# The Release of Metal Ions in the Gingival Fluid of Prosthodontic Patients

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Summary

*Corrosion of prosthetic restorations and simultaneous wear during usage leads to damage of the restoration and harmful reactions on the surrounding tissues or even on distant organs. The aim of this study was to identify the ions Au, Pt, Ag and Pd in the gingival fluid of patients with fixed prosthetic appliances and to determine the mass of ions in the obtained sample . The study included 20 subjects: of which 15 had fixed prosthetic appliances and the same level of hygiene, and 5 without have any prosthetic appliances, who served as a control group. The prosthetic appliances were made from alloy compounds: Au - Pt, Au - Pt, Ag - Pd, Ni - Cr, fixed in the mouths: from 1-14 days, and 3-8 years. Samples were obtained by placing adsorbent paper (AP) in the gingival sulcus (GS) of the abutment tooth for a period of ten minutes. AP with samples gingival fluid (ST) were analysed by means of a mass spectrometer with fused plasma (ICP-MS). The results were statistically analysed by the programme packet SPSS, with descriptive statistics and correlation of samples. In the whole sample statistically significant correlation was found for the concentrations of Ag, Au and Pt, while the concentration of Pd was characteristic for each sample. Analysis of the results in the control group did not reveal statistically significant correlation for the same inter-relations. In the sub-group Ag-Pd alloys statistically significant correlation was established between the concentrations of Ag, Au and Pd, and the most marked correlation found was between the concentrations of Au and Pd. Statistically significant differences were determined in the concentrations of Pt between the subgroups with new appliances, with the highest value in the group of Au alloy (P=0.023) in comparison with the control group. Statistically significant changes were determined in the values of Ag (P=0.011) and Pt (P=0.039), with a fall in the values of both metals in subjects with older prosthetic appliances.*

Key words: *corrosion, gingival fluid, prosthetic appliances.*

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## Introduction

In patients with prosthetic appliances electrochemical interaction occurs between the metal parts of the appliance and saliva rich in electrolytes, which results in corrosion of the surface of the appliance, the release of metal ions and the occurrence of corrosive products on the surface of the same (1). Simultaneously, wear of the appliance during function causes additional damage to the surface and thus closes the circle which leads to the deterioration of the prosthetic appliance (2). The course of the corrosive process will depend on the composition and metallurgical condition of the alloy used, the technological procedure used in the dental laboratory, i.e. the quality of the cast, the quality of the surface treatment, the presence of several alloys inside the oral cavity; mastication forces and local and systematic response of the host (3). Berzin (4) studied the presence of released ions from prosthetic alloys into surrounding tissue and found at least one out of four released elements (Ag, Au, Cu, Pd) in 84% of patients. In the case of an alloy containing silver, the beginning of the dissolution of metals was observed, such as Ga, In, Sn, Zn, while more refined metals, such as Pd i Ag, remained on the surface. Silver, owing to its electro-activity in contact with Cl creates on the surface a film $AgCl_2$. Electro-chemical testing of refined alloys frequently showed contradictory results (5, 6). The result of a study by Rinčić et al (7) is interesting, in which the release of ions from Co-Cr-Mo alloy was examined in *in vitro* conditions, where the ions Fe, Zn i Ni were found in high concentrations, particularly zinc ion, although it was not listed in the manufacturer's declaration.

## The aim of the study

The aim of this study was to examine the presence of ion metals, resulting from the corrosion of prosthetic alloys in the gingival fluid, in *in vitro* conditions. A search of relevant data bases resulted in several articles on the study of this problem performed *in vitro*, by simulating conditions in the oral cavity, and only a small number performed *in vivo*.

## Subjects and work methods

The study included 20 subjects (12 female and 8 male) ranging in age from 24 to 72 years; all patients from the Department of Prosthodontics, School of Dental Medicine University of Zagreb. Of this number 15 subjects had fixed prosthetic appliances and the same level of hygiene (GI) (Felton 2), and the other 5 (four female and one male) without prosthetic appliances and with GI Felton 1-2 served as the control group. In compliance with ethical principles in *in vivo* investigations (8, 9), all subjects were thoroughly acquainted with the course and aim of the study and possible risks. A questionnaire for each subject was completed by the same person who had conducted the examination, so as to minimise the possibility of mistakes. Apart from age, sex and interests, the questionnaire contained the following data: the place of the fixed appliance, the material from which the appliance was made, the duration of the appliance (1-14 days -new appliances; 3-8 years -old appliances) gingival index (GI) according to Felton (10).

Material from which the appliance was made:

- Gold-platinum alloy (18+8), Refinery precious metals Zagreb. According to ISO standards type IV. Composition: Au 75%, Pt 8%, Ag 9.5%, Cu 5.1%, other elements 1.5%.
- Silver-palladium alloy (Auropal SE) Aurodent, Celje, Slovenia. According to ISO (8891) standards type IV, registered with the Ministry of Health, Republic of Slovenia. Composition: Ag 64%, Pd 25%, Au 2%, Cu-8%, Zn<1%, not containing Ni, Be, In, Cd for manufacturers.
- Gold-platinum alloy (Dentor S) Aurodent Celje, Slovenia. According to ISO standards type IV, registered with the Ministry of Health, Republic of Slovenia. Composition: Au 75.5%, Pd 1.2%, Pt 4.4%, Ag 11% Cu 6.7%, Zn 1.2%.
- Nickel-chromium alloy (Wiron®99) Bego, Bremen, Germany. According to ISO 9693 standards type IV. Composition: Ni 65%, Cr 22.5%, Mo 9.5 %, Nb 1%, Si 1%, Fe 0.5%, Ce 0.5%, C max.0.02%, not containing Be for manufacturers specifications.

The method of taking a sample of gingival fluid (ST) with adsorbent papers (AP) was carried out

according to the protocol for sampling, developed for (ALT Corp.) Affinity Laboratory Toxicity testing (11).

The following equipment was used for taking the samples: AP (cellulose acetate paper) pore size 0.45μm (Sartorius, Goettingen, Germany), a glass container with a lid, disposable gloves, plastic tweezers and scales (Sartorius BP 210 D) with accuracy of five decimals. The adsorbent papers were chosen because of their favourable characteristics (great adsorptive capacity, dissolving without residuum) which differentiate them from others; e.g. endodontic paper-points. Each individual paper was placed in a glass container and hermetically sealed. The containers with AP were numbered from 1-20. The collective total mass was determined on the scales for each container with AP. Weighing was repeated after taking the ST sample. The difference in the mass before and after was the mass of the ST sample.

The abutment tooth and appertaining gingiva were air dried in order to remove any saliva, and the area was isolated with cellulose tissue all around, immediately before placing the AP in the GS. The AP was placed in the GS, apically guided up to slight resistance. The AP was left in the GS for 10 minutes. At the end of this period it was taken out and returned to a numbered glass container which was hermetically sealed. The container with the AP and the sample was weighed on the above mentioned scales. The method of taking samples in the control group of subjects was identical. The place of the sampling was not cleaned either mechanically or chemically, in order to avoid washing out the GS. When taking a sample of ST it is important not to touch the AP with unprotected hands at any moment during the preparation and only non-metal equipment should be used. For preparation of the sample a solution of HCl and $H^2O$ was used, ratio 1:1, "nanopure". First 2 ml of the solution was deposited on the filter paper, after which it dissolved without any residue. The amount was diluted to 10 ml of the total volume and placed in the spectrometer mass with the induced fused plasma. The measurement units of each were mg/l, which is equally ppm. The values are shown in the coordinated system as follows : Y axis was intensity, and X axis concentration. An analogous "coordinative" curve was done for comparison. A blind test was performed, using only water, with a metal in a specific concentration, in accordance with ICP standard, CERTI PUR, ICP multi-element standard, solution VI. In order to obtain data on the kinds and amounts of metal ions l released from the restorations, the prepared samples were analysed by means of the spectrometer mass with induced fused plasma (Inductively Coupled Plasma Mass Spectrometry) - model ELAN 9000, Perkin Elmer SCIEX instruments (12).

Statistical analysis was performed with the program packet SPSS, with descriptive statistics and correlation of samples (Spearman's coefficient correlation), analysis of samples in relation to the used alloy (Kruskal - Wallis test, Mann - Whitney - U test); and analysis of the samples in relation to duration of the cemented restorations (Kruskal - Wallis test, Mann - Whitney - U test). Nonparametric methods of analysis were used because of the small number of samples and abnormal distribution. During analysis for silver ion data were not analysed for two samples which markedly stood out from the other values, and it was assumed that the samples had been polluted or that an error had occurred in the methodology.

## Results

Data on the subjects (sex, age, bridge placement, GI, the amount of time expired from the fastening of the appliances and the material from which the appliances were made are shown in Table 1.

Analysis of gingival fluid samples determined the release of four kinds of ion metals: silver ion (Ag), gold ion (Au), palladium ion (Pd) and platinum ion (Pt) (Table 2).

The correlation of concentrations of some metal ions: Ag, Au, Pd and Pt is shown in Table 3 and Table 4. Table 3 shows the inter-relation in the whole sample from which it can be seen that the concentrations of silver, gold, and platinum are statistically significantly correlated, while the concentration of palladium. When the results in the control group (N=5) were analysed no statistically significant correlation was determined for the same interrelation. In the sub-group in which the alloy Ag-Pd (N=8) was used, statistically significant correlation

was determined between the concentrations of silver, gold and palladium, and the most pronounced correlation was between the concentrations of gold and palladium (Table 4).

As there was significant difference in the share of participants with new prosthetic appliances (within 1-14 days) between sub-groups with different used alloys (Ag-Pd alloy : gold alloy) data was separately analysed for the Ag-Pd alloy, depending on the subsequential period. and the values compared of released metal ions between the mentioned alloys for new prosthetic appliances in relation to the values in the control group. This analysis showed that statistically significant difference existed in the concentrations of platinum between the mentioned subgroup with the highest value in the group of gold alloy (P=0,023; Kruskal-Wallis ANOVA); (Table 5).

In order to determine the influence of the passing of time on the released mass of metals a subgroup with Ag-Pd alloys was analysed in which subjects had new (0-14 days) and old (3-8 years) prosthetic appliances in comparison with the control group. Statistical significant differences were determined in the values of silver (P=0.011) and platinum (P=0.039) with a fall in the value of both metals in subjects with older prosthetic appliances (Kruskal-Wallis ANOVA); (Table 6) .

## Discussion

In vivo investigations are difficult to realise. They demand very carefully selected and prepared patients, and interpretation of the obtained results is complicated because of the lack of uniformity of several factors. For example, the general health condition of the subjects (13), the method of treatment of the appliance in the dental laboratory (14), level of hygiene, individual attitude of the patient to their appliance, and more recently stress has been mentioned as a possible mediator of events in the organism (15). This study is one of a few *in vivo* investigations in this field. In the available literature the subject has not been investigated sufficiently. The object was to correlate in a large sample the amount of released ions in the gingival fluid of prosthetic patients with a series of parameters, such as the kinds of alloys, the presence of other materials in the mouth, pH values, specific nutritional and hygienic habits in determined age groups etc. and to compare this with subjects without prosthetic appliances, without fillings and with average values of the composition of the gingival fluid (16) and average values of pH saliva (17). However, the many problems which we came across limited the possibility of a larger sample, and thus better comparison of the parameters. The different antagonistic and synergistic effects of certain substances and processes in the human body can change the response of the organism and for this reason interpretation of data is frequently complex (18-23). Obstruction of important enzymes (creatinine, aldolase, alkaline phosphates, carbon-anhydrites, trypsin, chemotrypsin); disturbances of synthesised collagen (bones, cartilage), obstructions of thymidine in DNA and changed function of the cells of the immune system are some possible responses of the organism (24-28). Metal ions can interfere with the state of the metabolism, influencing the secretion of substances, such as cytokinins which have a key role in inflammatory processes (29). Consequently this may explain the different, often contradictory, results when nickel is tested (30, 31). It was demonstrated that Cu, Co, In, Zn significantly increase the cellular synthesis of prostaglandin E2, and inflammatory mediators stimulate arachidonic acid from the metabolism (28). *In vitro* investigations are therefore frequently more precise, uniform, and can be repeated. However, the problem arises of the simulation of conditions dominant in the mouth, particularly the composition of artificial saliva (6, 32). In his *in vitro* investigation Wataha (33) observed a different reaction of some elements a in the presence of proteins in the solution, Ag, Cu and Zn were released more in a physiological solution with 3% BSA, while in the case of Ni release did not depend on the kind of solution. International standards, summarised under the names ISO 10993 and FDA Blue Memorandum FDA (G95-1), which are based on ISO 10993-1, cite essential requirements which are set for appliances in order to ensure their biocompatibility. Selection of material is carried out with several kinds of tests. Such tests establish eventual cytotoxicity, sensitivity and irritation (34). Targeted investigations are related to possible systemic effects: ISO 10993-11, and potentially undesired effects of materials on organs and tissues remote

from the place of application, such as the liver, heart, kidneys and brain (34). This approach should prevent undesired effects of new alloys on patients, and also on the medical team. However, inflammation (35), discoloration and hyperplasia of the gingiva (36) in the surroundings of prosthetic appliances have been demonstrated. Diffusion of metal ions was determined from crowns in natural teeth (37), and allergy has become an increasing problem (38). The result of investigations on toxicity (17, 18) indicate significant mutual connection of specific elements, e.g. zinc in alloys, specific concentrations, such as, for example, carbon in Pd-Cu alloys or even the method of cold metal preparation (39).

Time elapsed since the fastening of the appliance is one more important factor in the intensity of the released ions. The greatest release of ions occurred in the first two weeks after fastening of the appliance, which was anticipated (Wataha) (3). The products of corrosion found in the gingival fluid in this study suggests the possibility of a connection with the finding of the same in saliva (40), and adjacent tissues (41). However, it is interesting to note the data on markedly low concentration of ions (lower than the control values) in the gingival fluid of prosthetic patients after 3-8 years. Whether the presence of fixed-prosthetic appliances, with irregularly shaped edges (42,43); or perhaps something else (44) induce greater secretion of gingival fluid and additional rinsing, remain questions for further study. The release of ions from an alloy does not have to be a proportional percentage of that element in the alloy. Namely, selective release exists which can enable elements present in traces to be released in large quantities (3), which was confirmed in this study. The released ions Au, Pd, Pt and especially Ag, found in this study, acquire greater significance in the context of Schmalz's range of cytotoxicity (39), as it is well known that small concentrations of ions can be pathological. The presence of silver determined in the samples of ST teeth with prosthetic appliances of Ni - Cr alloy can be explained by the amalgam fillings (17, 18) or the presence of Ag in the alloy.

## Conclusion

The release of ions Au, Pd, Pt and particularly Ag occurred in the case of all the alloys examined. Statistically significant correlations were found for the concentrations of Ag, Au and Pt in the group of prosthetic subjects, while analysis of the results in the control group did not determine correlation for the same inter-relations. In the sub-group of the alloy Ag-Pd correlation was found for the concentrations of Ag, Pd and Au. Within the same sub-group significant difference was found in the concentration of Pt, with the highest value in the group of alloy gold, and noticeably significant change in the value of Ag and Pt for subjects with new appliances; with a decrease in the value of both metals in subjects with older prosthetic appliances. Markedly low values of the concentrations of all ions were measured in samples of gingival fluid for old prosthetic appliances, even lower than the values of the control group.

In prosthetic dentistry it is necessary to use good quality alloys, of known composition and good biocompatibility. Correctly carried out technological procedure in the dental laboratory is another important condition in the therapy for prosthetic patients. Mistakes in any phase of the fabrication of the appliance can lead to structural irregularity which will result in poorer properties of the cast. The aggressive media in which the appliance is fastened, and wear during function, can additionally damage the appliance .