In vivo effects of dental casting alloys

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Abstract

OBJECTIVE: Corrosion products of different metallic alloys used in prosthetic dentistry often cause the development of a bluish-grey pigmentation of gingiva and oral mucosa. The aim of this study was to determine the content of metals in metallic pigmentations and evaluate the immune response to metals found in the oral cavity.

MATERIAL AND METHODS: The local tissue reactions were investigated clinically by electron microscopy and by energy dispersive x-ray microanalysis. An extensive anamnesis of the patients was recorded as well as earlier contacts with health care institutions. The immunological response to metallic components of dental alloys was evaluated in 34 patients by MELISA®, a modified test for lymphocyte proliferation. In addition, cytokines in culture media were determined in 10 persons by the Human Inflammation Antibody Array.

RESULTS: Dense particles containing metals were found in the matrix among collagen fibrils and in close proximity of the lamina basalis of the gingival epithelium. Particles were also localized intracellularly in fibroblasts, macrophages, and endothelial cells. Metallic depositions consisted predominantly of silver accompanied by selenium and sulphur. Twenty five out of 34 patients had increased lymphocyte reactivity (positive MELISA® test) to one or more metal components of dental restorations. A correlation between the positivity in MELISA® test and number of dental alloys in the oral cavity was also found. Twenty MELISA® positive patients suffered from serious health problems (various allergies, autoimmune diseases, Parkinson's syndrome etc.). Nickel and inorganic mercury were the most common sensitizers *in vitro*. The cytokine assay revealed that mercury chloride activated predominantly TH2 lymphocytes, while nickel chloride activated mainly TH1 lymphocytes.

CONCLUSIONS: Metallic pigmentations in the oral cavity demonstrate a corrosion process and may pose a risk in immunologically susceptible patients.

ORIGINAL ARTICLE

Abbreviations:

cpm	– count per minute
cp Ti	– commercially pure titanium
EDAX	– energy dispersive X-ray microanalysis
IFN	– interferon
IL	– interleukin
MELISA®	– memory lymphocyte immuno stimulation assay
SI	– stimulation index
TEM	– transmission electron microscopy
TNF	– tumor necrosis factor
RHP	– rhodamin

Introduction

Dental casting alloys release metal ions into the oral environment, and they have the potential to interact with oral tissues [6, 8, 14, 19, 20, 26, 28]. Blue-grey pigmentation (tattoo) may occur as a consequence [22]. Corrosion products can also be swallowed with further possibility of intestinal resorption. The amount and the type of metal elements released may not be directly related to the composition of alloys [19]. Due to their small size, metal ions are incomplete antigens (haptens) with a high immunogenic potential [2, 4, 7, 11, 18, 21–24].

Amalgam tattoos have been the subject of investigation of several studies [1, 3, 12, 15, 25], but similar studies concerning pigmentation around dental casting alloys are scarce.

It has been hypothesized that persons with metallic pigmentations of gingiva or oral mucosa might have a higher chance to become sensitized (allergic) to metal-based dental restorative materials. The aim of this study was to determine the metal composition of oral pigmentations by the means of transmission electron microscopy (TEM) with energy dispersive x-ray microanalysis (EDAX). Immunological response to metals was measured by a modified lymphocyte proliferation test, MELISA®. Released cytokines were detected by Human Cytokine Antibody Array.

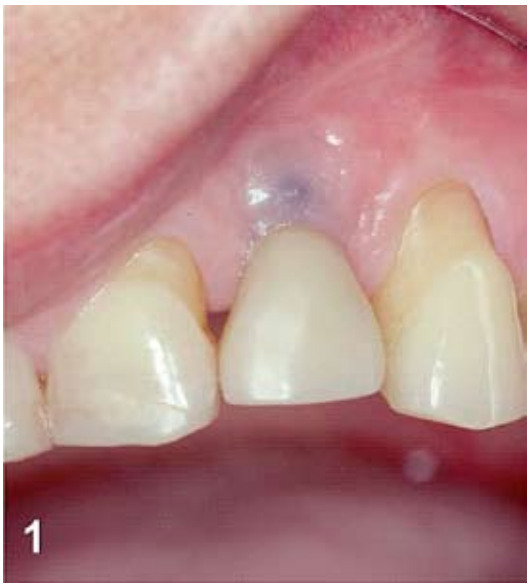

Figure 1. Example of pigmented oral mucosa. Tooth 22, restored with Ti implant, 15 months after implantation.

Material and Methods

Patients

Thirty-four patients (all referred by local dentists during the period 2003–2004) with metallic pigmentations were included in the study. They were 25 females and 9 males, 24 to 76 years old (mean age 55.8). In each patient an extensive anamnesis was recorded as well as earlier contacts with health care institutions. Patients were asked to complete a questionnaire regarding possible clinical metal hypersensitivity such as intolerance to metal earrings, wrist watches, and jeans buttons. The patients were informed about the purpose of the study and gave informed consent.

Examination of the oral cavity focused on the localization and number of pigmented areas and the number of restored teeth. A panoramic X-ray was also performed. The composition of dental alloys found in the oral cavity of the patient was verified from each individual dental record.

TEM and EDAX

Biopsies (about 1mm^3 were obtained from 15 patients with distinctively pigmented gingival areas adjacent to the metallic restorations. Mercury chloride ($HgCl^2$) and nickel chloride ($NiCl^2$) with distinctly pigmented gingival areas adjacent to the metallic restorations. The biopsy samples were surgically removed under local anaesthesia and immediately fixed in buffered 2.5% glutaraldehyde for 2 hrs. One half of the sample was fixed with buffered 2% OsO_4, in the second half OsO_4 was omitted. Standard method was used for further Epon 812 embedding and TEM processing. Semi-thin and ultra-thin sections were prepared using glass knives. The semi-thin sections were stained with toluidine blue. Ultra-thin sections were mounted onto standard copper grids or onto special plastic grids. Ultra-thin sections on copper grids were contrasted with uranyl acetate and lead citrate for control. After viewing in JEOL 100 B electron microscope, the samples with dense particles were analysed in Philips CM12/STEM electron microscope equipped with the EDAX DX4 X-ray analysis system in the STEM bright-field mode at 80 kV and spot size of 7.

Lymphocyte proliferation test

In a modified lymphocyte proliferation test, MELISA®, lymphocytes isolated by centrifugation on Ficoll gradient are partly depleted of monocytes before cultivation in 48 well cultures (1×10^6 lymphocytes per well) with various metal salts for 5 days [21, 24]. Control cultures

were cultivated in the absence of metal salts. After cultivation, lymphocyte cultures were pulsed with tritiated thymidine for 4 hrs, and radioactive DNA was harvested on a cell harvester. Incorporation of radioactivity was evaluated in liquid scintillation counter (Microbeta TRILUX, WALLAC, Denmark). The increase in ^{3}H-thymidine incorporation in metal-treated lymphocytes was expressed as a Stimulation Index (SI), which was defined as counts per minute (cpm) in metal-treated cultures / mean cpm in control untreated cultures. SI values ≥ 3 were regarded as positive. Statistical analysis of data was performed using Fisher's exact test for quadruple table.

Human Cytokine Antibody Array

The release of cytokines in cultures of 10 patients positive in MELISA® to $HgCl_2$ and $NiCl_2$ was examined by Ray Bio® Human Inflammation Antibody Array III (Ray Biotech, Norcross, GA, USA). The detection of cytokines in supernatants was performed after 3 days cultivation with metal salts or medium only. The membranes coated with antibodies against 40 cytokines were first incubated with blocking solution. Culture supernatants were then added and incubated for 2 hrs at room temperature. After removing of samples, the membranes were washed, processed with biotin-conjugated antibodies against cytokines, and incubated for another 2 hrs. Lastly, rhodamin (RHP)-conjugated streptavidine was added for 2 hrs. The reaction was visualized by detection buffer and luminescence.

Results

Thirty-one out of 34 patients reported various health problems which are summarized in Table 1. Twenty-one patients suffered from several kinds of allergic reactions. Three patients did not report any health problems.

Bluish-grey pigmentation of the soft oral tissues was observed in the vicinity of totally 57 teeth. The majority of teeth were devitalized and treated with posts and cores fabricated from a silver alloy (Table 2, alloy B) and with amalgam fillings (alloy A).

Subsequently, these teeth were reconstructed in four cases with resin-based material crown and in 53 cases with fixed crowns and bridges made from dental casting alloys (see Table 2). Mesially, distally, or occlusally from the teeth with adjacent pigmented tissues, amalgam fillings or other appliances, made from materials mentioned in Table 2, were usually found.

The number of alloys varied from 2 to 7, with a mean of 3.6. A maximum of 10 pigmented areas were found in one patient. The pigmentations occurred most frequently in the gingival mucosa (at the vestibular or oral site), rarely in the alveolar mucosa and buccal mucosa or in the floor of the mouth. In one case oral mucosa was pigmented when intraosseal titanium (Ti) oral implant was used (Fig. 1; Table 2, alloy K).

Stratified squamous epithelium with underlying *lamina propria gingivae* was seen on semi-thin sections.

Table 1. Medical history and results in MELISA® positive (+) and negative (-) patients.

	Patients	
	MELISA®+	MELISA®–
Number of patients	25	9
Allergies (medications)	7	1
(cosmetics)	4	1
(molds, pollen, dust)	3	
(food)	4	
(insect sting)	3	
Skin reaction on jewellery, buttons, earrings	7	
Astma bronchiale	2	
Scleroderma	1	
Sjögren's syndrome	1	
Endocrine diseases (struma, diabetes mellitus)	3	
Hypertrophy of prostate gland	1	1
Status post hysterectomy	2	2
Status post cholecystectomy	1	1
Chronic fatigue syndrome (CFS)	2	
Neurovegetative disorders	3	
Parkinson's syndrome	2	
Repeated migraine		1
Recurrent aphtous ulcer	1	1
Recurrent herpes labialis	1	
Periodontitis	1	2
Leukoplakia of buccal mucosa	1	
Desquamative gingivitis		1
Metal taste in the oral cavity	3	
Candidosis	1	
Gastritis	1	
Ulcus duodeni	1	
Inflammatory diseases of urinary system	1	
Gout		1
Coxarthrosis	2	1
Arthrosis of genus	2	
EBV reactivation	1	
Hypertension	2	2
Persistently high count of monocytes (20–40%)	1	
Persistently increased erythrocyte sedimentation rate	1	
Glaucoma	1	
Otosclerosis, otitis, hypacusia, tinnitus	4	
Without health problems	1	2

* Some patients suffered from multiple illnesses and allergies
Comment: Bold printed items occurred mainly in MELISA® positive patients

Table 2. Total number of reconstructed teeth in MELISA® positive (+) and negative (–) patients
Composition of alloys is also shown.

Alloys used for reconstruction	Approximative composition of alloys in % wt	Total number of reconstructed teeth	
		MELISA®+	MELISA®–
A (amalgam)	35Ag, 13Sn, 2Cu, 1.5Hg - alloy + 50Hg	15	6
B (Koldan)	90Ag, 9Sn	26	9
C (Konstrulit)	84Ag, 15.5Cu	1	
D (Herador)	85.5Au, 10Pt, 1.4Pd, 1In	1	
E (Aurix L)	65Au, 20Ag, 10Cu, 3Pd, 1Pt, 1Zn	19	8
F (Aurosa)	45Ag, 20Au, 20Pd, 14Cu	3	
G (Palargen)	57Ag, 40Pd, 2Zn	3	
H (Oralium)	63Co, 28Cr, 6Mo	4	1
I (Wiron 99)	65Ni, 23Cr, 10Mo, 1Nb	12	4
J (stainless steel wire)	73Fe, 19Cr, 8Ni	1	
K (Ti implant)	cpTi	1	

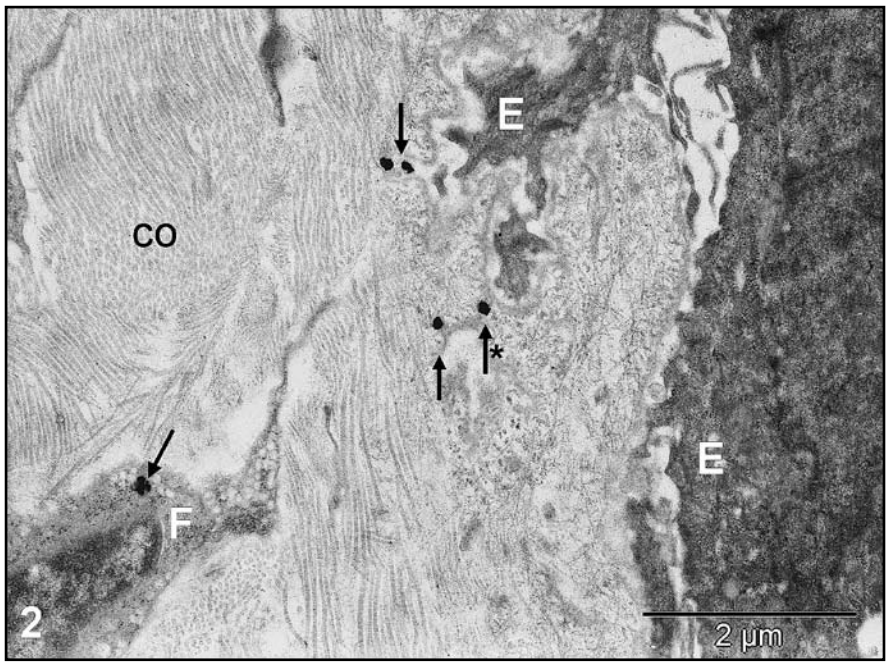

Figure 2. Dense particles in close proximity of lamina basalis of gingival epithelium.
TEM, ultra-thin section.
E – epithelial cell,
F – fibroblast with vacuolated cytoplasma,
co – collagen fibrils.
Arrows indicate dense particles.

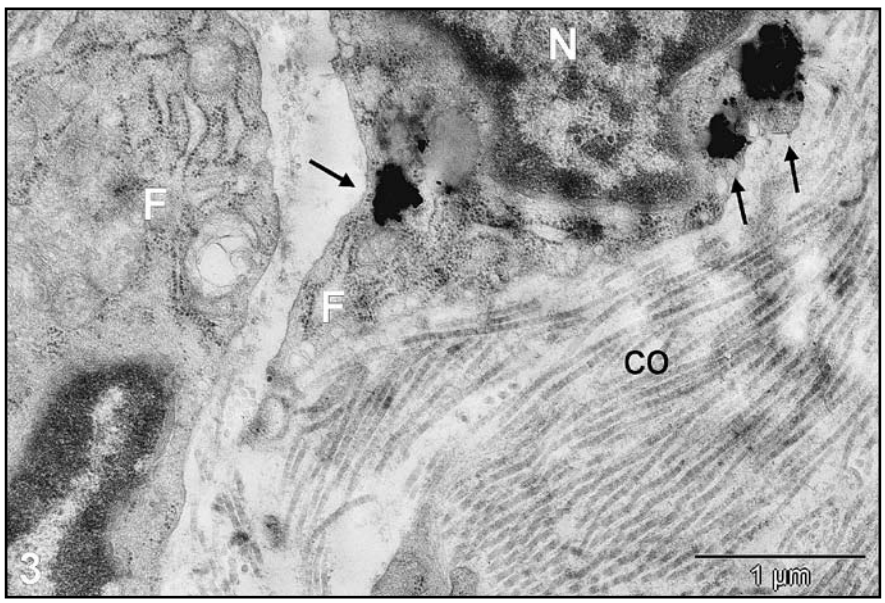

Figure 3. Fibroblast with dense particles and lipid vacuole (arrows).
TEM, ultra-thin section.
F – fibroblast,
N – nucleus,
co – collagen fibrils.

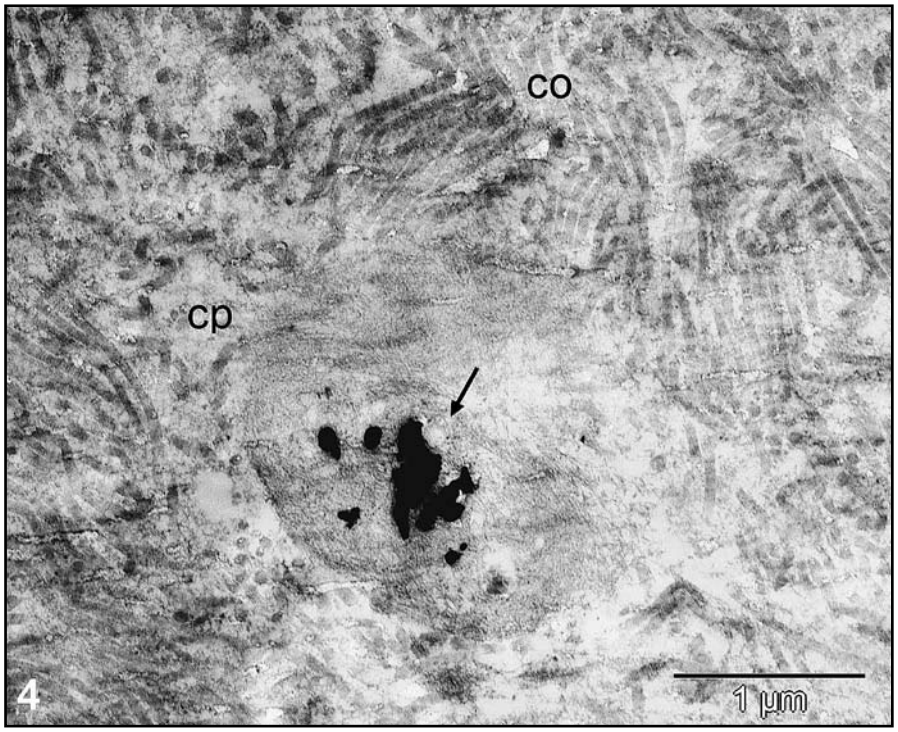

Figure 4. Damaged cell process (cp) with massive metal inclusions (arrow) surrounded by collagen fibrils (co). TEM, ultra-thin section.

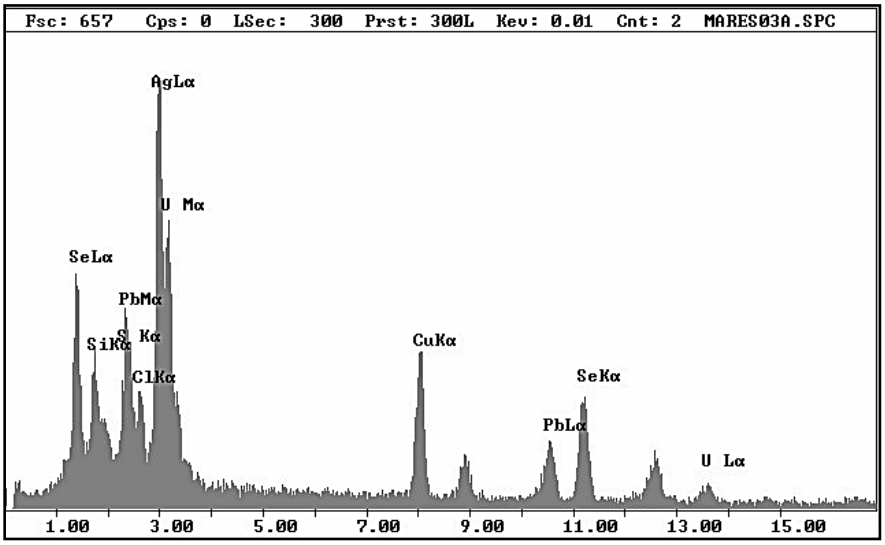

Figure 5. Characteristic X-ray spectrum recorded from typical electro-dense particle in the ultra-thin section (see particle marked with asterisk in Fig. 2) with the content of Ag, S, Se. The other elements recorded in spectrum originate from sample preparation process (Cl, U, Pb, Si) or from support grid (Cu). Background spectrum recorded near the analysed particle. x – axis: energy in kV; y – axis: counts

Dense particles of various size and location were seen on ultra-thin sections in the *lamina propria*. These particles occurred alone or in clumps, extracellularly or intracellularly in the tissue. Extracellular appearance of dense particles was seen in the matrix among collagen fibrils. These particles were also found in the close proximity of the *lamina basalis* of gingival epithelium but never in the epithelium itself (Fig. 2). Dense particles were located intracellularly in fibroblasts, as shown in Figure 3, rarely in macrophages and endothelial cells. Especially fibroblasts often displayed various signs of cell damage such as vacuolated cytoplasm, occurrence of cytoplasmic lipid vacuoles, or a loss of typical cell structures with distinct remnants of cytoskeleton only (Fig. 4). Numerous macrophages with phagosomes were found in the vicinity of the capillaries. Regardless of whether dense particles were diffusely spread or occurred in lumps, increased numbers of mast cells were found in the tissue by TEM.

The results of EDAX microanalysis confirmed that this type of analysis could be performed on ultra-thin sections contrasted with lead citrate and uranyl acetate with the use of copper grids. Particles localized in the pigmented tissue were found to contain at least one of the following elements: silver (Ag), zinc (Zn), aluminium (Al), Iron (Fe), chromium (Cr), copper (Cu), nickel (Ni), tin (Sn), sulphur (S), selenium (Se), calcium (Ca), phosphorus (P), and magnesium (Mg). Most frequently,

Table 3. Lymphocyte responses to metal salts *in vitro*

Metal	Number of patients	Stimulation Index		Percent of patients with positive results
		Mean	SE	
Hg	33	12.55	3.35	57.6%
Ni	28	5.84	1.23	53.6%
Cd	28	4.00	0.73	35.7%
Ti	25	3.20	0.83	28.0%
Pd	29	3.53	0.98	24.1%
Au	28	2.68	0.51	14.3%
Sn	31	2.41	0.55	12.9%
Ag	33	2.92	1.05	12.1%
Pt	22	2.13	0.32	9.1%
Co	26	2.28	0.62	7.7%
Cu	29	2.01	0.30	6.9%
Cr	26	2.23	0.77	3.8%

SE = standard error

Table 4. Clinical findings related to lymphocyte responses to metals salts *in vitro*

A. Number of pigmented areas related to the results of MELISA® test

MELISA® test	Number of patients	Number of pigmented areas	
		1	2 or more
+	25	56.0 %	44.0 %
–	9	33.3 %	66.7 %

B. Number of dental alloys related to the results of MELISA® test

MELISA® test	Number of patients	Number of dental alloys	
		2–3	4 or more
+	25	✱ 56.0 %	✱ 44.0 %
–	9	88.9 %	11.1 %

✱ significant difference ($P < 0.05$) according to Fisher's exact test for quadruple table

Table 5. *In vitro* production of cytokines by lymphocytes after stimulation with $HgCl_2$, $NiCl_2$, and in control cultures

	IFNγ	IL-1α	IL-6	IL-10	TNFα	TNFβ	sTNFRII
$HgCl_2$	xx	xxx	xx	xx	xx	xx	xxx
$NiCl_2$	xxx	xxx	xx	xxx	xxx	xxx	x
Control	xx	xx	x	x	xx	xx	xx

x – low production, xx - moderate production, xxx - high production
Only activated cytokines are shown

particles with only one metal element were identified. In most cases, the metallic depositions consisted predominantly of Ag, accompanied by Se or S or with both accompanying elements (Fig. 5). The diameter of the Ag particles varied from 10 to 310 nm. In a patient with one oral Ti implant, the metal was found in the alveolar mucosa (Fig. 1).

Twenty five patients showed a positive response in MELISA® to at least one of the metals present in own metal restorations while the lymphocytes of 9 patients were not stimulated *in vitro* (Table 4B). As shown in Table 3, the responses to mercury (Hg), Ni, cadmium (Cd), Ti, and palladium (Pd) were most frequent. Further, the number of dental alloys present in patients with positive responses was significantly higher than in patients who did not react to metals *in vitro*. In contrast, the degree of lymphocyte proliferation did not correlate with the number of pigmented areas (Table 4A).

Regarding the effect of metal salts on cytokine production, $NiCl_2$ stimulated production of IFN-γ, IL-1α, IL-6, IL-10, TNF-α and TNF-β, while $HgCl_2$ stimulated production of IL-1α, IL-6, IL-10 and s TNF RII (Table 5).

Discussion

It is well known that many patients do tolerate metal restorations well, without any visible pigmentation. Bluish-grey pigmentation occurred most frequently in the vicinity of devitalised teeth. A wide diversity of materials is used for reconstruction of devitalized teeth, such as posts and amalgams. They are often covered by metal crowns or bridges which may cause corrosion. Factors influencing this process, among others, acidity and the bacterial spectrum in oral cavity; metallurgical state of the appliance; quantity and composition of oral debris; food composition; poor oral hygiene; and, last, but not least, the time after the dental treatment [10, 20, 26, 27]. The location of the restoration was also important, since subgingival restorations have worse conditions as supragingival ones.

The dense particles observed by TEM were localized essentially in the same manner as described in amalgam tattoos by Veron *et al* [25] and Pritz & Ellinger [15]. Additionally, lipid vacuoles in some fibroblasts with metal deposits were also found. Accumulated metal particles in cells with loss of organelle structure and distinct cytoskeleton only were seen as well (Fig. 4), suggesting degenerative processes in the cells. The occurrence of macrophages and mast cells in the lamina propria gingivae indicated immunological reactivity around the pigmentations.

Results of EDAX microanalysis revealed that the majority of particles found in the lamina propria contained most frequently Ag and rarely other metals such as Cu, Sn, Zn and Ti. Interestingly, particles with the same metal composition as original alloys used for prosthodontic reconstructions were never found. This fact, together with size and location of the particles, indicates that corrosion was more probable etiological factor (mechanism) than tattooing of the tissue by instruments rotating in high speed [3, 15].

The finding of Ag as most frequent metallic inclusion is not surprising, since silver is a part of many alloys such as gold alloys, amalgams, and other materials. The presence of Ag was always accompanied by sulphur with or without Se. S is one of the basic elements of organic substances in the tissue, and Ag sulphide is formed due to its high affinity to Ag. Aoyagi and Katagiri [1] also detected the relationship between silver and S in paraffin sections of amalgam tattoos using electron-probe microanalysis. The same is probably true in the case of Se (according to its position in the table of elements). The occurrence of Se in the particles had to be of endogenous origin, since no medication with Se was reported by patients. The importance of Se in detoxification of heavy metals is often discussed in association with amalgam tattoos and argyria [13, 16, 17].

Titanium was identified in the tissue even when it was absent in dental alloys. This phenomenon can be explained by the abundant use of Ti in cements and other dental materials. Interestingly, as reported by Herlofson & Barkvoll [9], lauryl sulphate in toothpastes might damage oral mucosa, and Ti, present in toothpastes as well, might penetrate to tissue via sulcular and attachment epithelium. Last but not least, Ti dioxide (E171) is also used in many food and pharmaceutical products.

Copper, Fe and Zn were identified in dried samples of tissue not adjacent to metal restoration by Garhammer [5]. The author concludes that these elements might play a role as endogenous elements e.g. being part of metal-binding metaloproteinases or metalothioneins.

Twenty-five patients exhibited positive lymphocyte response to metals. Many of those patients reacted to several metals, predominantly to Hg, Ni, Cd, Pd, and Ti. These patients had also significantly higher number of dental alloys in comparison with the rest of the patients. Our results are in agreement with Valentine-Thon who reported Ni, Ti, Cd, Au and Pd to be the most frequently sensitizing metals [24]. In our patients, inorganic mercury was the most frequent sensitizer, probably because of the widespread use of amalgam fillings in the Czech population.

Based on anamnestic data, majority of MELISA® positive patients with oral pigmentations suffered from allergies as well as various autoimmune and endocrine diseases. The study of cytokines in metal-treated cultures revealed that Hg activated predominantly TH2 lymphocytes, while Ni activated TH1 lymphocytes. Taken together, a local reaction in the oral cavity could be accompanied by systemic reaction through the presence of lymphocytes sensitized to metal ions. Metal-induced inflammation could affect the overall immunological reactivity of these patients and could predispose them to the development of immunologically-mediated diseases such as allergy and autoimmunity. It would be optimal

to study a group of patients with the same diseases but without manifestations of oral pigmentations. However, we were not able to examine such a group, since patients without pigmentations were not interested to participate in our study.

Conclusion

Metallic pigmentations caused by corrosion of metal alloys in the oral cavity may pose a risk for metal-susceptible individuals. Therefore, we propose some important rules:

(i) to make a careful and thorough anamnesis,
(ii) to reduce the number of dental alloys in the patient's mouth as a prevention of hypersensitivity and oral pigmentations, and
(iii) in indicated cases to perform the MELISA® test to determine the possible immunological response to metals.

In at-risk patients, it is prudent to avoid metal appliances and recommend metal-free materials.

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