Dental Materials Journal 26(1): 96−104, 2007

Metallic Pigmentation of Human Teeth and Gingiva: Morphological and Immunological Aspects

Zora VENCLÍKOVÁ[1], Oldřich BENADA[2], Jiřina BÁRTOVÁ[2], Luděk JOSKA[3] and Lubor MRKLAS[1]
[1]Institute of Dental Research, General Faculty Hospital, Prague, Czech Republic
[2]Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[3]Department of Metals and Corrosion Engineering, ICT Prague, Czech Republic
Corresponding author, Zora VENCLIKOVA; E-mail: ZVenclikova@seznam.cz

Recieved August 23, 2006/Accepted October 17, 2006

The composition of metallic pigmentations in gingiva and dental roots was determined by means of transmission electron microscopy with energy dispersive x-ray microanalysis. The systemic immune response to the metals found in the oral cavity was evaluated in 10 patients by using a modified lymphocyte proliferation test. Immunological results were compared with a group of five controls without metallic materials and pigmentation. Dense particles of various shapes and sizes, as well as of diverse extracellular and intracellular localization patterns, were detected in the pigmented lamina propria gingivae. Metallic deposits consisted predominantly of silver accompanied by selenium or sulfur or both. Besides, Ag, Au, Cr, Ni, Fe, Hg, Cu, and Ti were identified in dentinal tubules of teeth reconstructed with dental alloys. Nine patients with metallic pigmentations had a positive lymphocyte proliferative response to one or more metals present in their own metal reconstructions. Results of this study thus indicated that dental alloys−by virtue of their corrosion process—might pose a significant risk to immunologically susceptible patients.

Keywords: Metallic pigmentations, EDX microanalysis, Lymphocyte proliferation

INTRODUCTION

Dental alloys are composed of a wide array of materials−from precious alloys containing gold, platinum, and palladium to non-precious alloys containing Ti, Cr, Co, Mo, Ni, and Fe. The corrosion properties of an alloy are influenced by several factors: metal composition and metallurgy of the alloy, buffering capacity and composition of saliva and crevicular fluid, microbial activity of dental plaque, alimentation habits, oral hygiene, *etc.*[1-5].

Corrosion products released in the oral cavity from the surfaces of dental alloys can be swallowed with the saliva, with further possibility of intestinal resorption. Alternatively, it could be due to crevicular corrosion in the area of gingival sulcus or corrosion of metal post and core reconstructions. In both of these cases, corrosion products diffuse into adjacent tissues and blue-gray pigmentation of gingiva and root dentin may develop[6]. The mechanism of biological interactions occurring between corrosion products, metals, and human tissues is still not fully understood. Notwithstanding, in studies related to contact sensitivity and hypersensitivity reactions, three possible fronts are generally considered: skin surface (associated with direct contact, piercing, *etc.*), mucosal surfaces of gastrointestinal tract (associated with consumed food), and mucous membrane of oral cavity (associated with amalgam fillings and dental alloys).

Although most humans tolerate metals well, adverse immune reactions appear in some individuals. Metals in direct contact with the skin can lead to contact dermatitis[7-9], while corrosion products of dental alloys in the oral cavity and gastrointestinal tract can give rise to local reactions[10-12]. The immunological consequence is cellular, delayed hypersensitivity occurring either locally or systemically. Physiological interaction of foreign materials (including metal ions) with mucosal tissue can lead to oral (mucosal) tolerance induction[13-15]. However, a minority of humans evince an intense reaction to metal dental reconstructions. Immunological response may occur locally, including oral discomfort and local reactions of varying intensities (e.g., stomatitis, lichenoid reaction). This local reaction could be accompanied by a systemic reaction, such as one characterized by the presence of cells sensitized to the metal ions. This state of delayed hypersensitivity could influence the overall immunological reactivity of the affected patients, thereby predisposing them to the development of immunologically mediated diseases, e.g., autoimmune diseases[16].

Pertaining to local tissue reactions and the long-term adverse effects, amalgam tattoos have been the subject of several studies[17-20]. On the other hand, similar studies concerning pigmentation around dental casting alloys are scarce. The aim of this study, therefore, was to determine the metal composition of oral pigmentations in gingiva and dental roots and to evaluate the systemic immune response to the metals found in the oral cavity.

MATERIALS AND METHODS

Patients and controls

Eleven patients (all referred by district dentists during the period of 2005-2006) with metallic pigmentations in oral cavity were included in the present study. They were six females and five males, aged 22 to 74 years (mean age was 58.8 years). For each patient, an extensive case history as well as earlier contacts with health care institutions were recorded. The patients were also asked to fill in a questionnaire regarding possible clinical metal hypersensitivity such as intolerance to metal earrings, wrist watches, and jeans buttons. After being informed of the purpose of this study, they gave their informed consent. One patient, however, rejected blood taking.

Control group consisted of five persons (two females and three males, mean age was 38.3 years) without substantial health problems and pigmentations in the oral cavity.

Examination of the oral cavity focused on the localization and number of pigmented areas as well as the number of restored teeth, and a panoramatic X-ray was performed. The composition of dental alloys found in the oral cavity of the patient was verified from the individual's dental record.

Transmission electron microscopy (TEM) with energy dispersive x-ray microanalysis (EDX)

Ten biopsies of pigmented gingiva (about 1 mm^3) were obtained from nine patients with distinctly pigmented gingival areas adjacent to the metallic restorations. Details on metal restoration, exposure duration, and number of biopsies are given in Tables 1 and 2.

The biopsy samples were surgically removed under local anesthesia and immediately fixed in buffered 2.5% glutaraldehyde for two hours. One half of the sample was postfixed in buffered 2% OsO_4, but with the second half OsO_4 was omitted. A 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 on standard copper grids or special plastic grids, where those on copper grids were contrasted with uranyl acetate and lead citrate for control.

After viewing with a JEOL 100B electron microscope, selected samples with dense particles were examined with a Philips CM12/STEM electron microscope equipped with an EDAX DX4 X-ray analysis system. Analysis was carried out in the STEM bright-field mode at 80 kV, spot size of 10 nm, and low background C2 aperture (70 μm) using DXAuto application. The strategy of EDX spectra acquisition was as follows. First, the image of an ultra-thin section of gingival tissue with electrondense particles was recorded in scanning transmission electron microscopy (STEM) bright-field (BF) mode at a resolution of 1024×800 pixels. The EDX spectra were subsequently recorded in spot mode (10-nm spot) from selected particles with a live time of 300 seconds. Each STEM BF image was duplicated, and each measured particle was marked in this copy immediately after spectrum recording. In this manner, the original image was designated for

Table 1 List of alloys and analyzed biopsies.

Alloy	Inserted period of alloy(s)(exposure time) in years	Number of biopsies
Amalgam	20	1
Alloy A + Alloy C	3	1
	10	2
	6	1
Alloy A + acrylic	5	2
	7	1
Amalgam + Alloy E	2	1
Ti implant	1	1

Table 2 Metal composition of the examined amalgam, alloys, and Ti implant.

		Au %wt.	Pd %wt.	Ag %wt.	Cu %wt.	Sn %wt.	Hg %wt.	Zn %wt.	mixing ratio alloy:Hg
Alloy I group	alloy A			90		9		1	
	alloy B			84	16				
	alloy C	65	4	20	10			1	
	alloy D	20	20	45	14				
amalgam				70	4	22	4		1:1
		Cr %wt.	Co %wt.	Mo %wt.	Ni %wt.	Fe %wt.	Ti %wt.	Nb %wt.	
Alloy II group	alloy E	23		10	65			1	
	alloy F	28	63	6					
	alloy G (wire)	19			8	73			
Ti implant							99.5		

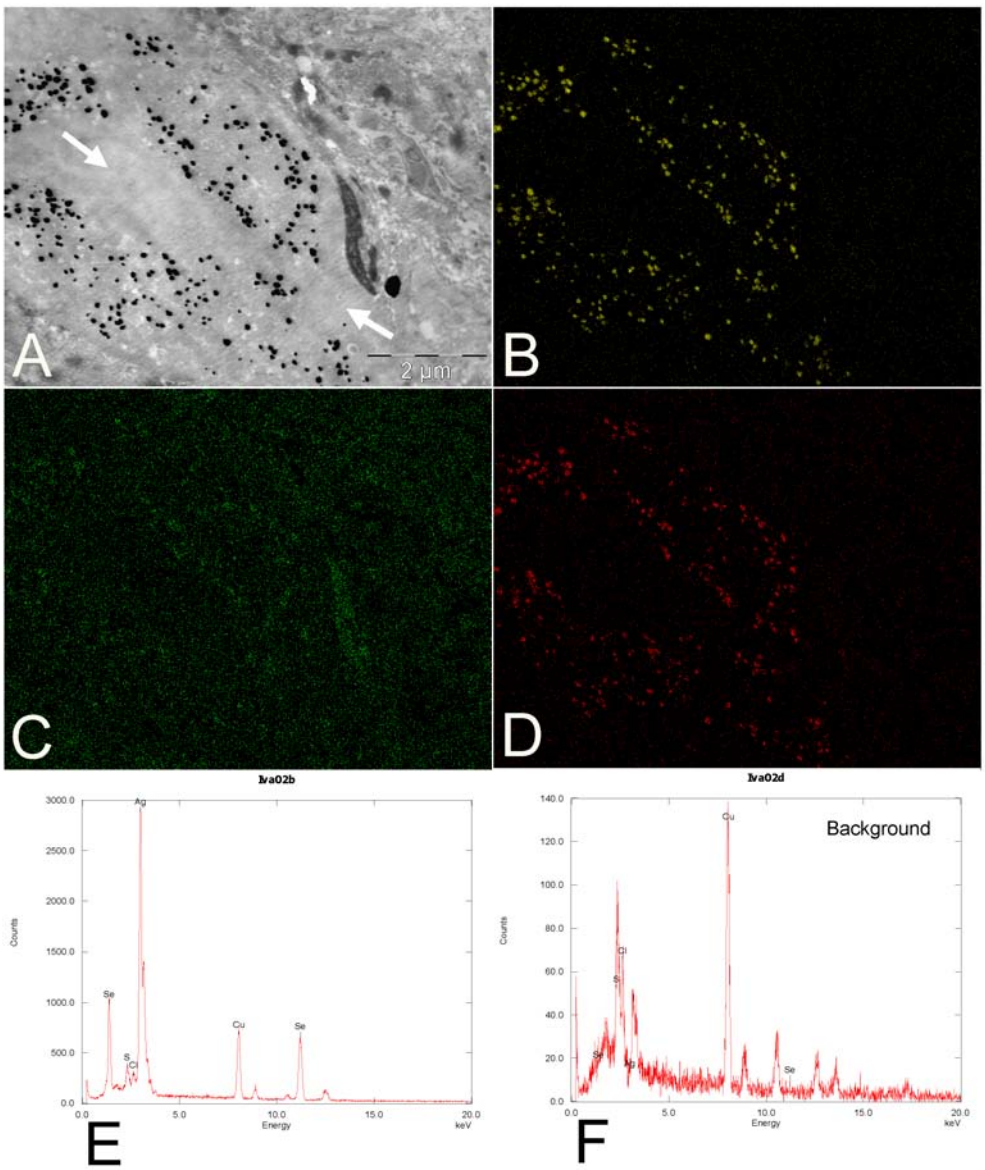

Fig. 1 Analysis of pigmented gingiva. A: STEM image of contrasted ultra-thin section. Damaged fibroblast with loss of organellar structure and presence of many dark ovoid particles; B-D: X-ray mappings of **Ag** (B), **S** (C), and **Se** (D); E: Characteristic X-ray spectrum from one selected dark particle; F: Background spectrum (control).

morphology description and its duplicate copy was marked with labeled particles of corresponding spectrum records.

Area scanning analyses were performed with iDX module of EDAX DX4 software package. A matrix of 256×200 pixels was used for acquisition of images and elemental X-ray maps, while a matrix of 512 × 400 pixels was used optionally for high-resolution data. Area scanning analyses were carried out at 80 kV with spot size of 10 nm, dwell time of 50 ms, and time constant of 40 ns. Recording time was about one hour for low-resolution images (256 × 400 pixels) and about four hours for high-resolution (512×400 pixels) images.

Three nearly eliminated teeth with pigmented roots were also examined in two patients. The alloys used for the tooth reconstructions were as follows: Tooth 1 - amalgam (20 years), alloy A (12 years), alloys D, F, and G (four years); Tooth 2 - alloys B and C (six years); Tooth 3—alloys A and D (five years). These teeth were surgically removed under local anesthesia, immediately fixed in 2.5% glutaraldehyde for four hours, and subsequently demineralized in 0.5 M EDTA (pH 7.4) at room temperature for eight weeks. Further post-fixation and processing on small-block specimens of sectioned demineralized

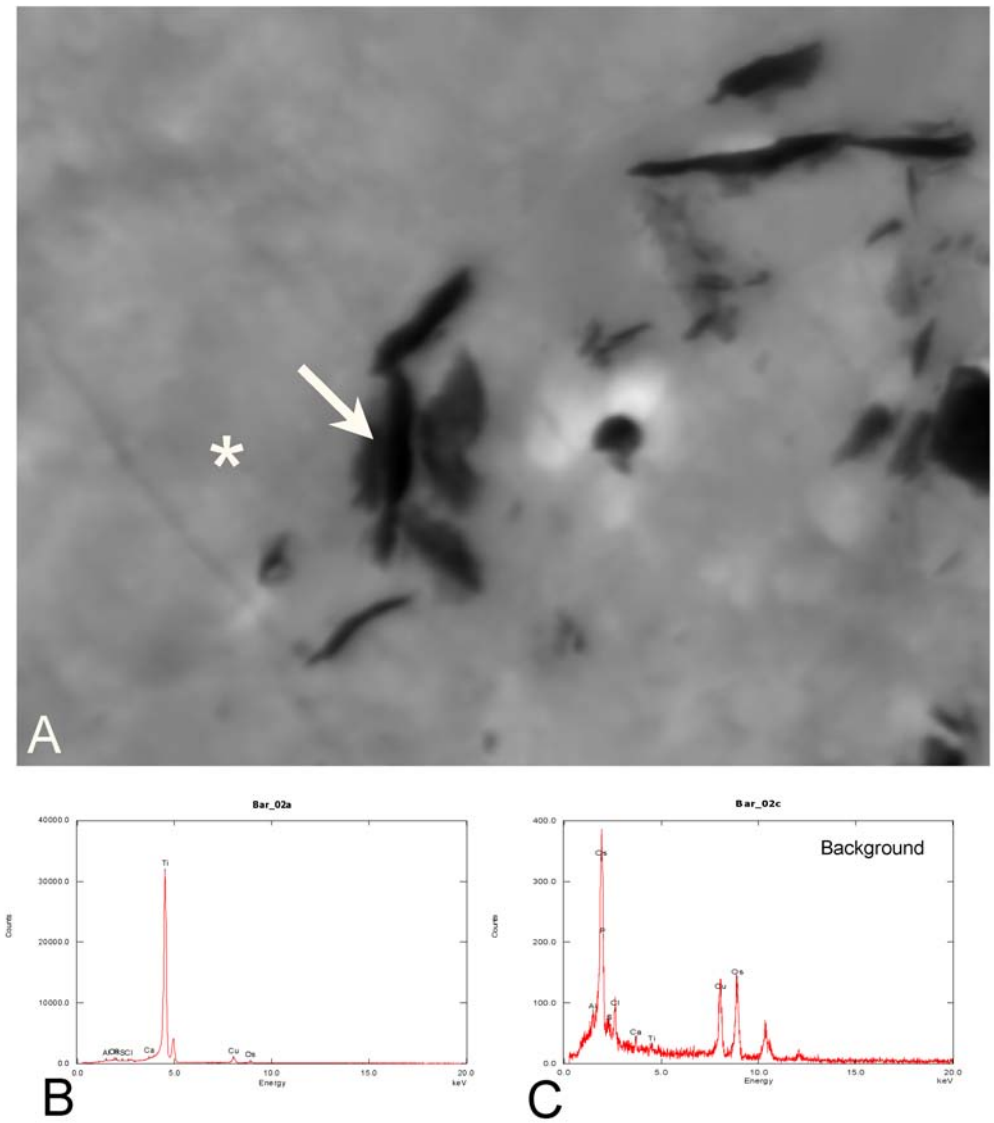

Fig. 2 Analysis of pigmented alveolar mucosa. A: STEM image of an osmium postfixed sample with needle-like dark deposits; B: Typical **Ti** X-ray spectrum from dark particle (arrow); C: Background control spectrum (asterisk).

roots were the same as for the soft tissue. Thirty to thirty-five small blocks were thus prepared and examined from each pigmented root.

Response of peripheral blood lymphocytes to metal ions

In a modified lymphocyte proliferation test using MELISA® [21,22], lymphocytes isolated by centrifugation on Ficoll gradient were partly depleted of monocytes before cultivation in 48-well cultures (1×10^6 lymphocytes per well) with various metal salts for five days. Control cultures were cultivated in the absence of metal salts. After cultivation, lymphocyte cultures were pulsed with ^{3}H-thymidine for four hours and radioactive DNA was harvested with a cell harvester. Amount of incorporated radioactivity was evaluated with a liquid scintillation counter (MicroBeta Trilux, Wallac, Denmark). 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 divided by mean cpm in control untreated cultures. SI value ≥ 3 were regarded as positive. Statistical analysis of data was performed using Fisher's exact test for quadruple tables.

RESULTS

Patients

Blue-grey pigmentation of the oral soft tissues was

Table 3 Occurrence of individual elements in analyzed particles in percents.

Element	Alloy A+Alloy C	Alloy B	Amalgam
Ag*	63.5	49.5	33.3
Ti	1.6	17.2	11.1
Sn	4.8	0.0	44.4
Cu	0.0	2.2	11.1
Fe	25.4	10.8	0.0
Al	7.9	8.6	0.0
Mg	3.2	1.1	0.0
Ca	0.0	8.6	0.0
Zn	7.9	0.0	0.0
Cr	1.6	0.0	0.0
Mn	1.6	0.0	0.0
Si	11.1	6.5	0.0
Se*	31.7	46.2	22.2
S*	46.0	37.6	11.1
Total number of analyzed particles	63	93	9

The numbers in columns headed by alloy codes used for dental restoration and amalgam represent the frequency of particles (in per cents) containing the specific element. In other words, 63.5 % of 63 particles found in discolored gingival tissue in patients with metallic restorations made of alloy A and alloy C contained silver, etc. Details on elements marked with an asterisk are given in Table 4.

Table 4 Detailed data on particles containing Ag.

Alloy A+Alloy C			Alloy A+(acrylic)			Amalgam	
Particle composition	Particle counts	～%	Particle composition	Particle counts	～%	Particle composition	Particle counts
Ag	1	1, 6	Ag	0	-	Ag	0
Ag, S	19	30, 1	Ag, S	3	3, 2	Ag, S	1
Ag, Se	10	15, 9	Ag, Se	11	11, 8	Ag, Se	2
Ag, S, Se	10	15, 9	Ag, S, Se	32	34, 4	Ag, S, Se	0

observed in the vicinity of nine teeth and one Ti implant. Seven teeth were endodontically treated with cast post and core reconstructions (Table 2, alloy A). These teeth were subsequently reconstructed in three cases with acrylic crowns and in four cases with crowns made from alloy C. In the remaining two teeth, cement and amalgam fillings were used as restorative materials. In one of these teeth, alloy E was used for the fixed appliance (Table 2). Amalgam tattoo developed in the form of a pigmentation patch.

All examined teeth with pigmented roots were reconstructed with cast post and core and crown. Alloys B and C were used in one tooth while alloys A and D were employed in the remaining teeth. Amalgam fillings or other appliances, made from materials mentioned in Table 2, were usually found mesially, distally, or occlusally from the teeth with adjacent pigmented tissues.

Stratified squamous epithelium with underlying *lamina propria* was seen on semi-thin sections. Exceptionally large and dark aggregations, probably of metal origin, were observed in biopsies stained with toluidine blue. Dense particles of various shapes and sizes, as well as of diverse localization patterns, were detected in *lamina propria* on ultrathin sections. 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 and in close proximity of the *lamina basalis* of gingival epithelium. An increased number of mast cells and macrophages was also found in the tissue near the dense deposits. On the other hand, intracellularly localized particles were found mainly in fibroblasts, and rarely in macrophages and endothelial cells. In particular, fibroblasts—with dense inclusions—often displayed various signs of cell damage, such as the occurrence of lipid vacuoles in the cytoplasm with

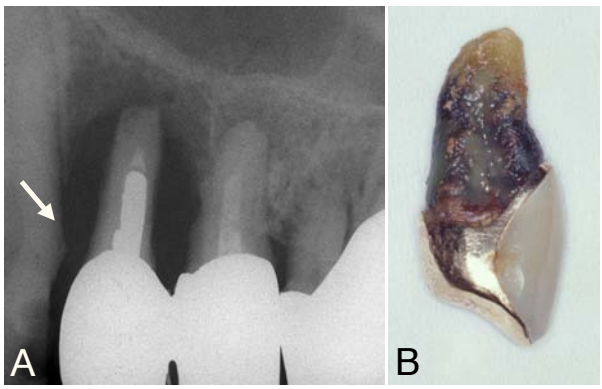

Fig. 3 A: Dental radiograph of nearly eliminated tooth (arrow); B: Same tooth after extraction exhibiting remarkable darkened part.

characteristic spherical shape and a virtually absent structure, enlarged mitochondria, or loss of typical cell structure with distinct remnants of cytoskeleton (Fig. 1A). Dense particles of ovoid shape were typical of silver content (Figs. 1A and 1B), while particles of needle-like shape were characteristic of Ti-containing inclusions (Figs. 2A and 2B).

Detailed results of EDX microanalysis of eight gingival pigmentations are summarized in Tables 3 and 4. With one titanium implant, the metal was found in dark alveolar mucosa.

Particles containing silver, sulfur, selenium, and silicon were detected in one special case, when the crown was fabricated from alloy E with underlying amalgam filling.

Dental radiograph of a nearly eliminated tooth revealed that a dark part of the root corresponded with the length of the metallic post (Figs. 3A and 3B). Dentin with abundant dentinal tubules was seen on semi-thin sections. Dense particles were observed in dentinal tubules of pigmented roots on ultra-thin sections. EDX microanalysis showed the presence of copper in dentinal tubules in the tooth reconstructed with alloys B and C (Fig. 4A) and of silver near the root surface. Both elements fitted the compositions of alloys B and C (Table 2), which were used for prosthodontic restorations. Similarly, gold, chromium, nickel, iron, mercury, copper, and titanium were identified in the dentinal tubules of the tooth reconstructed with alloys A and D and fitted with removable appliances fabricated from alloys F and G (Table 2).

Results indicated that the nine patients with oral metallic pigmentations had a positive lymphocyte proliferative response (SI $\geq$ 3) to one or more metals present in their own metal reconstructions. One patient with oral pigmentations did not react *in vitro*. As shown in Table 5, the responses to Hg, Ni, Cd, Ti, and Pd were most frequent. Based on anamnestic data, various kinds of allergic reaction (four patients), autoimmune thyreoiditis, endocrine diseases (goiter, diabetes mellitus), asthma bronchiale, Parkinson's syndrome, neurovegetative disorders, recurrent aphthous ulcers, ulcus duodeni (two patients), inflammatory disease of urinary system, prostatic hypertrophy, coxarthrosis, arthrosis genus, glaucoma, tinnitus, and hypertension were reported by seven patients with positive SI. Some patients suffered from multiple illnesses. Two patients with positive SI did not report any health problems. Gout and hypertension were recorded in the anamnesis of one patient with negative SI.

Controls

In the group of control persons (without oral pigmentations and metallic reconstructions), intact dentition was recorded in three individuals. One to three composite fillings were used as a restorative material in two persons. Weak positive lymphocyte proliferative responses to Ni and Mo were noted in two persons (Table 5). In terms of medical history, four persons were healthy (two persons were blood donors) and one person reported gout and periodontitis.

DISCUSSION

Interaction between dental alloys and the oral cavity results in the release of corrosion products which may cause problems if they accumulate in the human body. They can be deposited locally in the soft tissue, teeth, or alveolar bone as a blue-gray pigmentation, or swallowed with further possibility of intestinal resorption. According to Geurtsen[23], knowledge about the mechanism of biological interactions between metallic dental restorations and oral or systemic tissues is still very fragmentary. Therefore, there is an imperative need to clarify the release of cations from metallic dental restorations in the oral environment and determine the biological interactions of released metal components with oral and systemic tissues.

As shown in Tables 3 and 4, metallic inclusions found in the gingiva in the present study composed mainly and frequently of silver. Silver is a basic component of amalgam as well as the main constituent of many dental alloys used for prosthetic reconstructions in our patients. These factors thus accounted for its frequent occurrence in the tissue. Furthermore, our results corresponded with those of Garhammer *et al.*[24], whereby silver was frequently identified in dried samples of tissue adjacent to metal restorations. The presence of silver in our patients was always accompanied with sulfur or selenium or both. Sulfur is one of the basic elements of organic

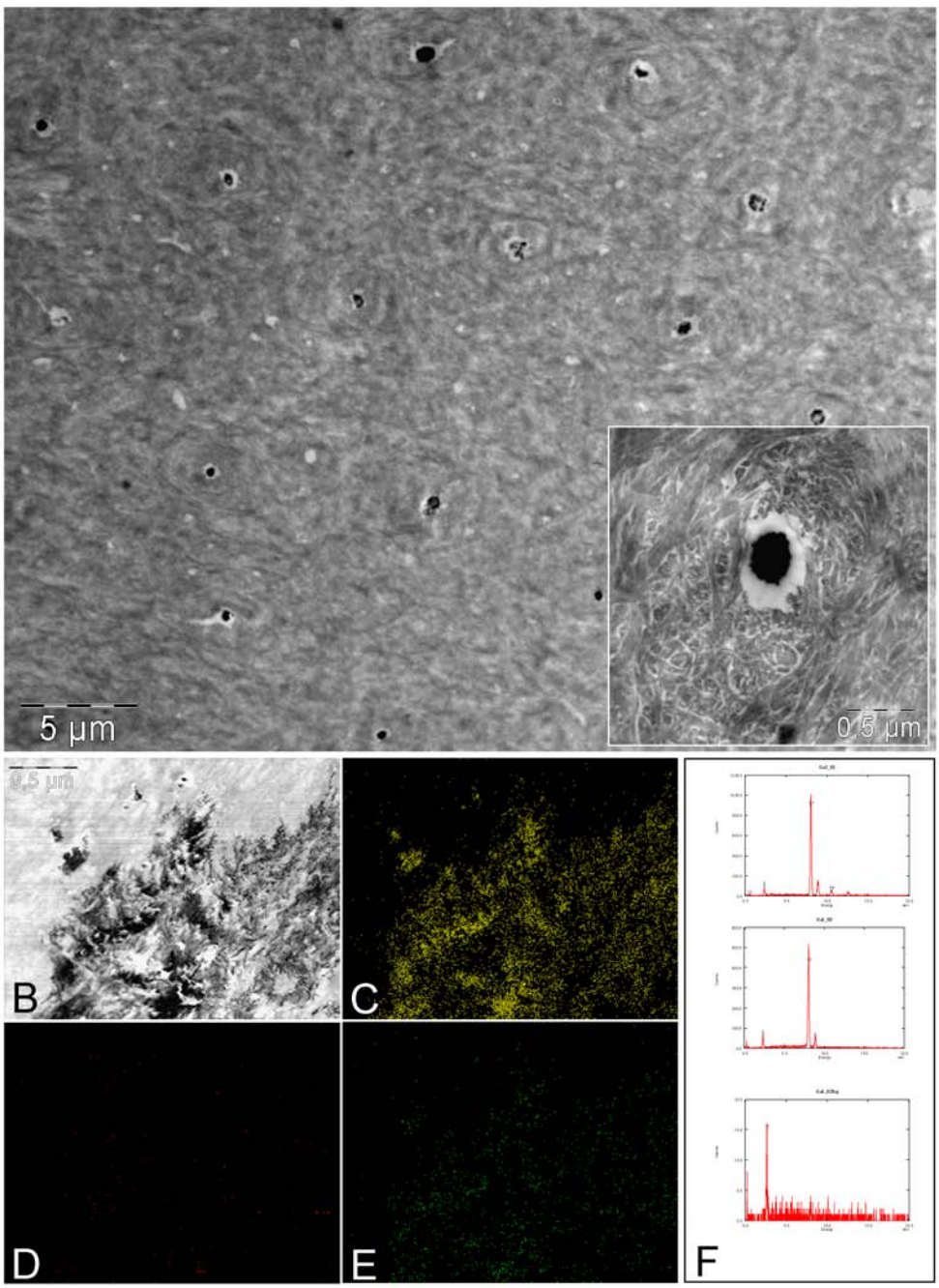

Fig. 4 Analysis of decalcified dentin from dark area of the tooth shown in Fig. 3B. A: STEM image of dentinal tubules filled with dark material in ultra-thin contrasted section. The insert shows a detail of one tubule; B: STEM image of ultra-thin non-contrasted section; C: X-ray copper map; D: X-ray chlorine map; E: X-ray phosphorus map; F: Typical X-ray spectrum of dark material in dentin tubules shown in A (upper), X-ray spectrum of sample shown in B (middle), and that of control background (bottom).

substances in the tissue, and silver sulfide was formed due to its high affinity for silver. The same was probably true for selenium (in view of its position in the periodic table of elements). Selenium present in the particles had to be of endogenous origin, since no medication with selenium was reported by the patients. The importance of selenium in the detoxification of heavy metals is often discussed in association with amalgam tattoos and argyria[25-27]. Similarly, the finding of silver sulfide was also reported in studies related to amalgam tattoos and metallic pigmentations[19,20].

Titanium was detected in the alveolar mucosa of a patient with endosteal Ti implant, which was fabricated from cp Ti. Most of the titanium needle-like deposits (Fig. 2A) were found in the extracellular matrix around fibroblasts and macrophages. In a study on biodegradation of titanium implants, similar

Table 5 Reactivity of lymphocytes to metal ions in 10 patients and five controls.

ion	Hg^{2+}	Pb^{2+}	Cd^{2+}	Ti^{4+}	Ni^{2+}	Cr^{3+}	Ag^{+}	Mo^{6+}
patients	7	3	4	3	6	1	1	0
controls	0	0	0	0	2	0	0	1

Only positive results (SI ≥3) are shown

finding was reported by Jonas *et al.*[28] after osteosynthesis of upper and lower jaws with titanium miniplates and screws. Likewise, titanium oxide particles embedded in avascular encapsulation around Ti plates were mentioned by Marx and Stern[29]. Interestingly, titanium was also identified in the tissue even when it was absent in dental alloys. This phenomenon can be explained by the junctional leakiness of epithelium and the frequent use of TiO_2 in cements, composites, acrylics, and toothpastes for color adjustment. TiO_2 is known as titanium white and is also used as a food additive (E171).

Additionally, lipid vacuoles and enlarged mitochondria in some fibroblasts with metal deposits were found on ultra-thin sections. Accumulated metal particles in cells with a loss of organellar structure but a distinct cytoskeleton were seen as well (Fig. 1A). Both these signs suggested that degenerative processes occurred in the cells. The presence of macrophages and mast cells in the *lamina propria of gingivae* indicated certain immunological reactivity around the pigmentations. This finding differed from the data of Aoyagi and Katagiri[20] concerning amalgam tattoos, whereby observed Ag_2S particles did not induce any host reaction. Similarly, very weak chronic inflammation with only a sprinkling of histiocytes and lymphocytes was observed by Jonas *et al.*[28] in the soft tissue surrounding Ti miniplates.

Metal deposits found in the dentinal tubules corresponded with either cast post and core restorations (Ag, Cu) or with the alloys used for the fabrication of the crown and removable appliance (Au, Cr, Ni, Fe). Some of them (Ti, Hg) could be present in connection with previous restorative and endodontic treatments. Physiologically, dentinal tubules are filled with the cell processes of odontoblasts. This state represents a moist milieu highly suitable for the development of the corrosion process. It was highly probable that such pigmented roots could serve as the reservoir and release source of metal ions in the human body.

The possible roles of local reaction and systemic immune response were tested with lymphocyte proliferative response. The results indicated that nine patients with oral metallic pigmentations had a positive lymphocyte proliferative response (SI≥3) to Hg, Ni, Cd, Ti, and Pd. Positive responses to only Ni and Mo were recorded exclusively in the group of control persons (Table 5). In our patients, inorganic mercury was the most frequent sensitizer, probably due to the widespread use of amalgam fillings in the Czech population. The detection of nickel in examined and control groups might point to an exogenous origin of Ni other than dental alloys, such as atmosphere (environment), tap water, foodstuffs, and cigarettes. As for the positive SI responses to Cd, Ti, and Pd, these results were in agreement with the data of Valentine-Thon and Schiwara[22], who reported Cd, Ti, and Pd to be among the most common sensitizing metals that frequently induce positive allergic reactions. As for the single positive response to Mo in one control person, no explanation could be offered presently.

Based on anamnestic data, five out of nine SI-positive patients with oral pigmentations suffered from allergies, autoimmune and endocrine disorders. A local reaction in the oral cavity may cause systemic reaction by sensitizing lymphocytes to metal ions. Metal-induced inflammation could affect the overall immunological reactivity of these patients and therefore predispose them to the development of immunologically mediated diseases such as allergy and autoimmune disease.

At present, *in vitro* lymphocyte transformation test and modified lymphocyte proliferation test, MELISA®, along with dermal, intradermal, and intraoral *in vivo* tests have been used to detect the allergenic potency of metals or casting alloys. Since there are several drawbacks in the traditional patch test—including the risk of actively sensitizing patients, the development of fast and reliable new tests would be very beneficial. On this note, innovative methods have been introduced recently. For example, by means of microarray technology, a total of 26 differentially expressed genes were identified as diagnostic markers for contact sensitivity[9]. Besides, detection of cytokines produced by mononuclear cells in patients with hypersensitivity to nickel and mercury chlorides was also used[30].

According to our experience supported by a previous work[6], the development of oral pigmentations was closely connected with the occurrence of corrosion of dental alloys. To circumvent this problem, the prudent choice of dental material (in conformity with the results of careful and thorough anamnesis) is necessary. When doubt prevails concerning a patient's tolerance to metal alloys, it is advisable to carry out a reliable immunological test to determine the actual immune response. Then, in patients with

metal allergies and autoimmune diseases, it is recommended to avoid metal appliances and to employ inert metal-free materials instead.

ACKNOWLEDGEMENTS

This project was supported by a grant from the Czech Ministry of Health, IGA NR-9124-3. Special thanks are due to Prof. Helena Tlaskalová, MD, PhD, for providing supervision to this study.

REFERENCES

1) Wataha JC, Malcolm CT. Effect of alloy surface composition on release of elements from dental casting alloys. J Oral Rehabil 1996; 23:583-589.
2) Ionescu G. Zahnlegierungen: Elektrochemische und biologische Materialprufung. Forsch Komplementarmed 1996; 3:72-77.
3) Wataha JC, Lockwood PE, Khajotia SS, Turner R. Effect of pH on element release from dental casting alloys. J Prosthet Dent 1998; 80:691-698.
4) Staffolani N, Damiani F, Lilli C, Guerra M, Staffolani NJ, Belcastro S, Locci P. Ion release from orthodontic appliances. J Dent 1999; 27:449-454.
5) Okazaki Y, Gotoh E. Comparison of metal release from various metallic biomaterials *in vitro*. Biomaterials 2005; 26:11-21.
6) Joska L, Venclikova Z, Bystriansky J, Novak P. Corrosion processes leading to the development of metallic pigmentation of the gingiva. Ces Stomat 2002; 102:197-203.
7) Büdinger L, Hertl M. Immunologic mechanisms in hypersensitivity reactions to metal ions: an overview. Allergy 2000; 55:108-115.
8) Gawkrodger DJ, Lewis FM, Shah M. Contact sensitivity to nickel and other metals in jewelry reactors. J Am Acad Dermatol 2000; 43:31-36.
9) Hansen MB, Skov L, Menné T, Olsen J. Gene transcripts as potential diagnostic markers for allergic contact dermatitis. Contact Dermatitis 2005; 53:100-106.
10) Garhammer P, Schmalz G, Hiller KA, Reitinger T, Stolz W. Patients with local adverse effects from dental alloys: frequency, complaints, symptoms, allergy. Clin Oral Investig 2001; 5:240-249.
11) Schmalz G, Garhammer P. Biological interactions of dental cast alloys with oral tissues. Dent Mater 2002; 18:396-406.
12) Thornhill MH, Pemberton MN, Simmons RK, Theaker ED. Amalgam-contact hypersensitivity lesions and oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95:291-299.
13) Mortz CG, Lauritsen JM, Bindslev-Jensen C, Andersen, KE. Nickel sensitization in adolescents and association with ear piercing, use of dental braces and hand eczema. The Odense Adolescence Cohort Study on Atopic Diseases and Dermatitis (TOACS). Acta Derm Venereol 2002; 82:359-364.
14) Marigo M, Nouer DF, Genelhu MCS, Malaquias LCC, Pizziolo VR, Costa ASV, Martins-Filho OA, Alves-Oliveira LF. Evaluation of immunologic profile in patients with nickel sensitivity due to use of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2003; 124:46-52.
15) Setcos JC, Babaei-Mahani A, Silvio LD, Mjor IA, Wilson NH. The safety of nickel containing dental alloys. Dent Mater 2006 (in press).
16) Bigazzi PE. Textbook of Autoimmune Diseases, Lippincott Williams & Wilkins, Philadelphia, 2000, pp.753-782.
17) Forsell M, Larsson B, Ljungqvist A, Carlmark B, Johansson O. Mercury content in amalgam tattoos of human oral mucosa and its relation to local tissue reactions. Eur J Oral Sci 1998; 106:582-587.
18) Pritz W, Ellinger A. Ultrastructurelle Untersuchungen an inkorporierten Amamgampartikeln in Geweben der Mundhole. Dtsch Zahnarztl Z 2000; 55:686-690.
19) Lau JC, Jackson-Boeters L, Daley TD, Wysocki GP, Cherian MG. Metallothionein in human gingival amalgam tattoos. Arch Oral Biol 2001; 46:1015-1020.
20) Aoyagi H, Katagiri M. Long-term effects of Ag-containing alloys on mucous tissue present in biopsy samples. Dent Mater J 2004; 23:340-347.
21) Stejskal VDM. Human hapten-specific lymphocytes: Biomarkers of allergy in man. Drug Information Journal 1997; 31:1379-1382.
22) Valentine-Thon E, Schiwara HW. Validity of MELISA for metal sensitivity testing. Neuro Endocrinol Lett 2003; 24:57-64.
23) Geurtsen W. Biocompatibility of dental casting alloys. Crit Rev Oral Biol Med 2002; 13:71-84.
24) Garhammer P, Schmalz G, Hiller KA, Reitinger T. Metal content of biopsies adjacent to dental cast alloys. Clin Oral Investig 2003; 7:92-97.
25) Matsumura T, Kumakiri M, Ohkawara A, Himeno H, Numata T, Adachi R. Detection of selenium in generalized and localized argyria: report of four cases with X-ray microanalysis. J Dermatol 1992; 19:87-93.
26) Rechmann P. Nachweis von Quecksilber und Selen in Amalgamtatowierungen. Dtsch Zahnarztl Z 1994; 49:151-153.
27) Sato S, Sueki, H, Nishijima A. Two unusual cases of argyria: the application of an improved tissue processing method for X-ray microanalysis of selenium and sulphur in silver-laden granules. Br J Dermatol 1999; 140:158-163.
28) Jonas L, Fulda G, Radeck C, Henkel KO, Holzhüter G, Mathieu HJ. Biodegradation of titanium implants after long-time insertion used for the treatment of fractured upper and lower jaws through osteosynthesis: Element analysis by electron microscopy and EDX or EELS. Ultrastruct Pathol 2001; 25:375-383.
29) Marx RE, Stern D. Oral and maxillofacial pathology: A rationale for diagnosis and treatment, Quintessence Publishing, Chicago, 2003, pp.705-707.
30) Venclikova Z, Benada O, Bartova J, Joska L, Mrklas L, Prochazkova J, Stejskal VD, Podzimek S. *In vivo* effects of dental casting alloys. Neuro Endocrinol Lett 2006; 27:25-32.