RELEASE OF PALLADIUM FROM BIOMECHANICAL PROSTHESES IN BODY FLUIDS CAN INDUCE OR SUPPORT PD-SPECIFIC IFNγ T CELL RESPONSES AND THE CLINICAL SETTING OF A PALLADIUM HYPERSENSITIVITY

A. CRISTAUDO, V. BORDIGNON[1], F. PETRUCCI[3], S. CAIMI[3], M. DE ROCCO, M. PICARDO[2], P. CORDIALI FEI[1] and F. ENSOLI[1]

Department of Allergy, [1]Laboratory of Clinical Pathology and Microbiology, [2]Laboratory of Skin Physiopathology, San Gallicano Dermatology Institute, Rome; [3]Department of Environment and Primary Prevention, Istituto Superiore di Sanità, Rome, Italy

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The increased use of Palladium (Pd) for biomedical applications, which has more than doubled in the last ten years, appears to be associated with an increased frequency of adverse reactions to Pd. The aim of this study is to investigate the relationship between the implant of a biomechanical apparatus containing Pd and the setting of a hypersensitivity to Pd by determining the levels of the metal released in biological fluids, assessing the effects of Pd on peripheral blood mononuclear cell (PBMC) cytokine production and exploring the clinical setting of skin sensitization. Of a total of 3,093 subjects examined in 2006, sensitization to Pd alone or in association with nickel (Ni) was observed in 1.6% and 13.03% of the individuals, respectively. Of these, a group of six subjects positive to Pd and negative to Ni at patch testing were selected on the basis of the oral clinical symptoms in order to measure both the levels of Pd in biological fluids and the degradation of the dental prostheses. Specific Pd measurements were carried out on salivary fluid, urine and serum samples by High Resolution Inductively Coupled Plasma-Mass Spectrometry. In addition, the degradation of the dental prostheses was assessed by both a "leaching test" and an analysis of the micro morphology of orthodontic prostheses. The induction of IFN-γ production by Pd was assessed in PBMC by the ELISpot assay. Skin sensitization to Pd was evaluated by patch testing and clinical examination. Ten healthy subjects were comparatively tested as controls. We found a specific induction of an IFN-γ response by Pd in PBMC collected from all the subjects positive to Pd at patch testing. On the contrary, control subjects did not show any response to Pd as assessed by IFN-γ ELISpot assay or by skin testing. Remarkably, the levels of Pd in all biological samples (saliva, sera, urine) were significantly higher in Pd-sensitized patients than in those collected from controls, reaching the highest concentrations in the urine. The leaching studies gave additional evidence that the dental appliances can release measurable levels of Pd in saliva. Oral clinical symptoms in patients with Pd dental prostheses were associated with measurable levels of Pd in the biological fluids, the induction of Pd-specific IFN-γ responses in PBMC and the clinical evidence of skin sensitization to Pd. These data suggest that dental appliances may represent an active source of Pd in the body, and this, in turn, can favour the clinical setting of a hypersensitivity to this metal.

Key words: palladium, allergy, skin, body fluids, contact dermatitis, dental prostheses, patch test, ELISpot assay

Mailing address: Antonio Cristaudo, MD
Department of Allergy
San Gallicano Institute
Via Elio Chianesi 53,
00144 Roma, Italy.
Tel: ++39 06 5266 6906 Fax: ++39 06 5266 6025
e-mail: cristaudo@ifo.it

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Palladium, a metal of the platinum group, represents a widely-used component in most cast dental restorations and amalgams (dental alloys may contain up to 10% Pd) (1-4). Due to its relatively low cost and the relatively simple and cost-effective processing procedures, the use of dentistry alloys containing Pd has more than doubled during the past ten years (5). Indeed, Pd appears more resistant to the effects of mechanical wearing (6), and data on local (7) and systemic toxicity (8) confirm its safety for use in dental appliances. In addition, such an increasing demand of Pd is also attributable to the release of the European Nickel Directive 94/27/EC (9) which restricted the use of Ni in all industrial products for limiting the consumer's contact with a well-recognized allergen. This prompted the exploitation of different metal compounds for medical destinations such as the alloys used in orthopaedics and in dental appliances (10).

At present, Pd is considered a low-risk metal for use in dentistry on the basis of the reported very low rate of dissolution (6). Nevertheless, the frequency of dermatitis caused by Pd, which was previously considered a rare occurrence (3), has recently increased (1, 11-12). In addition, a significant fraction of patients with an allergic reaction to Ni sulphate has also been shown positive to Pd chloride at patch-testing (13). In the last decade, sensitivity to Pd has been described by several reports. The most common symptoms are generally related to a contact allergy of the oral mucosa: cheilitis, stomatitis, burning mouth, lichenoid reactions, oral-facial granulomatosis, or swelling of the lips and cheeks, dizziness, asthma and chronic urticaria (11, 14-16). In some reports, the clinical symptoms ceased after replacing the Pd-containing appliances with Pd-free constructions (17).

Epidemiological data indicate a sensitization rate of 8% in Europe (18), with similar frequencies at different locations, being 9.4% in England (19), and 8.3% in unselected eczema patients in Austria (12). In Italy, the results of a 10-year evaluation study (1991-2000) showed an increasing trend to sensitization to Pd chloride, that reached the highest frequency (9.7%) in the year 2000 (11). These data may indeed represent an indicator of an emerging medical problem. Therefore, supported by the evidence that some patients suffering from burning mouth syndrome had a positive patch test to Pd, starting from the year 1996, we included Pd in the routine patch testing panel for the clinical assessment of contact dermatitis. The results of this perspective screening indicated that of a total of 3,093 subjects examined at the end of 2006, sensitization to Pd alone or in association with Ni was 1.6% and 13.03%, respectively.

In fact, several studies *in vivo* indicate that dental alloys are prone to corrosion and can release metal components in the oral cavity (20-21). Based on this evidence, in this study we evaluated a group of six subjects affected by a suspected adverse reaction to dental prostheses caused by sensitization to Pd to determine whether the degradation of the dental prostheses is associated to Pd hypersensitivity as assessed by patch testing and by the determination of T cell immune response to Pd using the ELISpot technique. The degradation of the dental prostheses was assessed by analysis of the micro morphology of orthodontic prostheses as well as by a "leaching test". In addition, the presence of Pd concentrations in body fluids was measured by a High Resolution Inductively Coupled Plasma-Mass Spectrometry (HR-ICP-MS) technique, a well established and very powerful analytical method for the determination of trace and ultra-trace elements in environmental and biological samples (22). Since polyatomic and isobaric interference could represent a bias for the analysis of very low Pd levels in such matrices, an interference study was performed to quantify such bias and apply due corrections.

MATERIALS AND METHODS

Patients

Among the subjects consecutively patch tested at the Department of Allergy in 2006, six patients with oral symptoms including gingivitis, taste irritation, dry mouth, and burning mouth associated to the presence of metal restorations, except amalgams, and with a positive patch test to Pd were selected for this study. Detailed medical history, including any previous metal exposure and use of metal dental prostheses, was collected. In one case, we were able to collect the biological specimens before and one month after the removal of the dental prostheses, respectively, having therefore the opportunity to compare the levels of Pd in the presence and in the absence of Pd prosthesis. Ten subjects with negative patch test to Pd and without any type of metal restorations or amalgams

were studied to provide background data for metal content determinations. All subjects gave informed consent.

Patients nos. 1, 4 and 5 were women, aged 57, 64 and 69, respectively, who came to our outpatient clinic suffering from a sense of burning and dryness in the mouth. The symptoms had started 1 to 3 years earlier, after having a fixed, palladium-based framework supporting 3 ceramic dental crowns (patients nos.1 and 5) or 5 ceramic dental crowns (patient no. 4) implanted. In all cases, the symptoms were initially localized at the tip of the tongue, becoming more intense with the time. The oral swabs performed to detect the presence of a *Candida albicans* infection were negative. None had any evidence of clinical depression or anxiety. Mouth-rinse, vitamins, anti-mycotic therapy and drugs for neuropathy did not improve the clinical condition. Blood tests did not reveal any additional alterations. Subjects nos. 1 and 4 refused to remove the dental alloy, while patient no. 5 consented to remove the palladium crowns, which were replaced with provisional acrylic crowns. This procedure was associated with a dramatic improvement of the symptoms.

Patient no. 2 was a 77-year-old woman who came to our clinic referring a chronic soreness of the lower lip. The patient had been asymptomatic until the previous year. At that time a full crown was placed on the mandibular canines to serve as abutment for a removable partial denture.

Patient no. 3 was a 71-year-old woman who reported a history of sensitization to common allergens such as pollen, but no previous sensitization to any metal. She developed a severe inflammation of the mucosa at sites in close contact with a porcelain partial denture alloy that she had worn for the previous 3 months. The fixed partial denture was removed and replaced by a provisional acrylic resin restoration with a progressive remission of all the clinical signs.

Patient no. 6 was a 59-year-old woman, with no history of allergy, who presented recurrent swelling and pain of the oral mucosa. Clinical examination showed a marked swelling and ulceration of the mucosa in close contact with a metal crown in place since 2005. The patient refused to remove the dental appliance.

Patch testing

The skin patch tests were performed using the European standard patch test series (Hermal Trolab, Reinbeck, Germany) supplemented with palladium chloride 1% ($PdCl_2$) in petrolatum, using Finn Chambers on Scampor (Epitest Ltd Oy, Tuusula, Finland). The allergens were placed on unaffected upper-back skin. Readings were performed on days 2 and 3. In subjects positive only to Pd we performed further two readings on days 4 and 7. A homogeneous redness and infiltration in the entire test area was classified as a 1+ reaction; homogeneous redness, infiltration and vesicles in the test area were classified as a 2+ reaction; homogeneous redness, infiltration and coalescing vesicles in the test area was a 3+ reaction. A 1+, 2+, or 3+ reading was interpreted as a positive response (23).

ELISpot assay

The setting of the immune response against Pd was further assessed *in vitro* by investigating the presence and quality of IFN-γ producing T cells elicited by metal compounds by the ELISpot assay as previously described (24). These studies were performed on the six patients with positive patch test to Pd and in control subjects (negative patch test to Pd).

Briefly, PBMC were isolated from 5 to 10 ml of heparinized blood, collected 48 h after skin testing, by standard Ficoll density-gradient centrifugation (Lympholyte-H solution Cederlane, Ontario, Canada) and washed twice with PBS. Cell aliquots were frozen in 90% heat inactivated Fetal Bovine Serum (FBS, Euroclone) and 10% DMSO (Dimethylsulphoxide, Sigma) and kept in liquid nitrogen until tested by the ELISpot assay. PBMC were plated at 2.5 $x10^5$ / well, to a final volume of 200 µl/well of complete medium. The metal salts used for *in vitro* assays were: nickel sulphate hexahydrate ($NiSO_4x6H_2O$, 20 µg/ml), palladium chloride ($PdCl_2$, 2.5 µg/ml) and rhodium acetate $Rh(CH_3COO)_2$, 5 µg/ml) (Merck AG, Darmstadt, F.R.G). Before use, metals were dissolved in sterile saline solution at 2 mg/ml (Bioindustria, Novi Ligure, Italy). These stock solutions were found negative for LPS contamination by the *Limulus* assay (BioWhittaker, Cambrex Company, USA). Red spots were analysed by the Automated ImmunoSpot Image Analyzer Software (AELVIS Technologies, TEMA Ricerche, Italy). The results were expressed as mean values of spot forming cells, for triplicate wells, detected upon stimulation.

Biological samples for Pd content analysis

Salivary fluid, urine and serum samples were collected for the analysis of Pd content in biological matrices. Saliva (1 ml) was collected in polyethylene (PE) tubes (Greiner, Frickenhausen, Germany) before breakfast, tooth brushing, and smoking, and this procedure was repeated after 2 days. The samples were treated with 1 ml of ultra-pure concentrated HNO_3 (Carlo Erba, Milan, Italy) in a microwave oven (FKV Milestone, Bergamo, Italy) for 2 h at 80°C. After digestion, samples were diluted up to 10 ml with deionised water (EasyPure, PBI International, Milan, Italy) and subsequently analysed. Serum and urine samples were collected in the morning in PE tubes and diluted 1:5 (v/v) with deionised water.

It should also be noted that the individual difference of saliva production, the regional pH value (in the surrounding area of the prostheses), diet (presence of proteins from food binding the released metals), personal habits (chewing gum use) and hygiene were all considered since these elements may influence the corrosion and metal ion release from the dental appliances (25). For these reasons, subjects with amalgams were excluded to avoid metal contamination from materials different from prostheses (26). Recommendation to avoid eating, tooth brushing or smoking (27) before saliva collection concurs in limiting the individual variability between subjects and in standardizing the procedure of saliva collection as consistently as possible.

Pd content analysis

Measurements of Pd content in all biological samples were performed by HR-ICP-MS. The technical characteristics and settings of the analytical instrumentation are summarized in Table I. The main disadvantage of this technique is the polyatomic and isobaric interference, which required a quantification and specific correction. Specifically, the analytical mass spectra were investigated at medium resolution (m/Δm 3000) to evaluate in detail molecular ions interference. Increasing concentrations of the interfering elements were added to each matrix to match and even exceed their expected ranges of concentration.

The calibration of the test was performed adopting the standard addition mode: diluted single-element standards were added to the analytical solutions of saliva, serum and urine. To compensate the instrumental drifts and matrix effects, Indium was added as internal standard (IS) to each sample.

Figures of merit

Limits of detection (LoDs) were calculated on the basis of the 3-σ criterion. Blanks for saliva, urine and serum were obtained from non-exposed subjects. The same samples, spiked as needed, were used for the within-series imprecision tests. Because of the lack of reference materials with certified levels of PGEs for the matrices under study, recovery tests were carried out to assess the accuracy of the method: a solution containing Pd was spiked to biological samples, with aliquots of 10, 50 and 100 ng l^{-1} of the analyte prior to pre-analytical treatment and then analyzed.

Leaching experiments

A leaching test on orthodontic prostheses was performed in order to evaluate the release of Pd in saliva. Two different metallic pivots and bridge-work crowns were left in contact with samples of saliva – obtained from two subjects of the control group not-exposed to Pd-containing prostheses – in a thermostatic bath at 37°C for 24 h. After incubation, saliva samples were digested and analyzed as mentioned above. Samples were tested in duplicate.

3D imaging of dental prostheses

Using computerized X-ray micro tomography, analysis of the external and internal micro morphology of the orthodontic prostheses was performed in order to examine their degradation. The hardware device used in this study was a desktop X-ray micro-focus CT scanner (SkyScan 1072, SkyScan bvba, Aartselaar, Belgium).

Statistical analysis

The unpaired *t* test was used to compare IFN-γ responses between groups of subjects. A P value < 0.05 was considered to be statistically significant. Statistical analysis was performed with GraphPad Software, version 4.00 (San Diego California USA).

RESULTS

Patch testing and clinical observations

The patch testing performed on 3,093 patients during the entire year 2006, revealed a mono-sensitization to Pd in 15 out of 928 positive individuals (1.6%), while 121 subjects (13.03%) had a positive patch test response to both Ni and Pd. Overall, the clinical evaluation of the 15 patients that had a positive patch test to Pd showed oral symptoms such as cheilitis, perioral dermatitis and periodontitis (5/15). Burning mouth syndrome is characterized by symptoms of pain or burning sensation of the tongue in the absence of any clinical evidence of eczema and/or blistering at clinical evaluation (5/15); glossodynia was also reported (5/15), sometimes associated with the burning mouth syndrome. At the clinical observation, the mucositis and stomatitis found in 4 of these patients were relevant. In 5 patients, hand dermatitis and diffuse eczema were prevalent. Anamnestic data showed that all patients monosensitized to Pd were wearing dental prostheses.

Among these subjects, six patients showing a positive patch test reaction to Pd and oral symptoms including gingivitis, taste irritation, dry mouth, and burning mouth associated to the presence of metal restorations, except amalgams, were selected for a more in depth biochemical, immunological and clinical exploration, including the measurement of

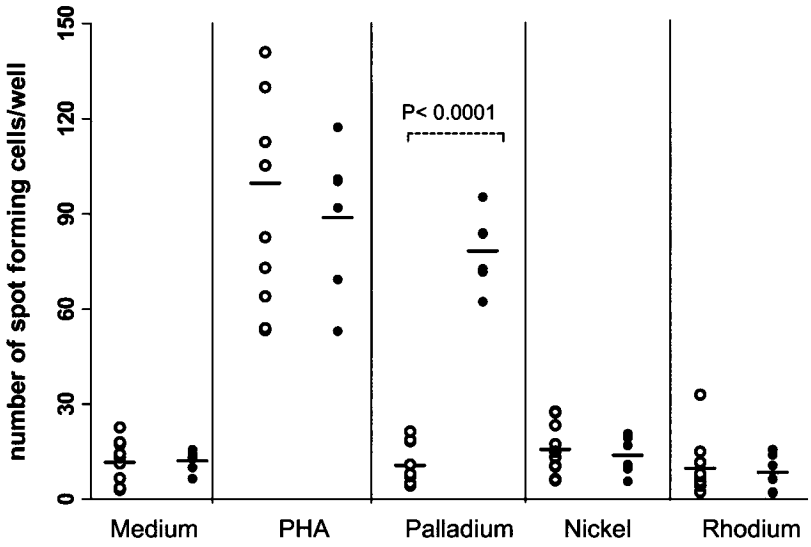

Fig. 1. *IFN-γ responses induced by metal ions or PHA. ELISpot assay was performed to assess IFN-γ producing PBMC (2.5 x10^5/well) in response to stimulation with $PdCl_2$ (2.5 μg/ml), $NiSO_4$ (20 μg/ml), $Rh(CH_3COO)_2$ (5 μg/ml) or PHA (1 μg/ml). The results were expressed as number of spot forming cells (SFC)/well. The mean values of triplicate wells were calculated for each subject. Black circle represents subjects with positive patch test to Pd and oral symptoms (six individuals); white circle represents healthy controls (ten individuals). The statistical analysis was performed using the unpaired t test.*

Pd released in biological fluids and the assessment of the T cell immune response against Pd. A detailed medical history of patients, including history of metal exposure and wearing of metal dental prostheses, is detailed in Materials and Methods. As mentioned, in one case we were able to collect the body fluids before and after the prostheses removal, therefore having the opportunity to gather comparative data.

Determination of Pd sensitization in vitro by assessing T cell immune response to Pd

Fig. 1 shows the basal levels of IFN-γ-producing cells as compared to those elicited by Pd salts or PHA in different tested subjects. Results are depicted as scatter plots of individual spot-forming cells. All six of the patients with a positive Pd patch test had a strong T cell-mediated IFN-γ immune response to Pd, which was highly specific since they did not raise any measurable response against Ni or Rh (P<0.0001). On the other hand, all subjects with negative patch test did not show any IFN-γ T cell response to Pd nor to Ni or Rh. It should be noted that a preliminary functional validation of such testing was performed by assessing the induction of IFN-γ-producing T cells by a standard stimulus (PHA). The

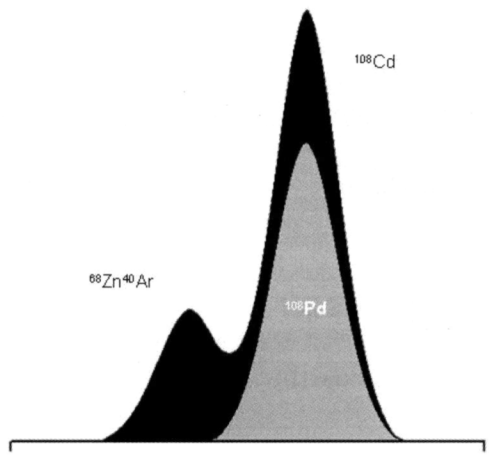

Fig. 2. *Interferences profile on mass ^{108}Pd at medium resolution mode (m/Δm=3000).*

data confirmed that PHA stimulated lymphocytes from all subjects, including patients and controls, significantly increased the number of IFN-γ-producing T cells as compared to their unstimulated counterparts (P<0.0001) with no differences among the individuals examined (patients and controls).

Table I. *High Resolution Inductively Coupled Plasma-Mass Spectrometry (HR-ICP-MS). Specifications on instrumental settings and data acquisition parameters.*

Apparatus	Element (Thermo-Finnigan, Bremen, Germany) equipped with the Guard Electrode device
RF power	1200-1250 W
Sample introduction	Nebulizer: concentric, Meinhard glass type; Spray chamber: Scott-type, water-cooled
Internal standard	^{115}In
Analytical Masses	^{105}Pd, ^{106}Pd, ^{108}Pd
Interface	Pt cones
Gas flow rates (l min^{-1})	Cooling, 14; auxiliary, 1.00; nebulizer, 1.10
Resolution (m/Δm)	Medium Resolution = 3000
Acquisition mode	Electric scan
Number of scans	15

Table II. *Range of Pd content (ng/L) in the different biological samples as assessed by HR-ICP-MS.*

Specimens	**Saliva** (5ng/L)*	**Serum** (12 ng/L)*	**Urine** (8 ng/L)*
Patients (n=6)	19-1140	12-180	250-580
Controls (n=10)	5-13	12-17	8-25

**Limit of detection*

PD Quantification In Body Fluids

Method validation and analytical performance

In order to validate the method for Pd determination in biological specimens, a specific "interference" study was performed to identify the most appropriate isotope mass of Pd in order to overcome the influence of mass interferences. Among Pd isotopes, a mass equal to 108 was considered optimal since its signal is hampered by ^{68}Zn ^{40}Ar and ^{108}Cd only (Fig. 2). Operating at medium resolution, the interference of the Zn polyatomic species can be physically shifted, whereas for the isobaric interference of ^{108}Cd on ^{108}Pd the use of a mathematical correction was deemed necessary as follows:

$$I\ ^{108}Pd = I\ 108 - [I\ ^{111}Cd \times IR]$$

where I means the intensity (counts) and IR is the isotopic abundance ratio between the masses ^{108}Cd

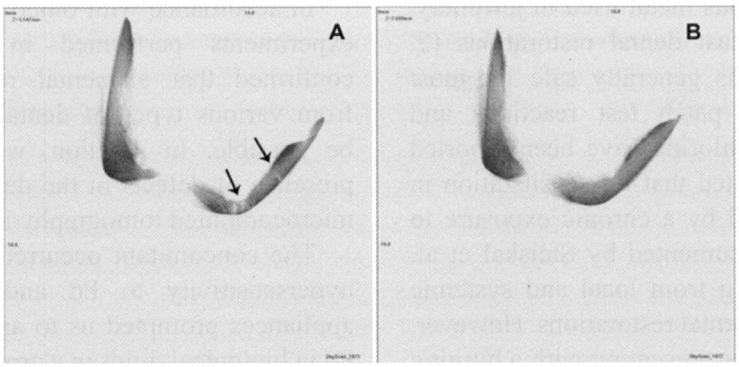

Fig. 3. *The Micro-CT 3D images provided direct evidence of the corrosion and the structural defects in the micro architecture of the prostheses as assessed in patient no. 5. Panel **A** represents the dental appliance obtained from the patient, while panel **B** shows a matched control, consisting of a brand-new dental appliance.*

and ^{111}Cd, chosen to quantify the Cd intensity.

Studies on the limit of detection (LoD), repeatability and recovery were performed in order to validate the measurements of Pd in biological fluids. The detection power achieved by HR-ICP-MS, equipped with a pneumatic nebulizer, was adequate to analyze the expected concentrations of Pd in the studied matrixes. In particular LoDs of 5, 12 and 8 ng l^{-1} was reached for saliva, serum and urine respectively. The reliability of the test was further proven by the repeatability obtained on ten measurements well within the limits of confidence. At a concentration of 50 ng l^{-1}, precision varied from 5.2 for the digested solutions (saliva) to 6.8 and 7.9 for water-diluted solution (serum and urine). In the latter case, the presence of organic matter may account for a higher (although acceptable) instability in the analytical signal, which cannot be fully counterbalanced by the use of the internal standard. The results of the recovery studies were consistent with the added amounts for all matrixes (10, 50 and 100 ng l^{-1}). In saliva the recovery varied from 95 to 105%, in serum from 98 to 103% and in urine, from 96 to 105%.

Pd levels in biological specimens

Pd content in saliva, serum and urine samples from the six patients wearing dental appliances and from the control group is shown in Table II. Pd was detected in all biological samples. The levels were always higher in urine as compared to those found in saliva or in sera. In all the samples collected from the six patients, the levels of Pd were significantly higher than those collected from control subjects. Remarkably, one patient, who had the dental prostheses removed due to severe oral symptoms, showed a dramatic reduction of Pd content in saliva (250 ng l^{-1} *vs* 150 ng l^{-1}), serum (120 ng l^{-1} *vs* 50 ng l^{-1}) and urine (570 ng l^{-1} *vs* 280 ng l^{-1}) as compared to the levels measured before the removal.

Leaching experiments results

During the leaching experiments no colouring of saliva was noticed in any sample. Measurable levels of Pd were released in the healthy saliva by all the dental appliances with the following differences: the mean values of Pd released from the two metallic implanted pivots (730 ± 60 and 790 ± 50 ng l^{-1}) were higher than those released by the two bridge-work crowns (120 ± 30 and 190 ± 20 ng l^{-1}).

Microcomputed tomography results

The Micro-CT 3D imaging provided precise evidence of structural defects in the micro architecture of the dental prostheses obtained from the patient suffering from oral symptoms who decided to restore the dental appliance after participating in this study (Fig. 3). This analysis confirmed that the dental appliance can be susceptible to corrosion.

DISCUSSION

Hypersensitivity to different metals represents a frequently emerging problem because of a significant

morbidity. Pd is a precious metal used in jewellery, industry and in most cast dental restorations (2, 5). Even if this metal is generally safe for most medical uses, positive patch test reactions and hypersensitivity to Pd chloride have been reported (28). It has been suggested that Pd sensitization in humans may be induced by a chronic exposure to low doses of Pd, as documented by Stejskal et al. (29) in patients suffering from local and systemic symptoms attributed to dental restorations. However, contact allergy in patients presenting with a burning mouth syndrome have not been adequately assessed in previous studies, also considering that in earlier tests potentially relevant allergens such as Pd were not included, therefore in some cases the cause of sensitization might have remained unrecognized.

Despite it being well-known that hypersensitivity to Pd is generally related to a simultaneous sensitization to other metals such as Cobalt, Potassium dichromate ($K_2Cr_2O_7$) and Nickel (3, 11, 13, 23), in our experience the frequency of mono-sensitizations to Pd increased from 0.11%, considering the years 1996 and 1997, to 1.6% in 2006. This tendency was confirmed by the frequency of 1.8% of mono-sensitizations to Pd calculated on a total of 5350 subjects analysed in the years 2007 and 2008, suggesting that this metal, which has often been used as a substitute for Ni in the last ten years, is becoming a "novel" potential allergen.

Interestingly, in the present study, all patients mono-sensitized to Pd (positive with patch test and IFNγ ELISpot) had intra-oral symptoms and were wearing dental prostheses at the time of the clinical evaluation. This is consistent with previous case reports showing that restorative materials can be a cause of allergic contact mucositis (2, 30). Indeed, Pd is a very common component of various dental appliances, therefore the local mucosa, lateral tongue and gingiva in contact with the dental restoration represent the most proximal site that can be affected by contact allergic reactions (31). Even if the Au–Pt–Pd dental alloys are considered the most resistant materials to electrochemical and chemical corrosion, numerous parameters are not always controllable, such as the quality of the restoration and the lifestyle of the patients. In fact, the level of Pd biologically available in the form of Pd ions depends on the type of corrosive environment present in the mouth (25).

In accordance with others (26, 32), the leaching experiments performed in the present study, confirmed that elemental release of metal ions from various types of dental casting alloys could be possible. In addition, we also confirmed the presence of defects in the dental appliances by the microcomputed tomography imaging.

The concomitant occurrence of oral symptoms, hypersensitivity to Pd and presence of dental appliances prompted us to analyse the presence of Pd in biological fluids in a group of selected patients. The HR-ICP-MS offers a powerful approach for the simultaneous analysis of different elements in biological matrixes due to its very high sensitivity. In respect to other analytical techniques, such as ETV-AAS or ICP-AES, this method offers limits of quantification much lower than one order of magnitude, operating at the highest resolution. A set of appropriate interference studies and implementation of validation procedures allows the highest accuracy. Also, for these reasons, the levels of Pd found in this study are much lower than those previously found with other analytical techniques (26). In fact, problems of mass interference affecting the determinations obtained by ICP-MS are mostly solved by the HR-ICP-MS by operating at increasing steps of resolution.

The clinical assessment of Pd in saliva samples showed higher levels in the group of patient than in the control subjects, although Pd content in saliva showed little variations among the six patients. These results support data from Garhammer et al. (26), showing that components of dental alloys are released into saliva and to the adjacent soft tissue of the oral cavity.

Regarding serum concentrations, Pd levels were found at very low concentrations in patients and did not reflect those found in saliva and urine. These data offer an indirect confirmation of the results obtained in rats that, after receiving a sub-acute oral dose of Pd, did not show an increase in serum Pd concentration according to the administered dose (33).

The highest mean values of Pd levels were detected in urine samples. This result is in agreement with a previous study (34) and strongly suggests that also in humans Pd is subjected to a predominant kidney excretory pathway as described in small

animals (33).

In conclusion, oral clinical symptoms in patients with Pd dental prostheses were associated with measurable levels of Pd in the biological fluids, the induction of Pd-specific IFN-γ responses in PBMC and skin sensitization to Pd. These data indicate that dental appliances may represent an active source of Pd in the body, and this, in turn, can favour the clinical setting of hypersensitivity to this metal.

REFERENCES

1. Kielhorn J, Melber C, Keller D, Mangelsdorf I. Palladium: a review of exposure and effects to human health. Int J Hyg Environ Health 2002; 205:417-32.
2. Vilaplana J, Romaguera C, Grimalt F, Cornellana F. New trends in the use of metals in jewellery. Contact Dermatitis 1991; 25:145-8.
3. Vincenzi C, Tosti A, Guerra L. Contact dermatitis to palladium: a study of 2300 patients. Am J Contact Dermat 1995; 6:110-2.
4. Tobey RG. Palladium-silver: the alloy of the future. Trends Tech Contemp Dent Lab 1989; 6:31-3.
5. Cristaudo A, Sera F, Severino V, De Rocco M, Di Lella E, Picardo M. Occupational hypersensitivity to metal salts, including platinum, in the secondary industry. Allergy 2005; 60:159-64.
6. Gil FJ, Sanchez LA, Espias A, Planell JA. *In vitro* corrosion behaviour and metallic ion release of different prosthodontic alloys. Int Dent J 1999; 49: 361-7.
7. Mahler DB, Engle JH, Adey JD. Effect of Palladium on the clinical performance of amalgam. J Dent Res 1990; 69:1759-61.
8. Murdoch RD, Pepys J. Enhancement of antibody production by mercury and platinum group metal halide salts. Int Arch Allergy Appl Immunol 1986; 80:405-11.
9. Liden C. Legislative and preventive measures related to contact dermatitis. Contact Dermatitis 2001; 44: 65-9.
10. Vamnes JS, Lygre GB, Gronningsaeter AG, Gjerdtet NR. Four years of clinical experience with an adverse reaction unit for dental biomaterials. Commun Dent Oral Epidemiol 2004; 32:150-7.
11. Larese Filon F, Uderzo D, Bagnato E. Sensitization to palladium chloride: a 10-year evaluation. Am J Contact Dermat 2003; 14:78-81.
12. Aberer W, Holub H, Strohal R, Slavicek R. Palladium in dental alloys--the dermatologists' responsibility to warn? Contact Dermatitis 1993; 28:163-5.
13. Hindsen M, Spiren A, Bruze M. Cross-reactivity between nickel and palladium demonstrated by systemic administration of nickel. Contact Dermatitis 2005; 53:2-8.
14. Garau V, Masala MG, Cortis MC, Pittau R. Contact stomatitis due to palladium in dental alloys: a clinical report. J Prosthet Dent 2005; 93:318-20.
15. Hansen PA, West LA. Allergic reaction following insertion of a Pd-Cu-Au fixed partial denture: a clinical report. J Prosthodont 1997; 6:144-8.
16. Augthun M, Lichtenstein M, Kammerer G. Studies on the allergenic potential of palladium alloys. Dtsch Zahnarztl Z 1990; 45:480-2.
17. Prochazkova J, Sterzl I, Kucerova H, Bartova J, Stejskal VD. The beneficial effect of amalgam replacement on health in patients with autoimmunity. Neuro Endocrinol Lett 2004; 25:211-8.
18. Kranke B, Aberer W. Multiple sensitivities to metals. Contact Dermatitis 1996; 34:225.
19. Finch TM, Prais L, Foulds IS. Palladium allergy in a British patch test clinic population. Contact Dermatitis 1999; 41:351-2.
20. Kerosuo H, Moe G, Hensten-Pettersen A. Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 1997; 111:595-8.
21. Schmalz G, Garhammer P. Biological interactions of dental cast alloys with oral tissues. Dent Mater 2002; 18:396-406.
22. Bolann BJ, Rahil-Khazen R, Henriksen H, Isrenn R, Ulvik RJ. Evaluation of methods for trace-element determination with emphasis on their usability in the clinical routine laboratory. Scand J Clin Lab Invest 2007; 67:353-66.
23. Rietshel RL, Fowler JF Jr. Practical aspects of patch testing. In: Rietshel RL, Fowler JF Jr, eds. Fisher's Contact Dermatitis, 5th edn. Philadelphia: Lipincott Williams and Wilkins, 2001:9-26.
24. Bordignon V, Palamara F, Cordiali-Fei P, Vento A, Aiello A, Picardo M, Ensoli F, Cristaudo A. Nickel, palladium and rhodium induced IFN-gamma and IL-10 production as assessed by *in vitro* ELISpot-

analysis in contact dermatitis patients. BMC Immunol 2008; 15:9-19.
25. Kerosuo H, Moe G, Hensten-Pettersen A. Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 1997; 111:595-8.
26. Garhammer P, Hiller KA, Reitinger T, Schmalz G. Metal content of saliva of patients with and without metal restorations. Clin Oral Investig 2004; 8:238-42.
27. Grocholewicz K, Noceń I, Gutowska I. The influence of hygiene and health habits on the concentration of some ions in saliva of postmenopausal women. Ann Acad Med Stetin 2006; 52:25-9.
28. Rebandel P, Rudzki E. Allergy to palladium. Contact Dermatitis 1990; 23:121-2.
29. Stejskal VD, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A, Mayer W, Bieger W, Lindh U. Metal-specific lymphocytes: biomarkers of sensitivity in man. Neuro Endocrinol Lett 1999; 20: 289-98.
30. De Rossi SS, Greenberg MS. Intraoral contact allergy: a literature review and case reports. J Am Dent Assoc 1998; 129:1435-41.
31. Laine J, Kalimo K, Happonen RP. Contact allergy to dental restorative materials in patients with oral lichenoid lesions. Contact Dermatitis 1997; 36:141-6.
32. Jensen CS, Lisby S, Baadsgaard O, Byrialsen K, Menne T. Release of nickel ions from stainless steel alloys used in dental braces and their patch test reactivity in nickel-sensitive individuals. Contact Dermatitis 2003; 48:300-4.
33. Iavicoli I, Bocca B, Fontana L, Caimi S, Petrucci F, Bergamaschi A, Alimonti A. Distribution and elimination of palladium in male Wistar rats following subacute oral exposure. J Toxicol Environ Health A 2009; 72:88-93.
34. Violante N, Petrucci F, Senofonte O, Cristaudo A, Di Gregorio M, Forte G, Alimonti A. Assessment of workers' exposure to palladium in a catalyst production plant. J Environ Monit 2005; 7:463-8.