker Inc) supplemented with 10% heated-inactivated fetal calf serum (Hyclone), 0.1 mg/mL streptomycin (Invitrogen), 100 IU/mL Na-penicillin G (Invitrogen), 1% L-glutamine (200 mM; Merck), 1% β-mercaptoethanol, 1000 U/mL granulocyte-macrophage colony-stimulating factor (Novartis Pharma), and 20 ng/mL IL-4 (RandD systems lot AG270911A). After 6 to 7 days, immature dendritic cells were harvested and seeded on the surface of different dental cast alloys in the 24-well, flat tissue culture plates of polystyrene (Greiner Cellstar; Greiner Bio-One GmbH; 25×10^4 cells/well in a final volume of 1 mL).

THP-1 cells

A vial of cell passage 17 of THP-1 cells (American Type Culture Collection) was kept at -80°C until thawing. The cells were cultured in 75-cm^2 culture flasks (Greiner Cellstar; Greiner Bio-One GmbH) at a density of 10^6 cells /1 mL of RPMI 1640 medium (BioWhittaker Inc) containing

Table 2. Reported metal ion release in vitro from dental equipment and alloys

Metal	Type of Alloy (Name/Brand)	Methods and Conditions of Exposure[a] Device/Specimen Quantification	Fluid Volume	Duration	Release µg/cm²	ppb (µg/L)	nM	Reference[b]
Ti	Ti-6Al-4V Zimmer (Sulzer Orthopedics)	Dental implant Ø 22 mm×2 mm	MEM 100 mL	28 d	nd	nd	nd	26
	Ti (Ormco)	20 orthodontic brackets 4 buccal molar tubes	MEM 30 mL	30 d	?	5.4	112	27
Cr	Ni-Cr (Remanium CS)	Cast alloy specimen Ø 5 mm×3 mm	Artificial saliva 11.5 mL	60 d	0.01	0.8	15	28
	Ni-Cr (Vera Bond II)	Cast alloy specimen + electrolysis 40×20×3 mm	Artificial saliva 50 mL	2 d	0.17	66.5	1.279	29
	Co-Cr-Mo (Wironit)	Cast alloy specimen Ø 8 mm×3.2 mm	Artificial saliva pH 7.5 Artificial saliva pH 4 5 mL	6 wk	1.52 1.80	550.0 650.0	10.577 12.500	30
	Co-Cr (Remanium)	Cast alloy specimen + electrolysis 40×20×3 mm	Artificial saliva 50 mL	2 d	0.10	37.9	729	29
	Stainless steel (Ultraminitrim, Dentaurum)	20 orthodontic brackets 4 molar tubes	MEM 30 mL	30 d	?	9.0	173	27
	Stainless steel (Preform arch wires, Ortho-Organizers)	Orthodontic wire 0.017×0.025 inch 100 mm length	Artificial saliva 100 mL	28 d	0.51	11.0	212	31
	Stainless steel (American Orthodontics, 3M Unitek)	2 orthodontic wires 20 ortho brackets 2 ligatures	Artificial saliva flow, 0.5 mL/min 19.41 L (flow)	28 d	?	0.3	5	25
Co	Co-Cr-Mo (Wironit)	Cast alloy specimen Ø 8 mm×3.2 mm	Artificial saliva pH 7.5 Artificial saliva pH 4 5 mL	6 wk	1.74 4.04	630.0 1460.0	10.678 24.746	30
	Co-Cr (Remanium)	Cast alloy specimen + electrolysis 40×20×3 mm	Artificial saliva 50 mL	2 d	0.02	9.3	158	29
	Stainless steel (Ultraminitrim, Dentaurum)	20 orthodontic brackets 4 molar tubes	MEM 30 mL	30 d	?	1.4	23	27
	Ni-Cr (Remanium CS)	Cast alloy specimen Ø 5 mm ×3 mm	Artificial saliva 11.5 mL	60 d	0.28	21.1	358	28
Ni	Ni-Cr (Remanium CS)	Cast alloy specimen Ø 5 mm×3.2 mm	Artificial saliva 11.5 mL	60 d	1.10	82.9	1.405	28
	Ni-Cr (Vera Bond II)	Cast alloy specimen + electrolysis 40×20×3 mm	Artificial saliva 50 mL	2 d	0.53	207.0	3.508	29
	Stainless steel (Ultraminitrim, Dentaurum)	20 orthodontic brackets 4 molar tubes	MEM 30 mL	30 d	?	417.0	7,067	27
	Stainless steel (Preform arch wires, Ortho-Organizers)	Orthodontic wire 0.017×0.025 inch 100 mm length	Artificial saliva 100 mL	28 d	0.28	6.0	102	31
	Stainless steel (American Orthodontics, 3M Unitek)	2 orthodontic wires 20 ortho brackets 2 ligatures	Artificial saliva flow, 0.5 mL/min 19.41 L	28 d	?	1.0	16	25
Cu	Stainless steel (American Orthodontics, 3M Unitek)	2 orthodontic wires 20 ortho brackets 2 ligatures	Artificial saliva flow, 0.5 mL/min 19.41 L	28 d	?	1.6	0.25	25
	Pd-Cu (Orion Vesta)	Cast alloy specimen 2.06 cm²	Lactic acid/NaCl; pH 2.3 5 mL	7 d	2.62	1048.0	9850	4
	Pd-Ag (Orion Argos)	Cast alloy specimen 2.06 cm²	Lactic acid/NaCl; pH 2.3 5 mL	7 d	0.05	20.0	188	4
	Amalgam (Tytin; Kerr UK)	Discs Ø 10 mm×2 mm	0% H_2O_2 1% H_2O_2 20 ml	24 h	0.08 0.38	9.2 41.6	143 650	32
Zn	Au-Pt based (a.o. Biocclus 4)	Cast alloy specimen plate 10×10×0.5 mm	H_2O Sprite light; pH 2.8 20 mL	2 h	0.733 4.0	80.6 440.0	1240 6769	33
Pd	Pd-Cu (Orion Vesta)	Cast alloy specimen 2.06cm²	Lactic acid/NaCl; pH 2.3 5mL	7 d	0.67	276.0	2.613	4
	Pd-Ag (Orion Argos)	Cast alloy specimen 2.06 cm²	Lactic acid/NaCl; pH 2.3 5 mL	7 d	0.33	136.0	1,278	4

(*continued on next page*)

Table 2. (*Continued*) Reported metal ion release in vitro from dental equipment and alloys

Metal	Type of Alloy (Name/Brand)	Methods and Conditions of Exposure[a] Device/Specimen Quantification	Fluid Volume	Duration	Release µg/cm²	Release ppb (µg/L)	Release nM	Reference[b]
Au	Au-Pt based (Biocclus 4)	Cast alloy specimen 10×10×0. 5mm	H_2O Sprite light; pH 2.8 20 mL	2 h	0.019 0.136	1.9 15.0	10 76	33
Hg	Amalgam (Tytin; Kerr UK)	Discs Ø 10 mm×2 mm	0% H_2O_2 1% H_2O_2 2 0mL	24 h	0.02 3.28	2.7 360.0	13 1791	32

MEM, minimal essential medium; nd, not detectable. [a]Unless otherwise stated, experiments performed with polished alloys at 37°C, and at neutral pH. [b]References published from 2005 to 2015.

1% L-glutamine (2 mM; Merck), 0.1 mg/mL streptomycin (Invitrogen),100 IU/mL Na-penicillin G (Invitrogen), and 10% heat-inactivated fetal calf serum (HyClone calf serum; GE Healthcare). The THP-1 cells were maintained in logarithmic growth by passaging every 3 to 4 days. The THP-1 cells were seeded on the surface of different dental cast alloys in the 24-well, polystyrene plate (flat bottom, Greiner Cellstar; Greiner Bio-One GmbH) at a concentration of 25×10^4 cells per well. The THP-1 cells were then exposed for 24 hours in a final volume of 1 mL.

Alloy specimen preparation

Eight different dental cast alloys (10-mm diameter, 1-mm thickness) were used in this study (Table 1). Ni-Cr, Co-Cr, Pd-Cu, Pd-Ag, Ti-6Al-4V, and Au alloys were provided by the Department of Dental Materials Science (Academic Center for Dentistry, Amsterdam) and were cast according to the manufacturer's instructions, using the lost-wax technique, making use of phosphate-bonded graphite-free casting investment and individual ceramic crucibles per alloy; melting was done by means of a gas-oxygen torch.[4] Amalgam (Cavex non-Gamma-2; Cavex Holland BV) was presented in the form of capsules I spill: yellow/white 400 mg alloy/435 mg mercury. The content of the mercury capsules was mixed with an amalgamator (ProMix; Dentsply Sirona) to form a homogeneous mass with a smooth consistency that was condensed into the disk impressions. Stainless steel brackets frequently used in practice were also used (Dentaurum). Specimens were finished and polished using abrasive prepolishing and surface smoothing red and green disk-shape abrasive (Dentsply Sirona). All finishing and polishing processes were carried out in a similar way to simulate the preparation of the cast metal alloys for clinical practice. Acrylic resin disks were used (GC Tempron; GC Europe NV) as a nonmetal control).

Exposure to dental cast alloy and LPS

The specimens were rinsed in a detergent solution for 5 minutes and scrubbed and rinsed under tap water and distilled water for 5 minutes. The specimens were then dried and placed in 70% ethanol and sterilized in an autoclave (125°C for 60 minutes). On the day of culture, they were placed on the bottom of 24-well plates in 500 µL of culture medium with 25×10^4 MoDC or THP-1 cells per well placed on top of the specimens in a final volume of 1 mL. Cultures were performed in the absence or presence of LPS (*Escherichia coli* 055:B5; Sigma-Aldrich). Plates were incubated at 37°C in 5% humidified CO_2. Stimulation by LPS was chosen to be moderate (low dose of LPS) to allow for the evaluation of both stimulation and inhibition by the alloys (for MoDC, 0.5 ng LPS/mL, and for THP-1, 50 ng LPS/mL). Plates were incubated at 37°C in 5% humidified CO_2. After 24 hours, supernatants were collected and stored at -20°C until measurement.

To confirm whether cell activation was due to metal ion release, alloy supernatants obtained by submerging alloy specimens in 500 µL of culture medium (24-well plates; 24 hours; 37°C) were also evaluated. Supernatants (that is, alloy-exposed culture medium) were retrieved and stored under sterile conditions.

Dental casting alloy toxicity experiments

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction tests (Sigma-Aldrich) were used to measure the viability of the cells. Approximately 500 µL of cells (25×10^4/well) were plated in 24-well culture plates and exposed to 500 µL of cast alloy supernatants. After 24 hours of incubation, the supernatants were removed, and 500 µL of MTT solution (5 mg/mL) was added per well. MTT solution was freshly prepared, dissolved with H_2O, and filtered through a 0.22-µm filter. The plates were incubated in the dark at 37°C. After 2 to 3 hours of incubation, 500 µL of dimethyl sulfoxide (Merck) was added to each well, and, after agitation, the solution was measured using an enzyme-linked immunosorbent assay (ELISA) reader (ELx808; Bio-Tek Instruments Inc) at optical density of 490 nm. The optical density of the cells in the absence of metal was considered 100%. The viabilities of exposed cells were determined by using the following formula: [(OD experimental sample/OD of control cells) ×100%].

IL-8 evaluation

Production of IL-8 was measured in cell culture supernatants by using an ELISA kit (Peli-Kine ELISA; Sanquin) using 96-well microtiter plates (Nunc MaxiSorp; eBioscience) according to the manufacturer's instructions. Absorbance was measured at 450 nm. The IL-8

concentration in the supernatant was assessed by using calibration curves (lower detection limit: 15.4 pg/mL) and is expressed in picograms or nanograms per milliliter.

Data analysis

Data are medians of at least 5 independent experiments and the interquartile range (25th to 75th percentile). The statistical significance of the effects of various casting alloys on the secretion of IL-8 was analyzed by using the nonparametric Wilcoxon rank sum test. All statistics were performed using software (MedCalc v11.1.0; Mariakerke) (α=.05).

RESULTS

To study the immune stimulatory effects of dental cast alloys on innate cells, first, the cytotoxicity of 8 commonly used dental cast alloys (Table 1) for innate cells was determined after 24 hours of culture. The viability of both MoDC and THP-1 cells exceeded 80% in all alloy cultures, indicating that these alloys were not toxic to the cells (data not shown). Then, MoDC and THP-1 cells were exposed to specimens of these 8 alloys as well as to acrylic resin as a nonmetal control and to LPS as a positive control. IL-8 secretion was evaluated in the supernatants after 24 hours. As shown in Figure 1A, all dental cast alloys induced significant (Wilcoxon P<.01) IL-8 production in MoDC, whereas acrylic resin specimens did not. Au alloy provided the strongest stimulus, but potential differences among the metal specimens did not reach statistical significance. In order to further confirm the innate stimulatory capacity of cast alloys, THP-1 cells were studied using the same approach (Fig. 1B). Although overall IL-8 production levels were lower, robust production of IL-8 could be observed for most alloys but tended to be highest for Au and Pd-Cu alloys, supporting the earlier data. Further experiments were carried out to investigate whether the activation of THP-1 cells resulted from metal ion release into the supernatant or from direct cell contact with the alloys. Therefore, alloy-exposed medium specimens were prepared by submerging the alloys in culture medium for 24 hours and evaluating them for their stimulatory capacity. For most alloys, the supernatants contained sufficient stimulatory metal ions to induce significant, albeit low, IL-8 production. However, for Ni-Cr and stainless steel specimens, metal ion release under the present test conditions was apparently too low to activate THP-1 cells (Fig. 1C). Again, Au and Pd-Cu specimens stood out as having the most distinct innate immune triggering capacity.

Effects of cast alloys on LPS responsiveness

To evaluate whether cast alloys might have affected the host's reaction to bacterial TLR ligands such as endotoxin, LPS responsiveness of MoDC and THP-1 was evaluated in the presence of the 8 dental alloys. Figure 2A shows that LPS-induced IL-8 secretion by MoDC was significantly higher in the presence of gold alloy specimens, suggesting at least an additive effect of the stimuli. For the other alloys, significant potentiation of the LPS response by MoDC was not reached. In THP-1 cells (Fig. 2B), however, where the LPS response in itself is relatively low, significant increments of the LPS-induced IL-8 secretion were observed not only with specimens of the Au alloy but also with Pd-Cu, Pd-Ag, Ti, and amalgam specimens. In addition, supernatants collected from alloys submerged in culture medium were tested for their capacity to potentiate the LPS responsiveness of THP-1 cells. As can be seen in Figure 2C, Pd-Cu and Au alloy supernatants induced significantly increased LPS-induced IL-8 secretion, whereas the other alloys did not increase LPS responsiveness significantly.

DISCUSSION

Exposure to metals can have adverse health effects, depending on their concentration and chemical form.[23] As reported earlier, salts from several dentally applied metals can activate the innate immune system. Here, for the first time to the authors' knowledge, the immune stimulatory effects of alloy specimens on innate cells were demonstrated. The results shed a new light on clinical and experimental experiences with these metals, which are known for their capacity to activate both innate and adaptive immune responses.

In the present study, dental cast alloys as solid specimens were tested, reflecting the actual situation in the oral cavity. An alloy is a mixture of at least 2 metals, and dental alloys usually contain at least 4 metals and often 6 or more. More than 25 elements can be used in dental alloys, including Zn, Hg, Ni, Cu, Co, Au, Pd, Pt, Ag, Ti, and Fe.[9,26-33] The present study explored how far commonly used cast alloys could trigger innate responsiveness in MoDC and in model THP-1 cells. The results fit with the earlier findings that most metals used for dental alloys show innate stimulatory activity.[1,2] However, the strongest and most consistent IL-8 release was found with alloys containing Au and Pd-Cu. These findings might explain why oral exposure to these metals has been associated with distinct clinical problems.[10]

The stimulatory capacity of dental cast alloys could result from metal ion release but also from direct contact between the innate cells and the alloys. Several in vitro studies, listed in Table 2, reported on the ion release from alloys with largely varying results.[4,25-33] Similar variations were observed when metal release was evaluated in clinical fluids.[5] Metal ion release was influenced by the duration of exposure, the method of evaluation, the pH, and the brand, the composition, and the surface treatment of the alloy.[4,13] The huge variation in reported metal ion release data stresses the importance of testing

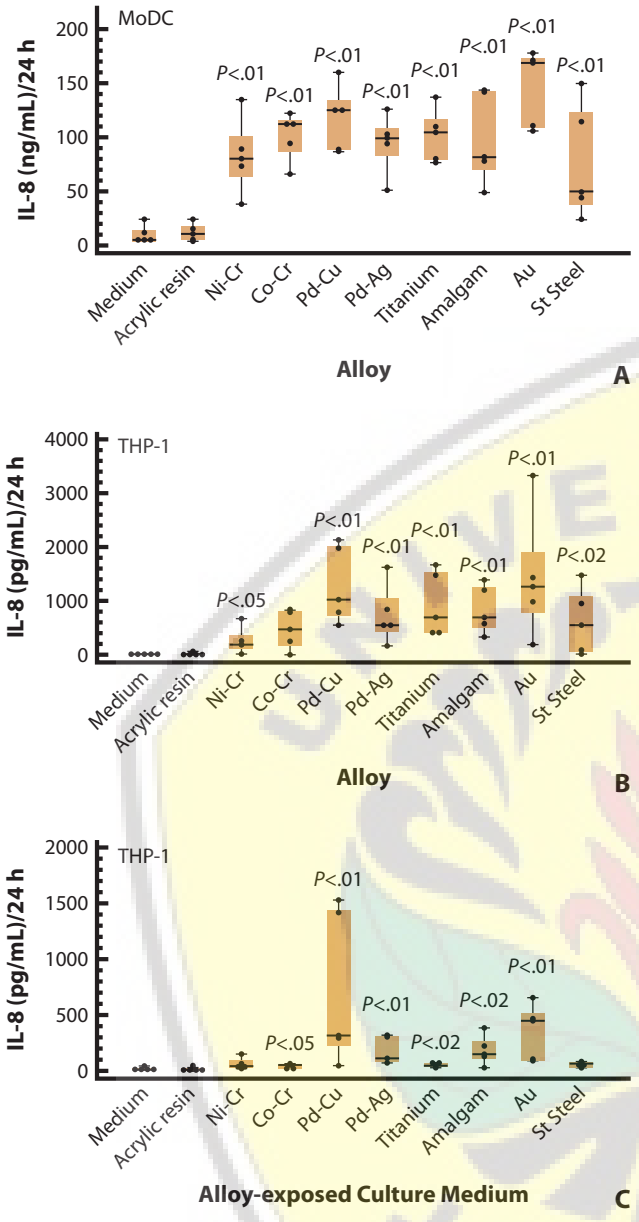

Figure 1. Cells exposed to alloys, medium, and resin controls. A, MoDC. B, THP-1 cells. C, THP-1 cells cultured in alloy-exposed culture medium (supernatant). *P* values for significant differences between IL-8 production (*P*<.05) from alloy exposure and that of medium control. MoDC, monocyte-derived dendritic cells.

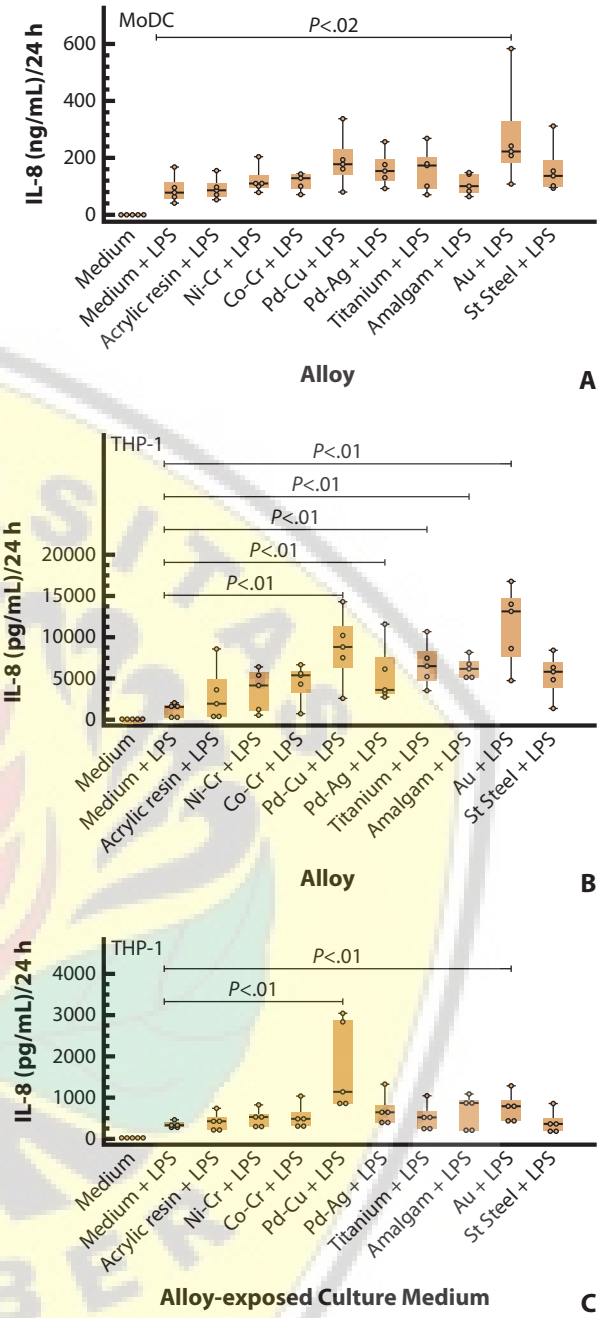

Figure 2. Cells exposed to alloys, medium, and resin controls. All cultures except for medium control contained low dose of LPS (0.5ng/mL for MoDC and 50 ng/mL for THP-1 cells). A, MoDC. B, THP-1 cells. C, THP-1 cells cultured in alloy-exposed culture medium (supernatant). *P* values for significant differences between IL-8 production (*P*<.05) for alloy exposure and that of medium control. MoDC, monocyte-derived dendritic cells.

the actual alloys themselves, instead of only their salt solutions, for immune stimulatory capacities. In none of these metal ion release studies, was the most effective innate stimulatory dose, as assessed in our previous studies[1,2] (ranging from 125 to 750 μM for most metals and 250 to 750 nM for Hg) reached. It is therefore remarkable that in the present experiments, for 6 of 8 alloys, the concentration of metal ions released into plain culture medium within 24 hours appeared to be high enough to induce significant IL-8 production by THP-1 cells, in particular for Pd and Au-based alloys. Actually, direct contact of innate cells with the alloys may also contribute to the innate activation, especially in the case of stainless steel and Ni-Cr alloy.

Stainless steel does trigger innate responses in both MoDC and THP-1 cells, most likely because of the Ni

content (13 to 15%). Indeed Ni was reported to be released from stainless steel (Table 2). However, in the present study, neither stainless steel nor the Ni-Cr alloy released detectable stimulatory ions in the supernatant within 24 hours, nor did they potentiate LPS responsiveness. In vivo conditions facilitating metal corrosion could, however, still result in increased Ni ion release and subsequent innate immune reactions. In dentistry, stainless steel crowns are commonly used for brackets and to treat primary teeth in children to prevent further caries until replacement by permanent teeth. In general, stainless steel is cost-effective since it will be removed naturally when the permanent teeth are approaching. The present in vitro results suggest that stainless steel can, however, stimulate inflammation by direct contact with mucosal innate cells. This would be in line with the incidental finding of local gingival inflammation at the site of oral stainless steel exposure, especially in individuals with nickel allergy.[11]

Acrylic resin specimens have been used as a putative negative control, as it is a nonmetal material. It has been widely used in dental research because of its low cost, ease of use, and diversity of indications. In agreement with our hypothesis, the results show that direct cell contact with acrylic resin does not stimulate innate cells.

In a previous study of the innate stimulatory capacity of low, nearly physiological metal concentrations, we showed that such low concentrations did not directly activate innate cells but could potentiate LPS responsiveness.[3] This study showed that in vitro exposure to most of the alloy specimens also potentiated the inflammatory response to bacterial endotoxin LPS. This was most obvious with the THP-1 cell line as responder cells, because the basal LPS response of these cells is relatively low compared with that of MoDCs. However, under the present "noncorrosive" conditions, only Pd-Cu and Au alloys released enough metal ions in the supernatant within 24 hours to potentiate LPS responsiveness. In aggregate, the present results suggest that the presence of Pd and Au based dental alloys in particular might facilitate antimicrobial immune responses and also lead to untoward local reactivity perceived as irritancy.

The use of actual dental cast alloys in in vitro studies instead of metal salt solutions provides an effective strategy for studying the potential immune stimulatory effects of orally applied metals. A major advantage of using alloy specimens in such a screening test is that the capacity to release metal ions into the environment is also taken into account. Although generally lower amounts of IL-8 are being produced than by MoDC, THP-1 cells are ideal responder cells in such a test system, in particular for studying effects of metal alloys on LPS responsiveness. Because actual metal release into the alloy supernatants was not measured in this study, further investigations into the innate stimulatory potential of low concentrations of transition metals released form dental cast alloys are warranted.

CONCLUSIONS

Based on the finding of this in vitro study, the following conclusion was drawn:

Use of an in vitro culture system to expose MoDC and THP-1 cells to different dental alloys, in particular when they contain Pd or Au, can trigger innate immune responses and strengthen the host's responsiveness to bacterial endotoxin.

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Noteworthy Abstracts of the Current Literature

Implant placement accuracy using dynamic navigation

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Purpose. The aim of this prospective study was to determine platform and angle accuracy for dental implants using dynamic navigation, a form of computer-assisted surgery. Three hypotheses were considered: (1) the overall accuracy for implant placement relative to the virtual plan is similar to that of static tooth-borne computerized tomography (CT)-generated guides; (2) the dynamic system is more accurate than freehand methods; and (3) there is a learning curve associated with this method.

Material and Methods. This study involved three surgeons placing implants in the mandible and maxilla of patients using a dynamic navigation system (X-Guide, X-Nav Technologies). Virtual implants were placed into planned sites using the navigation system computer. Post-implant placement cone beam CT scans were taken on all patients. For each patient, this scan was mesh overlayed with the virtual plan and used to determine platform and angular deviations to the virtual plan. The primary outcome variables were platform and angular deviations, comparing the actual placement to the virtual plan. Secondary analyses included determination of accuracy related to case experience and freehand placement of implants. Comparisons to published accuracy studies were made for implant placement using static guides.

Results. Accuracy deviations from the virtual plan were similar to those reported for static tooth-based guides using literature references as the comparison. The accuracy of dynamic navigation was superior compared to freehand implant placement. The three surgeons had similar accuracies after their learning curve was achieved. Proficiency based on case series was achieved by the 20th surgical procedure.

Conclusions. Dynamic navigation can achieve accuracy of implant placement similar to static guides and is an improvement over freehand implant placement. In addition, there was a learning curve to achieve proficiency.