

Original article

Reactivity to sodium tetrachloropalladate ($\text{Na}_2[\text{PdCl}_4]$) compared to PdCl_2 and NiCl_2 in lymphocyte proliferation tests

Background: For patch testing, replacement of the commonly used palladium dichloride (PdCl_2) by sodium tetrachloropalladate ($\text{Na}_2[\text{PdCl}_4]$) was recently demonstrated to improve test accuracy and show a significant correlation with nickel (Ni), supporting the concept of cross-reactivity between Pd and Ni. A promising alternative to metal allergy patch testing is the *in vitro* lymphocyte proliferation test (LTT).

Objectives: The aim of this study was to test whether $\text{Na}_2[\text{PdCl}_4]$ is also more sensitive for diagnosing Pd allergy with a standardized LTT.

Patients/methods: After determining optimal nontoxic and nonmitogenic concentrations for $\text{Na}_2[\text{PdCl}_4]$, blood samples from 105 patients with clinical suspicion of metal allergy were tested with an LTT called memory lymphocyte immuno stimulation assay for $\text{Na}_2[\text{PdCl}_4]$, PdCl_2 and NiCl_2 . Reaction profiles were analysed for concordant positive reactions.

Results: Using the conventional cut-off of stimulation index ≥ 3 , 74.3% showed a positive reaction to NiCl_2 , 15.2% to PdCl_2 and 28.6% to $\text{Na}_2[\text{PdCl}_4]$. All positive results to PdCl_2 were covered by $\text{Na}_2[\text{PdCl}_4]$. From the 30 positive reactions to $\text{Na}_2[\text{PdCl}_4]$, 26 (87%) were concordant for NiCl_2 reactivity.

Conclusion: In LTT, the use of $\text{Na}_2[\text{PdCl}_4]$ results in more positive reactions in Pd allergy testing which are in concordance with positive reactions to PdCl_2 and NiCl_2 .

J. Muris¹, C. J. Kleverlaan¹,
A. J. Feilzer¹, E. Valentine-Thon²

¹Department of Dental Materials Science, Academic Centre for Dentistry Amsterdam, Amsterdam, the Netherlands; ²Laboratory Center Bremen, Department of Immunology, Bremen, Germany

Key words: cross reactivity; LTT-MELISA; metal allergy; palladium; tetra chloropalladate.

Joris Muris, DDS
Academic Centre for Dentistry Amsterdam (ACTA)
Department of Dental Materials Science
Louwesweg 1
1066 EA Amsterdam
the Netherlands

Accepted for publication 2 November 2008

Metal allergy has been gaining interest in the last years. In dentistry, a great variety of metals resulting in *c.* 7000 different dental alloys are used to restore decayed teeth. Because of galvanic and crevice corrosion as well as the aggressive oral environment, all metals will ionize to some extent. The clinical impact from this metal exposure is mainly described in case reports. Palladium (Pd) allergy emerged in the literature after the introduction of Pd-containing dental alloys in 1973 (1). Palladium is mainly used in crown and bridge work and introduced due to rising gold prices. Furthermore, Pd and its alloys are used in jewellery and as catalysts in the chemical and automotive industries (2).

In literature, cross-reactivity between nickel (Ni) and Pd is described, although positive reactions exclusively to Pd have been reported (3). Concordant positive reactions to Ni and Pd may be explained by (i) sensitization to both metals (ii) contamination of the Pd patch test material with spurious amounts of Ni (although several studies have disputed this theory) (4) and (iii) the fact that Ni and Pd have similar chemistry and electron arrangements, which might be responsible for true cross-reactivity at the T-cell level (5–7). Hindsen et al. (8) provided evidence for

cross-reactivity of Ni and Pd *in vivo* by systemic administration. They yielded flare-up reactions on sites previously tested with Ni and Pd after oral Ni provocation. In their study, contamination was excluded by chemical analysis. Pistoor et al. and Moulon et al. (6, 7) provided evidence for cross-reactivity on the T-cell level between Ni and Pd, as well as copper (Cu), by *in vitro* tests. In both studies, Ni-reactive T-cell clones proliferated upon selective Pd and Cu stimulation. In most studies concerning cross-reactivity with patch testing, nearly all Pd reactors react to Ni as well (90–100%) (9), although a lower rate has been communicated lately by Fowler and Hayden (70%) (10). Because of the higher prevalence of Ni allergy compared to Pd allergy, true cross-reactivity seems not to be the case (1).

While metal allergies are in principle diagnosed with an epicutaneous or patch test, alternatives have been sought caused by certain drawbacks of patch testing, including its low sensitivity to certain metals such as Ni (11–13) and its potentially sensitizing character (14). Several *in vitro* tests have been described in the literature, of which the lymphocyte proliferation or transformation test (LTT) seems to be the most

promising alternative, especially for nondermal-related allergies (11, 12, 15–18).

Recently, sodium tetrachloropalladate ($\text{Na}_2[\text{PdCl}_4]$) and palladium dichloride (PdCl_2) were used as test salts for Pd allergy patch testing (19). It was found that $\text{Na}_2[\text{PdCl}_4]$ is more sensitive than PdCl_2 , most probably due to its better solubility in water because of its mononuclear structure, while the molecular structure of PdCl_2 is oligo- or polymeric and is therefore nearly insoluble in water (20, 21). In contrast to PdCl_2 , NiCl_2 is well soluble in water and is therefore a standard test material for Ni allergy testing in LTT and in patch testing.

As the solubility and the molecular structure of test allergens in *in vitro* tests is an important factor in antigen presentation, it was hypothesized for this study that $\text{Na}_2[\text{PdCl}_4]$ is also a more reactive test material than PdCl_2 for diagnosing Pd allergy by means of LTT. The reactivity to $\text{Na}_2[\text{PdCl}_4]$ was compared to the reactivity to, and the concordance with, PdCl_2 and NiCl_2 , respectively, in patients with clinical suspicion of Pd and Ni allergy.

Materials and methods

Patients

Blood from 105 samples submitted to the Department of Immunology, Laboratory Center Bremen, Germany accredited according to DIN EN ISO 15189 and DIN EN ISO/IEC 17025, for routine LTT-MELISA® (memory lymphocyte immuno stimulation assay) diagnosis of allergy to various metals including PdCl_2 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and NiCl_2 ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$; Sigma-Aldrich Chemie GmbH) was used. The LTT-MELISA® is a patented modification of the LLT using a standardized (17, 22) and validated (23) protocol. All patients had clinical suspicion of Ni and/or palladium allergy, rendering this a selected group of patients. Whenever sufficient lymphocytes were available, an extra LTT test was performed in parallel with $\text{Na}_2[\text{PdCl}_4]$ ($\text{Na}_2[\text{PdCl}_4] \cdot 3\text{H}_2\text{O}$; Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands; purity: 99.998%) as test allergen. All blood samples were submitted in CPDA monovettes (Sarstedt AG & Co., Nümbrecht, Germany) or ACD Solution A vacutainer tubes (Becton Dickinson GmbH, Heidelberg, Germany) and transported by normal post or by private courier to arrive in the laboratory usually within 24 h, at most 48 h, after the blood was drawn.

Peripheral blood mononuclear cells (PBMCs) were isolated on Ficoll Histopaque (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) immediately upon arrival in the laboratory and either used immediately or stored in 20% medium (RPMI-1640 containing HEPES; Life Technologies, Karlsruhe, Germany), 8 mg/l gentamicin (Sigma-Aldrich Chemie GmbH), 2 mmol/l L-glutamine (Biochrom seromed, Berlin, Germany) and 20% pooled, heat-inactivated human AB serum (Cambrex Bio Science Verviers S.p.r.l., Verviers, Belgium) overnight at 4°C prior to set up.

LTT-MELISA®

The LTT-MELISA® was performed in the Laboratory Center Bremen essentially as described by Stejskal et al. (17) and modified for routine clinical testing by Valentine-Thon (23, 24). Briefly, 1×10^6 PBMCs (monocyte reduced by plastic adherence) in 1 ml of

10% medium were pipetted into the wells of a 24-well cell culture plate precoated with two to three serial (1 : 2) dilutions of metal salt solutions in the following initial concentrations: PdCl_2 , 125 µg/ml (0.71 mM); $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 100 µg/ml (0.42 mM); $\text{Na}_2[\text{PdCl}_4] \cdot 3\text{H}_2\text{O}$, 125 µg/ml (0.36 mM). Activated monocytes produce prostaglandins (25), which negatively affect lymphocyte activation (26). During preparation procedures the number of monocytes increases. In order to normalize this number and to exclude possible negative effects of produced prostaglandins, partial monocyte depletion is carried out. The concentrations used were determined according to standard procedures applied in LTT to reveal nontoxic and non-mitogenic reactions. All salts used were of 'pro analysi' grade, and the diluent was sterile double-distilled water. To dissolve PdCl_2 in 10 ml of water, 250 µl of 30% HCl was needed. No HCl was needed to dissolve $\text{Na}_2[\text{PdCl}_4]$ in water. Solubility was macroscopically checked. The plates were incubated for 5 days at 37°C with 5% CO_2 . Three negative controls (lymphocytes in 10% medium without antigen) and one positive control (lymphocytes in 10% medium plus 2 µg/ml pokeweed mitogen; Sigma-Aldrich Chemie GmbH) were incubated on each plate.

After 5 days, 600 µl of cell suspension from each well was transferred to a new 24-well plate, and the cells pulsed for 5 h with 3 µCi methyl- ^3H -thymidine (Amersham Buchler, Brunswick, Germany; specific activity 185 GBq/mmol). The radioactivity [counts per minute (cpm)] was measured in a liquid scintillation counter (1450 Microbeta Trilux, Wallac Distribution GmbH, Freiburg, Germany).

A reaction was considered positive when one or more of the test concentrations gave a stimulation index (SI) ≥ 3 , calculated as follows:

$\text{SI} = \text{cpm in test well divided by the average cpm in negative control wells.}$

A SI < 2 was considered negative, SI ≥ 2 but < 3 was interpreted as a 'possible sensitization', and a SI ≥ 3 was interpreted as positive, i.e. evidence of sensitization.

Cells from the 5-day cultures were additionally analysed morphologically after staining cytospin preparations with rapid differential haematology staining solutions (Dade Behring AG, Marburg, Germany). Only tests in which the radioactively positive results showed the presence of lymphoblasts and radioactively negative results showed only viable, small lymphocytes (noncytotoxicity and nonmitogenic stimulation) were accepted as valid.

Statistical analysis

For statistical analysis, the obtained SI values were converted to binary values. To evaluate concordance ($\text{Na}_2[\text{PdCl}_4]$ with PdCl_2 and NiCl_2), the Spearman's rank order correlation (SIGMASTAT 3.0; SPSS Inc., Chicago, IL, USA) was used. $P < 0.05$ was considered significant.

Results

The mean SI of 105 patients classified in four different groups are summarized in Table 1. As cut-off values of LTT results to discriminate between a positive and a negative test reaction are still debated, the results are presented separately using two different cut-off values: SI ≥ 3 and SI ≥ 5 . The average cpm of the negative controls was 792, while the positive pokeweed controls showed in nearly all cases a SI > 30.

Table 1. The mean stimulation index (SI) and the SD in parentheses of 105 patients classified in four different groups

	NiCl ₂	PdCl ₂	Na ₂ [PdCl ₄]
SI < 3	1.6 (0.6) [n = 27]	1.3 (0.6) [n = 89]	1.6 (0.7) [n = 74]
3 ≤ SI < 5	3.8 (0.6) [n = 17]	3.8 (0.8) [n = 3]	3.6 (0.5) [n = 13]
SI ≥ 3	23.6 (35.9) [n = 78]	12.4 (14.0) [n = 16]	11.5 (20.1) [n = 30]
SI ≥ 5	29.1 (38.8) [n = 61]	14.4 (14.9) [n = 13]	17.6 (25.4) [n = 16]

Stimulation index ≥ 3

Twenty-three samples (21.9%) did not show a positive proliferation reaction to any of the investigated metal salts, while 78 cases (74.3%) showed a positive proliferation reaction to NiCl₂, 16 (15.2%) to PdCl₂, and 30 (28.6%) to Na₂[PdCl₄] (Fig. 1). All positive results to PdCl₂ were covered by Na₂[PdCl₄]. Twenty-six of the 30 samples positive to Na₂[PdCl₄] (87%) showed concordant reactions to NiCl₂. Furthermore, four exclusively Pd-positive (Ni-negative) reactions were observed towards Na₂[PdCl₄], of which one was also positive to PdCl₂. Spearman's rank order correlation between Na₂[PdCl₄] and PdCl₂ was 0.640. Although the correlation is not very strong, it is significant ($P < 0.05$). No statistically significant correlation was found between Na₂[PdCl₄] and NiCl₂.

Stimulation index ≥ 5

Forty-two samples (40.0%) did not show a positive reaction to any of the investigated metal salts, while 61 cases (58.1%) showed a positive proliferation reaction to NiCl₂, 13 (12.4%) to PdCl₂ and 16 (15.2%) to Na₂[PdCl₄] (Fig. 2). Interestingly, not all the positive reactions to PdCl₂ are covered by Na₂[PdCl₄] when using this cut-off value. Three samples (1.9%) showed positive proliferation towards PdCl₂ and NiCl₂, but not to Na₂[PdCl₄]. Furthermore, one sample reacted positively only to Na₂[PdCl₄]. Finally, one sample was negative to NiCl₂, but positive to PdCl₂ and Na₂[PdCl₄]. Spearman's rank order correlation between Na₂[PdCl₄] and PdCl₂ at

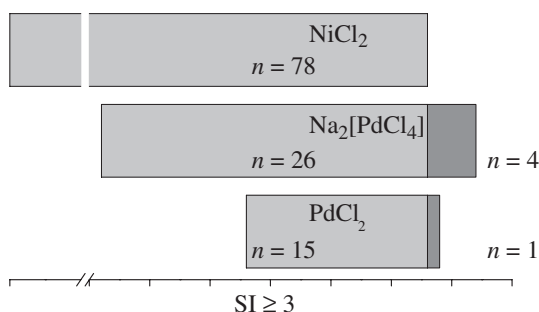


Figure 1. Positive reactions to the materials investigated from 105 patients when cut-off value is SI ≥ 3. Areas of vertical overlap represent concordant reactions.

Reactivity to Na₂[PdCl₄] compared to PdCl₂ and NiCl₂

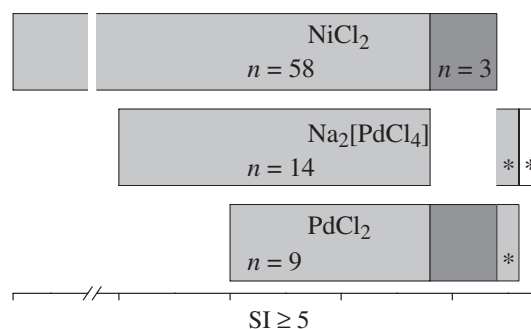


Figure 2. Positive reactions to the materials investigated from 105 patients when cut-off value is SI ≥ 5. Areas of vertical overlap represent concordant reactions (* represents one sample only).

cut-off value ≥ 5 is not strong (0.645) but still significant ($P < 0.05$). Again, no statistically significant correlation was found between Na₂[PdCl₄] and NiCl₂.

Discussion

The aim of this study was to evaluate the reactivity of Na₂[PdCl₄] as a test material when LTT-MELISA[®] is used on blood samples derived from Pd and Ni allergy susceptible patients, as the patients were submitted for testing based on clinical suspicion of a Ni and Pd allergy. No attempt was made to investigate the clinical relevance of positive LTT-MELISA[®] results, as detailed anamnestic data concerning exposure and patch test results were not available on all of these patients. However, there are several studies confirming the clinical relevance of LTT-MELISA[®] published in the past (24, 27). These publications showed a decrease in SI after removal of the insulting antigen, mostly dental restorative materials. Lymphocyte transformation test in general seems to be promising, especially for the detection of nondermally induced metal sensitization, as described for bronchial or oral mucous membrane contact with beryllium, titanium and other dental metals (24, 28), as well as for orthopaedic implant-related sensitization (15, 16).

Compared to patch test results in general (29), a great number of Ni and Pd-positive reactions are found in this study. This is mainly explained by the inclusion of patients with a specific suspicion of a Ni and Pd allergy. In the patch testing study (19) as mentioned before, positive reactions to Pd increased from 1.8% to 14%, because of the use of Na₂[PdCl₄] compared with PdCl₂. This drastic increase is most likely explained by two advantages of Na₂[PdCl₄] compared with PdCl₂. First, Na₂[PdCl₄] is better soluble in water because of its mononuclear structure, as PdCl₂ forms oligo- or polymeric structures in water (20, 21). Secondly, because of the smaller molecular volume of Na₂[PdCl₄], skin penetration will be easier compared to PdCl₂. With

respect to Ni, a comparative study of Ni chloride, bromide, iodide and dioctanoate showed that not the polarity but the molecular volume was the decisive factor for membrane diffusion (30).

The use of $\text{Na}_2[\text{PdCl}_4]$ compared with PdCl_2 in LTT nearly doubled the positive reactions to Pd, from 15.2% to 28.6% (cut-off value ≥ 3). As skin penetration is not required in LTT, this may explain the less pronounced increase in positive reactions. All positive reactions to PdCl_2 are covered by $\text{Na}_2[\text{PdCl}_4]$. Except from four positive reactions to $\text{Na}_2[\text{PdCl}_4]$, all were concordant to NiCl_2 (87%). These findings are in line with the former reports on concordant reactions between Ni and Pd. Although from these results, it seems that $\text{Na}_2[\text{PdCl}_4]$ is more sensitive than PdCl_2 for Pd allergy testing; more research is needed to elucidate the clinical relevance of these positive reactions.

The lack of statistically significant correlation between Ni and Pd-positive reactions can be explained by the remarkably high number of positive reactions to Ni. Almost all Pd-positive subjects reacted positively to Ni as well (87%; SI ≥ 3), whereas more than half of Ni reactors did not react to Pd. A similar trend was observed in patch testing, although less pronounced (19). A likely explanation is that to obtain a positive reaction in LTT, the subject has to be exposed to the relevant allergen to have sufficient specific T-cells in the blood circulation. When a current exposure is lacking or is insufficient, not enough T-cells might be circulating to give rise to a positive test result. As exposure to Pd is much more limited than to Ni in daily life, fewer positive reactions to Pd would be expected. On the other hand, as in patch testing nearly all positive reactions to Pd are concordant to Ni, but not the other way around as a result of the much higher prevalence of Ni allergy itself. Indeed, these results do not confirm cross-reactivity between Ni and Pd as stated by Aberer earlier (1). Thus, Pd allergy might be caused by

concomitant sensitization, and clinical relevance might be overlooked by the lack of knowledge on chronic Pd exposure as for example because of Pd-containing dental restorations.

When the cut-off value is ≥ 5 , 3 PdCl_2 -positive reactions, concordant to NiCl_2 but not to $\text{Na}_2[\text{PdCl}_4]$, were found. It looks like $\text{Na}_2[\text{PdCl}_4]$ might be more sensitive especially in the area of weak to moderate positive reactions in LTT-MELISA[®] and probably in all LTTs. The most appropriate cut-off will be clearly that which is most clinically relevant.

In summary, the results show that $\text{Na}_2[\text{PdCl}_4]$ clearly induced more positive reactions than the commonly used PdCl_2 , not only in patch testing but also in LTT as well. This is most probably caused by the better solubility in water of $\text{Na}_2[\text{PdCl}_4]$. The larger difference in patch testing is probably as a result of the additional advantage of the better skin penetration kinetics of $\text{Na}_2[\text{PdCl}_4]$ caused by the smaller molecular volume. All positive reactions to PdCl_2 were covered by $\text{Na}_2[\text{PdCl}_4]$ (cut-off ≥ 3). Furthermore, nearly all samples with positive reactions to $\text{Na}_2[\text{PdCl}_4]$ show concordant positive reactions to NiCl_2 (87%). Although the results do not support the theory of cross-reactivity between Ni and Pd, they are in line with other studies *in vitro* and *in vivo*. Further research on $\text{Na}_2[\text{PdCl}_4]$ as a test allergen in LTT is recommended in order to obtain information on the clinical relevance of these positive Pd reactions and the true sensitivity of $\text{Na}_2[\text{PdCl}_4]$ in Pd allergy testing.

Acknowledgments

The authors are grateful for the excellent technical assistance of Ms Gudrun Emmermacher, Ms Ursel Köster and Ms Bianca Schüttpehlz.

References

1. Aberer W, Holub H, Strohal R, Slavicek R. Palladium in dental alloys – the dermatologists' responsibility to warn? *Contact Dermatitis* 1993;**28**:163–165.
2. 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**:417–432.
3. Orion E, Matz H, Wolf R. Palladium allergy in an Israeli contact dermatitis clinic. *Contact Dermatitis* 2003;**49**:216–217.
4. Wahlberg JE, Boman AS. Cross-reactivity to palladium and nickel studied in the guinea pig. *Acta Derm Venereol* 1992;**72**:95–97.
5. Santucci B, Cannistraci C, Cristaudo A, Picardo M. Multiple sensitivities to transition metals: the nickel palladium reactions. *Contact Dermatitis* 1996;**35**:283–286.
6. Pistor FH, Kapsenberg ML, Bos JD, Meinardi MM, von Blomberg ME, Scheper RJ. Cross-reactivity of human nickel-reactive T-lymphocyte clones with copper and palladium. *J Invest Dermatol* 1995;**105**:92–95.
7. Moulon C, Vollmer J, Weltzien HU. Characterization of processing requirements and metal cross-reactivities in T cell clones from patients with allergic contact dermatitis to nickel. *Eur J Immunol* 1995;**25**:3308–3315.
8. Hindsen M, Spiren A, Bruze M. Cross-reactivity between nickel and palladium demonstrated by systemic administration of nickel. *Contact Dermatitis* 2005;**53**:2–8.
9. Bordel-Gomez M, Miranda-Romero A, Castrodeza-Sanz J. Isolated and concurrent prevalence of sensitization to transition metals in a Spanish population. *J Eur Acad Dermatol Venereol* 2008;**22**:1452–1457.
10. Fowler JF, Hayden JM. Palladium patch reactivity. *Exog Dermatol* 2003;**2**:277–278.
11. Menne T. Nickel allergy – reliability of patch test. Evaluated in female twins. *Derm Beruf Umwelt* 1981;**29**:156–160.

12. Rustemeyer T, von Blomberg BM, van Hoogstraten IM, Bruynzeel DP, Scheper RJ. Analysis of effector and regulatory immune reactivity to nickel. *Clin Exp Allergy* 2004;**34**: 1458–1466.
13. Skoglund A. Value of epicutaneous patch testing in patients with oral, mucosal lesions of lichenoid character. *Scand J Dent Res* 1994;**102**: 216–222.
14. Bruze M, Conde-Salazar L, Goossens A, Kanerva L, White IR. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis* 1999;**41**:241–250.
15. Thomas P, Bandl WD, Maier S, Summer B, Przybilla B. Hypersensitivity to titanium osteosynthesis with impaired fracture healing, eczema, and T-cell hyperresponsiveness in vitro: case report and review of the literature. *Contact Dermatitis* 2006;**55**: 199–202.
16. Hallab NJ. Lymphocyte transformation testing for quantifying metal-implant-related hypersensitivity responses. *Dermatitis* 2004;**15**:82–90.
17. Stejskal VDM, Cederbrant K, Lindvall A, Forsbeck M. Melisa – an in-vitro tool for the study of metal allergy. *Toxicol In Vitro* 1994;**8**:991–1000.
18. Everness KM, Gawkrödger DJ, Botham PA, Hunter JA. The discrimination between nickel-sensitive and non-nickel-sensitive subjects by an in vitro lymphocyte transformation test. *Br J Dermatol* 1990;**122**:293–298.
19. Muris J, Kleverlaan CJ, Feilzer AJ, Rustemeyer T. Sodium tetrachloropalladate ($\text{Na}_2[\text{PdCl}_4]$) as an improved test salt for palladium allergy patch testing. *Contact Dermatitis* 2008;**58**: 42–46.
20. Sham T. X-ray absorption near edge structure (xanes) studies of the distribution of low-lying unoccupied states in PdCl_2 . *Chem Phys Lett* 1983;**101**: 567–572.
21. Gaikwad AV, Rothenberg G. In-situ UV-visible study of Pd nanocluster formation in solution. *Phys Chem Chem Phys* 2006;**8**:3669–3675.
22. Stejskal VD, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A et al. Metal-specific lymphocytes: biomarkers of sensitivity in man. *Neuroendocrinol Lett* 1999;**20**:289–298.
23. Valentine-Thon E, Schiwara HW. Validity of MELISA for metal sensitivity testing. *Neuro Endocrinol Lett* 2003;**24**:57–64.
24. Valentine-Thon E, Müller K, Guzzi G, Kreisel S, Ohnsorge P, Sandkamp M. LTT-MELISA is clinically relevant for detecting and monitoring metal sensitivity. *Neuro Endocrinol Lett* 2006;**27**(Suppl. 1):17–24.
25. Humes JL, Bonney RJ, Pelus L, Dahlgren ME, Sadowski SJ, Kuehl FA Jr et al. Macrophages synthesis and release prostaglandins in response to inflammatory stimuli. *Nature* 1977;**269**:149–151.
26. Roth MD, Golub SH. Human pulmonary macrophages utilize prostaglandins and transforming growth factor beta 1 to suppress lymphocyte activation. *J Leukoc Biol* 1993;**53**:366–371.
27. Stejskal V, Hudecek R, Stejskal J, Sterzl I. Diagnosis and treatment of metal-induced side-effects. *Neuro Endocrinol Lett* 2006;**27**(Suppl. 1):7–16.
28. Muller K, Valentine-Thon E. Hypersensitivity to titanium: clinical and laboratory evidence. *Neuro Endocrinol Lett* 2006;**27**(Suppl. 1):31–35.
29. Kanerva L, Rantanen T, Aalto-Korte K, Estlander T, Hannuksela M, Harvima RJ et al. A multicenter study of patch test reactions with dental screening series. *Am J Contact Dermatitis* 2001;**12**:83–87.
30. Hostynek JJ. Factors determining percutaneous metal absorption. *Food Chem Toxicol* 2003;**41**:327–345.