ORIGINAL ARTICLE

The mechanism of gingiva metallic pigmentations formation

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Abstract The occurrence of blue-grey areas in the soft tissue represents one of the problems affecting patients whose teeth have been restored using metallic materials. It is generally accepted that it is caused by mechanical penetration of a metallic material into the soft tissue. Several facts indicate that this mechanism is not general. The aim of the study was to determine an alternative mechanism of the origin of pigmentations, based on the corrosion interaction of metallic materials used in prosthodontics with the oral environment. The study was comprised of an analysis of pigment particles, determination of exposure conditions of metallic materials in vivo and laboratory evaluation of corrosion properties of the studied materials. Particles containing silver, sulphur and/or selenium could be seen in ultra-thin sections in the lamina propria gingivae. Comparison of the corrosion laboratory results with the results of in vivo measurements indicated the intensification of corrosion under these conditions. Amalgams and silver-containing alloys used for teeth restorations may release silver under the conditions of the oral cavity.

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O. Benada Institute of Microbiology Acad. Sci. CR v.v.i., Videnska 1083, 142 20 Prague 4, Czech Republic The formation of soluble silver compounds in the sulcular area or in a crevice between the crown and the cast post-andcore reconstruction facilitates their transport to the soft tissue and subsequent deposition.

Keywords Pigmentation · Gingiva · Silver compounds · Electrochemistry · Microscopy

Introduction

The occurrence of blue-grey areas in the soft tissue of the mouth represents one of the problems affecting some patients whose teeth have been restored using metallic materials. The size of these areas ranges from mere spots and local stains up to continuous pigmented areas surrounding the restored tooth. This phenomenon has been known for a relatively long time, and it is described in literature on dental amalgams as the so-called amalgam tattoo [3, 4, 25, 34]. As generally believed, it is caused by mechanical penetration of a metallic material—mostly amalgam—into the soft tissue in the course of its treatment in the oral cavity. This mechanism is noted in a number of works [5, 12, 16, 24, 26, 27]. Analyses of the pigmented tissues reveal the presence of Ag, Hg, Cu, Fe, Zn, S, Se, Ca, P and Si [1, 2, 7, 8, 12, 21, 29, 31].

However, the occurrence of pigmentation has been also observed in other than amalgam-related cases. Yamada and Sato reported the presence of silver-containing stains in the vicinity of crowns based on a gold alloy [35]. The presence of silver, gold, copper and palladium in the tissue adjacent to dental alloys has been noted by Garhammer et al. [11], while the presence of silver and copper in the root of a tooth covered with a crown made of a gold-based alloy is documented in the article [13]. Pigmentations with a dominant content of silver accompanied by a number of other elements (Zn, Ti, Al, Fe, Cr, Cu, Ni, Sn, S, Se, Ca, Si, P, Mg) and formed in the close vicinity of teeth restored with cast posts-and-cores made of an Ag–Sn alloy and crowns made of resin or a gold-based alloy have been observed by Venclikova et al. [32]. On the other hand, metal deposits found in dentinal tubules corresponded with material used for endodontic and prosthodontic treatment [33].

These findings together with the well-known clinical fact that pigmentations may also occur in the tissue of a tooth root, on the mucous membrane of the face or at the bottom of the oral cavity [15, 20] as well as problem analysis published by Rechmann [28] indicate that the mechanism of their origin from particles of materials penetrating into the soft tissue during dental treatment is not general.

The aim of the presented article, which originated in the framework of a study of the immune reaction in patients with teeth restored using a broader range of metallic materials, was to determine an alternative mechanism of the origin of pigmentations, based on the interaction of metallic materials used in prosthetics with the oral cavity environment.

Materials and methods

The study was comprised of an analysis of pigment particles in tissue preparations, determination of exposure conditions of metallic materials in vivo, experimental evaluation of corrosion properties and determination of the rate of silver release from the studied materials.

Biopsies

Nine biopsies of about 1 mm³ were obtained from eight patients with distinctly pigmented gingival areas adjacent to metallic restorations. In eight cases, pigmentations developed after prosthodontic treatment with alloy S covered by alloy G or polymer (Table 1). The only pigmentation was possible to denote as an amalgam tattoo. The teeth with amalgam fillings were restored before prosthodontic treatment. The composition of dental alloys was verified in individual dental records of each examined person. Patients were informed about the purpose of the study, and they gave their informed consent. The biopsy samples were removed surgically under local anaesthesia and immediately fixed in buffered 2.5% glutaraldehyde for 2 h. One half of the sample was post-fixed with a buffered 2.5% OsO4 solution, and in the second half, the use of OsO_4 was omitted. Standard methods were used for further Epon 812 (Serva, Heidelberg, Germany) embedding and transmission electron microscopy processing. Semi-thin and ultrathin sections were prepared using glass knives. Semi-thin sections were stained with toluidine blue, and ultra-thin sections were mounted onto standard copper grids or onto special plastic grids. Ultra-thin sections on copper grids were also stained according to Reynolds [30] for the control. After viewing in the electron microscope JEOL 100B (JEOL, Tokyo, Japan), the samples with dense particles were analysed by a CM12/STEM electron microscope equipped by EDAX DX4 (EDAX, Mahwah, NJ, USA) analysing system in the scanning transmission electron microscopy bright-field mode (FEI, formerly Philips EO, The Netherlands).

Electrochemical measurements in vivo

The electrochemical potentials were measured using a Hi8314 device (Hanna Instruments, Kehl am Rhein, Germany). A sterile platinum electrode with an area of 0.8 mm^2 was used for the redox potential measurement in combination with a silver/silver chloride reference electrode RE 401 (Theta, Prague, Czech Republic). It was inserted in a sterile micropipette plastic tip filled with a diluted solution of potassium chloride in de-mineralized water [19], closed with agar. During the open circuit potential of metal alloys in the mouth measurement, electric contact was ensured by a common dental instrument made of stainless steel, with a ball-shaped tip adapted for electrical connection to the voltmeter. Prior to every measurement, the instrument tip was insulated by wax Ceradent M (Dental, Prague, Czech Republic) in the length of at least 20 mm. This treatment [22], tested in a laboratory, allowed for contacting materials in the oral cavity without affecting their open circuit potential in any way. On touching the

Table 1 Composition of the studied materials (amalgams-composition of alloys+mixing ratio with mercury)

	Au (%wt)	Pd (%wt)	Ag (%wt)	Cu (%wt)	Sn (%wt)	Hg (%wt)	Zn (%wt)	Mixing ratio, alloy/Hg
alloy G	65	4	20	10			1	
alloy S			90		9		1	
amalgam A			60	28	12			1.35:1
amalgam B			70	4	22	4		1:1
amalgam C			70	15	12	3		1:1
amalgam D			43	30	25	2		1:1

metal surface with the instrument, the wax film was distorted by pressure, and an electric contact with the material was established. The wax layer at the same time insulated the tip from the surrounding environment, thus eliminating the possibility of influencing the measurement by the material of the instrument.

The measurements proceeded as follows: at first, the redox potential of the patient's saliva was measured under the tongue, and then open circuit potentials of the restored teeth metallic components were scanned one by one. The whole cycle was repeated four times. The measurement was conducted in 31 persons with a total of 52 amalgam fillings and 40 crowns made of a gold-based alloy. Statistical significance of the difference in open circuit potentials of the studied alloys was evaluated using the Student's *t* test on the significance level of P=0.05.

Laboratory study

Two alloys and four amalgams (one low-copper and three high-copper ones) were chosen for the experimental corrosion behaviour study. A gold-based material (identified as G in the text below) had been used in the examined group of patients for dental crowns, while a silver-based alloy (identified as S in the text below) had been used for post-and-core in several cases. A survey of the materials' composition is given in Table 1.

Amalgam specimens were prepared according to a standardized procedure [6], with amalgamator Capmix (3 M ESPE, Seefeld, Germany) used for the mixing. Four cylinders of 4 mm in diameter were used for the measurement, conductively attached (Ecobond 57C, Grace Speciality Polymers, Columbia, MO, USA) to a brass support and vacuum cast in epoxy resin (Epoxicure Resin, Buehler, Düsseldorf, Germany). The alloys were in the form of discs of 16×3 mm. The measurement was conducted on specimens in an as-received state. The specimens' surface was wet ground (up to P1200 paper) prior to exposure.

Laboratory measurements were done in a model saliva solution of the following composition: 20.1 mmol/l KCl, 17.9 mmol/l NaHCO₃, 3.6 mmol/l NaH₂PO₄, 5.1 mmol/ l KSCN, lactic acid 10.0 mmol/l, pH ~6.8, temperature $37.0\pm0.5^{\circ}$ C [19]. The oxygen content was minimized (nitrogen/10% vol. CO₂, 2 h) or standardized (air/10% vol. CO₂, 2 h). To simulate the effect of a change in the crevice environment aggressiveness on the corrosion behaviour of the amalgams and the silver alloy, the measurements were conducted in a model electrolyte with an increased concentration of chloride and thiocyanate ions.

Potentiodynamic curves were measured in a model solution with minimized oxygen content. After the open circuit potential stabilization (2 h), the curves were scanned using a PC4/Fas potentiostat (Gamry Instruments, Warminster, PA, USA) at a potential change rate of 1 mV/s. The silver/silver chloride electrode Theta RE401 was used as the reference. The results were recalculated and are presented in a standard hydrogen electrode scale.

Exposure tests

Exposure measurements with a subsequent analysis were conducted in a model solution containing oxygen, in a 12-ml plastic weighing bottle for 16 h. After the exposure, the electrolyte was quantitatively transferred into plastic volumetric flask, stabilized with a solution of diluted nitric acid (1:1 volume ratio) Merck Suprapur (Merck, Darmstadt, Germany), and analyzed by a mass spectrometer with inductively coupled plasma Elan 6000 (Perkin Elmer, Waltham, MA, USA). All laboratory utensils were cleaned in nitric acid (1:1). The applied chemicals had a purity guaranteed for trace analysis.

Results

Particle analysis

Dense particles of different size could be seen in ultra-thin sections in the lamina propria gingivae. These particles occurred alone or in clumps, extra-cellularly or intracellularly in the tissue. Particles were also found in the close proximity of the lamina basalis. The results of the conducted analyses are summarized in Table 2. The diameter of silver particles ranged between 10 and 310 nm, and all of them, regardless of their size, were of an ovoid shape. The analysed particles most often contained silver, accompanied with sulphur or selenium or both. The majority of particles found in the lamina propria contained only one metallic element, but in some of them, two or three other metallic components were identified. The presence of Ca, Si and P was also noted in some cases. No particles of an elemental composition that would be at least roughly similar to that of the alloy used for the tooth restoration were ever found anywhere.

Characterization of metallic materials and saliva in vivo

The open circuit potentials of materials in vivo and the redox potentials of the saliva are summarized in Fig. 1. In the course of the measurement, open circuit potentials of materials used in teeth with adjacent pigmentation and in those showing no problems were collected separately. The subsequent analysis showed that the values recorded in both groups did not show any statistically significant differences, and consequently, the data were processed in Tooth two stad by

Table 2	Results	of	the	pigmentation	grains	anal	ysis
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Allov G (crown) allov	S (cast post-and-core)	Polymer (crown) allo	ov S (cast post-and-core)	Amalgam		
Composition of particles	Number of particles	Composition of particles	Number of particles	Composition of particles	Number of particles	
Ag	1	Ag	0	Ag	0	
Ag+S	19	Ag+S	3	Ag+S	1	
Ag+Se	10	Ag+Se	11	Ag+Se	2	
Ag+S+Se	10	Ag+S+Se	32	Ag+S+Se	0	
Si	2	Si	5	Sn	3	
Ti	1	Ti	16	Ti	1	
Fe	6	Fe	6	Fe	1	
Si+Al	2	Al	3	Sn+Cu	1	
Si+Mg	2	Ca	6			
Fe+Al	1	Cu	2			
Fe+Sn	3	Mg	1			
Fe+Zn	3	Si+A1	4			
Fe+Zn+Cr	1	Fe+Ca	2			
Fe+Zn+Al	1	Fe+Si	1			
Fe+Mn+Si+Al	1	Fe+Si+Al	1			

an accumulated form. The difference between the open circuit potentials of the amalgams and those of gold-based alloys was statistically significant. The average redox potential of the saliva determined during the measurements conducted within this study was 0.27 V. The maximum value representing the theoretical maximum potential attainable by a metallic material in the oral cavity was 0.34 V.

Laboratory data

Corrosion behaviour of metallic materials in environments with varying oxidizing ability can be characterized by dependencies



Fig. 1 Open circuit potential of metallic materials in vivo and redox potential of saliva

between potential and current—potentiodynamic curves. The potential can be understood as an oxidizing ability of the environment; the value of the current corresponds to the rate of electrochemical reactions and relates thus to the corrosion rate and release of corrosion products. The potentiodynamic curves of the studied materials as shown in Fig. 2 indicate their largely different corrosion behaviour. The curve of amalgam A is typical of high-copper materials while that of amalgam B is typical of low-copper ones. The curves of materials C and D, which have a similar course as that of amalgam A, were omitted for the sake of clarity.

The rate of silver release from alloy S and from the amalgams is shown in Fig. 3. The strong effect of local changes in the crevice environment concentration on the corrosion behaviour of amalgam A and alloy S is evident from Fig. 4.

Discussion

This study was aimed at the explanation of cases when the formation of pigmentations from particles of alloys inserted mechanically in the mucous membrane during a tooth restoration is improbable. This, generally accepted, mechanism cannot be in many situations excluded. However, the shape of the particles, together with the fact that no pigmentation grains of a composition that would be at least approximately comparable with that of the applied alloys or amalgams were found during this study, indicate the possible existence of an alternative mechanism. Intracellular localization of the particles also testifies to their



Fig. 2 Potentiodynamic curves—amalgams and alloys (model saliva deaerated, 37°C)

origin in the cell, and the assumed existence of an alternative mechanism is also supported by the areal character of some pigmentation in the oral cavity. The hypothesis of pigmentation creation by alternative mechanisms was formerly published by Rechman [28].

The results of the open circuit potential measurement were in good agreement with the values published by Muller et al. [23] and Yontchev et al. [36]. The redox potential of the saliva was in the range of 0.139–0.342 V. The open circuit potentials of the amalgams were below this range, while the values obtained for the gold-based alloy did reach this range. However, the average value of the open circuit potential of the gold-based alloy was



Fig. 3 Release of silver (average \pm standard deviation, 16-h exposure, model saliva aerated, 37°C)



Fig. 4 Potentiodynamic curves—influence of the electrolyte composition (aerated solution, 37°C)

statistically significantly lower (at p=0.05) than the redox potential of the saliva. For the purpose of considerations relating to the electrochemical behaviour of materials in the oral cavity, the presented results show that a potential at the level of 0.34 V may be considered limiting.

The measurement of the open circuit potential of metallic materials in vivo proved that this value may range within broad limits for all groups of materials. The comparison of the potentiodynamic curves' shape with the region of the open circuit potentials of the amalgams and alloys in vivo indicates the danger of intensification of corrosion processes in this area, particularly in the case of a low-copper amalgam. At potentials mentioned, this material shows a breakdown of the passive layer [17]. This phenomenon not only results in an increase in the rate of mercury release [18], but it may be also assumed that it facilitates the course of corrosion processes and the release of corrosion products. As for high-copper amalgams (material A is shown as an example), the curves do not show any essential change in the range of open circuit potentials in vivo.

On the curves of the alloys, the corrosion potentials (corrosion potential is minimum of the curve) are shifted by more than 500 mV in the positive direction as compared with the amalgams. Significant in this case is the silverbased alloy (alloy S) sharp corrosion rate increase, which occurred at potentials slightly beyond the limit of values attainable in the oral cavity.

An alternative mechanism of the pigmentation formation, i.e. its origin by a manner other than the dental material penetration in the soft tissue during dental treatment, must explain the formation of soluble and thus transportable corrosion products for the most noble and most frequent component of pigmentations—silver. In the

work of Joska et al. [14], it is stated, based on an analysis of the electrochemical behaviour of palladium-silver alloys and a thermodynamic calculation for the silver model saliva system, that silver forms soluble chloride and thiocyanate complex compounds even in the range of potentials that were determined by way of in vivo measurement within this study. An equilibrium calculation made for the pure element did not take into account the effect of the multicomponent character of alloys used in dentistry. The calculation for real systems is often hardly possible due to inaccessibility of thermodynamic data. However, experimental determination of the rate of silver release confirmed that it did occur. According to the results, the amount of released silver did not depend on its concentration in the solid phase. Higher rates were recorded for materials with a more positive open circuit potential, i.e. under conditions mostly facilitating the course of an anodic dissolution reaction. A lower rate measured for amalgam D might be due to the formation of a copper-based layer blocking the surface [9, 10]. During laboratory exposure, the open circuit potentials of the studied materials stabilized on more negative values than in the case of in vivo measurements. The differences were probably due to a decreasing content of the least noble, i.e. most corrosion-active, components during long-term exposure in the oral cavity, as compared with materials whose surface was ground immediately before the laboratory measurement. The shift of corrosion potential to more negative values results in the case of metals corroding in an active state in the decrease in corrosion rate. In view of this fact, the rates of silver release in vivo could be higher than the values obtained under model conditions.

The occurrence of gingiva pigmentations in patients who were subject to our broader clinical observation could not be attributed to any particular kind of material. Prosthetic works in the nearest vicinity of the pigmentations were made of a whole range of materials used for teeth restoration. In this study, pigmentation was found even in the case of a tooth treated by cast post-and-core made of silver alloy covered by a polymeric crown. Gold- and palladium-based alloys as well as dental amalgams contain silver, which is released by way of corrosion processes. The transport of corrosion products from the free surface of a crown or filling to the tissue is, however, improbable. The transfer of corrosion products to the mucous membrane is more likely when the crown is subgingival. Yet, even this consideration fails in the case of pigmentation in which silver was detected but occurred around a tooth restored with a crown made of a material that did not contain this element.

According to dental records of the studied group of

patients, the crowns around which pigmentations occurred

had been cemented on amalgams or on a silver (S) alloy.

Under normal conditions, the material below the crown is not in contact with the oral environment. However, the analysis of the state of extracted teeth treated with prosthetic works proved that crevices were formed between the crown and the tooth in the course of exposure [13]. This way, the oral environment got to the filling material used for the tooth restoration, and the soluble compounds formed by the corrosion process could transfer to the soft tissue.

The corrosion of the material in the crevice, which was in this case represented by the space between the crown and the tooth, may differ from the corrosion of the surface freely accessible for the electrolyte. The limited possibility of the corrosion products transport from the crevice leads to an increase in their concentration. This results in the origin of a local positive charge, which is compensated by the migration of anions (in this case chloride and thiocyanate ions), and consequently, the corrosion aggressiveness of the environment increases. A comparison of the potentiodynamic curves of the low-copper amalgam (B) in electrolytes with an increased concentration of chloride and thicyanate ions clearly reveals a distinct current increase shift by 200 mV in the negative direction. The same phenomenon was noted for the S alloy, with the shift in the negative direction being 130 mV. At real values of the redox potential of the environment, the exposure of both materials under crevice corrosion conditions results accordingly in further intensification of corrosion processes and release of soluble corrosion products. The course of the high-copper amalgams' potentiodynamic curves practically did not change with growing aggressiveness of the environment. It may be assumed that the amount of silver released to the environment with a higher aggressiveness will not increase in this case. Nevertheless, as results from our measurements, these materials act as a source of silver too.

Conclusion

Dental amalgams and silver-containing alloys applied in teeth restoration may release silver in the form of soluble compounds under the conditions of the oral cavity. This phenomenon is not necessarily critical provided that the corrosion products are transferred by the saliva to the digestive tract. The formation of soluble silver compounds in the sulcus area or in a crevice between the crown and the cast post-and-core facilitates their transport to the soft tissue and subsequent deposition of silver and possibly also other metals in the soft tissue. The use of materials with a more significant silver content for post-and-core is therefore unsuitable in view of the danger of pigmentation creation, even if the material containing silver is not in a direct contact with oral cavity environment after the application. Acknowledgements The study has been worked out as part of grant NR9124/3 (Ministry of Health, Czech Republic).

Conflict of interest The authors declare that they have no conflict of interest.

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