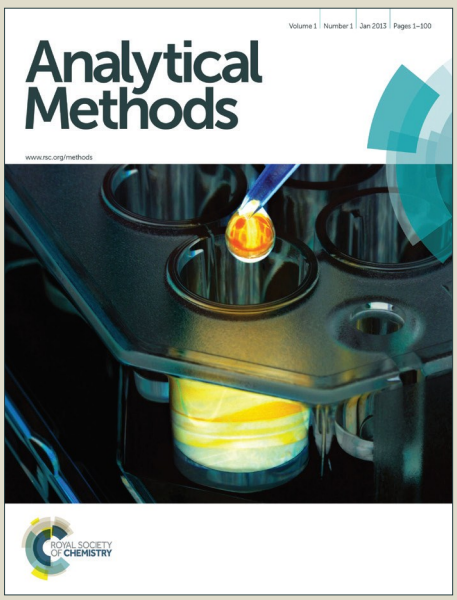
Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

In-vitro determination of titanium and other metals released from intraosseous dental implants to the mucosa

Iwona Rykowska[2], Krzysztof Makuch[1], Wiesław Wasiak[2]

[1]*Clinic of Geriatric Dentistry, Karol Marcinkowski University of Medical Sciences, Bukowska 70, 60-812 Poznań*
[2]*Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89b, 61-614 Poznań, POLAND*
Fax: + 480618291555
E-mail: obstiwo@amu.edu.pl, wasiakw@amu.edu.pl, macuch@poczta.onet.pl

In-vitro determination of titanium and other metals released from intraosseous dental implants to the mucosa

Keywords: dental implants, titanium (metal) ion, ICP-OES

Abstract

The main goal of the study was to determine the compounds released from titanium into the overlying oral mucosa and the other metals found in two-stage dental intra-osseous implants. The determined concentration of the metals was as follows: titanium from 1.7 to 13.1 μg/g, nickel 10.0 – 0.8 μg/g, aluminum 5.3 – 100.5 μg/g, zinc 16.1 – 118.8 μg/g, and copper 0.3 – 13.6 μg/g, respectively. Additionally, we also present the results of experiments carried out to determine the release of metal ions from two implant systems (Astra and Dentium) into a solution of lactic acid (1%). The quantitative analysis of the metal ions was performed by means of an emission spectroscope with inductively coupled plasma ICP-OES.

Studies were also carried out on the surfaces of different implant systems, including: Osteoplant, Astra, Dentium, SKY, Neoss as well as Biomet. The research involved the use of scanning electron microscopy to determine the chemical composition and quality of these systems.

1. Introduction

The introduction of new biomaterials to the dental market provides intensive development of modern implantology [1-2].

The chemical composition of metallic implants should provide a homogeneous structure in terms of the distribution of elements in the whole volume to be biocompatible. It is also expected to be of good corrosion resistance in the environment of tissues and body fluids. The rate of osseo-integration is influenced by many different factors. One of them is the degree of purity and the nature of the surface layer of the implant.

John C. Wataha [3] introduced the term of biocompatibility as a capability of some substances to provoke an appropriate biological response in given application [4], proving that there was no material completely biologically inert. The so-called biocompatible substances disposed into tissues exert some actions on them and vice versa - body tissues affect these substances. Common reactions in such a system depend on the capabilities of an organism, as well as the substance and its function.

The changes in the properties of the material may occur during its use and the forces acting on it. The reaction of the body can also be affected. The changes in the organism-material system may contribute to deterioration of the patient's health. Summarizing, the body's response to the functions and the time of deposition of the materials used in the medical treatment is a dynamic process [5]. The chemical composition of metallic implants should provide a homogeneous structure in terms of the distribution of elements in the whole volume to be biocompatible. The implants are also expected to be of good corrosion resistance in the environment of tissues and body fluids.

Steinemann studied the cytotoxicity of pure metals defining the relationship between polarization resistance and the reaction of tissues. According to his research, titanium, niobium, tantalum, zirconium, and platinum were highly biocompatible elements, and vanadium was considered an element of high toxicity [6].

Dental implants are usually made from commercially pure titanium or titanium alloys. These materials exhibit good mechanical properties, including high bio-tolerance of corrosive resistance in the oral environment, which makes them competitive in relation to other currently used metallic biomaterials.

Recent literature suggests that titanium alloys are not inert materials when used for medical purposes although that was the belief until recently. For example, they may induce dermatitis herpetiformis. Titanium implants in the body may cause not only local but also global reactions. Scientists have currently come up with the hypothesis that there is a correlation between the amount of compounds released as a result of corrosion and implant treatment failure. This has raised recent doubts regarding biocompatibility of titanium.

The surface chemical composition of titanium implants also affects the hydrophilicity of the surface. Highly hydrophilic surfaces seem more desirable than hydrophobic ones in view of their interactions with biological fluids, cells and tissues [7-9]. The biological properties of titanium depend on its surface oxide film. Several mechanical and chemical treatments have been used to modify the surface morphology and properties of titanium dental implants. One possible method of improving dental implant biocompatibility is to increase surface roughness and decrease the contact angle.

The widely used biomaterials, including titanium and its alloys, manifest a range of physicochemical properties which determine the way they are exploited. According to the literature, titanium dental implants considered biocompatible with the human body may, under certain conditions, cause inflammatory or allergic reactions. In some cases they may lead to the healing process being poor as well as to atopic dermatitis, and a general deterioration of the patient's health. It has been noted that the sensitivity to titanium materials used in the oral cavity in the form of intra-osseous implants may be due to galvanic and electrochemical corrosion, resulting in the release of titanium ions. These processes may contribute to the incidence of gingival discoloration, swelling, gingivitis, stomatitis, skin rash, erythema [10-12].

In the last 40 years, descriptions of cases suggesting side effects of the use of implants, including those made of pure titanium, have appeared [1,2,6]. They have usually comprised reactions such as metalosis [13] or fistulas, often in the form of eczema, rash, or itch surrounding the implant, thus suggesting allergy [14-16].

Recently, some researchers have been reporting allergic reactions to titanium implants. On the other hand, people who are allergic to other metals such as nickel and chromium, often tolerate titanium, and the reasons for the occurrence of an allergic reaction are their individual genetic characteristics, health status, diet, or the current phase of the biological rhythm. SEM analysis allows for very accurate determination of the chemical composition in the micro structure of the material being tested. It showed that the implant systems are not made of pure titanium, but an alloy of many elements, and various coatings and modifications of varied chemical composition applied to the implant surface. The chemical composition should be chosen so as to prevent unwanted destruction of the implant, sometimes resulting in an allergic reaction, and irritation of the body.

An implant, introduced into the body, is exposed to corrosion, and thus has the effect of stimulating or inhibiting the activity of the enzyme proteins, affecting metabolic and immunologic disorders. Pathological changes may occur in different tissues or organs depending on the quality and quantity of the metallic elements. The penetration of microelements into the surrounding tissue may be only a local impact, but also a general observation. Therefore, attention should be paid to the relationship between the

physicochemical properties of the implant and the biological environment, with all the effects typical for bio-electric processes.

The order of toxic metals determined sequentially by Venable and Stuck [33], and Frank and Zitter [34] was as follows:

- cobalt, magnesium and its alloys, iron, aluminum bronzes were considered to be toxic,
- zinc, silver, cerium, nickel, aluminum compounds, and various types of steel were determined to be of medium toxicity,
- gold, aluminum, titanium, stainless V2A and V4A, Vitallium alloy were found to belong to the group of non-toxic metals.

Frank and Zitter [34] also separated, with the clinical course of corrosion and metalosis, three characteristic waveforms:

− *silent* – initiation of corrosion starts immediately after implantation, and the development of corrosion does not cause immediate morphological changes in the surrounding tissues,

− *sharp* – rapid initiation of corrosion and its further development provoking changes in the tissue and detectable spectrally and histologically,

− *discreet* – tissue changes manifest themselves only after removal of the implant.

Previous research regarding the area of biocompatibility of titanium and other materials used in the implants was carried out on hard-tissue materials, usually extracted from animals [17-21]. Fewer studies, however, were performed to determine the response of human tissue and, especially, soft tissue to titanium implants.

Titanium content in the soft tissues surrounding the titanium implant was investigated by Jǒrgenson et. al [22]. It was shown that the tissue in direct contact with a titanium plate used for osteosynthesis of a skull in four patients contained from 7.92 to 31.80 $mg \cdot g^{-1}$ dry weight titanium.

In the 1980s some research was undertaken on vanadium-free titanium alloys. The medical community disclaimed penetration of vanadium from the implant to the surrounding tissues. Vanadium is considered a toxic element that causes the formation of inflammatory and allergic reactions, and neurogenic disorders. It also exhibits a high tendency towards corrosion [23-24]. Similarly to vanadium, aluminum also shows adverse effects on the human body. However, implants should not contain toxic elements [25]. The content of trace elements in the human body, except for iron, is very low. Corrosion of the implant placed within the body causes a release of the biomaterial to the tissue surrounding the implant, influences the increase of the total content of microelements, and thus may interfere with their natural presence in the body.

Kumazawa et al. [26] performed some in vitro and in vivo research into morphological and functional neutrophils and the impact of the ions of vanadium, nickel, and titanium. Nickel ions exerted the most devastating effect on neutrophils and showed the highest cytotoxicity. The stimulatory effect was related with ions of vanadium and titanium, but did not cause tissue damage.

The rate and quality of osseo-integration in titanium implants are related to their surface properties. The surface composition, hydrophilicity and roughness are parameters that may play a role in implant–tissue interaction and osseo-integration. Also, the size of the molecules matters. Inflammation, both in vitro and in vivo, appeared at a size less than 10 microns, while for 40 microns and above the metal molecules were characterized as biocompatible [27].

In the present study, the main goal was to determine titanium and other metals concentration in the mucosa covering two-stage intra-osseous dental implants. All the implants were made from titanium alloys of class IV. The research material consisted of segments of mucosa of 4 mm in diameter taken during the exposure of the two-stage implants after 4-6 months of healing. The samples were taken from 10 patients: 7 women and 3 men, of different age and status. Additionally, it also presents the results of experiments carried out to determine the release of metal ions from two implant systems (Astra and Dentium) into a solution of lactic acid (1%). This solution is characterized by its similarity to the reaction that is found in the oral cavity. As stated in [28], lactic acid [29, 30] and artificial saliva [31, 32] provides similar experimental results, however, the first compound is more popular and easy to obtain. The quantitative analysis was performed by means of an emission spectroscope with inductively coupled plasma ICP-OES.

Studies were also carried out on the surfaces of different implant systems, including: Osteoplant, Astra, Dentium, SKY, Neoss as well as Biomet. They involved the use of scanning electron microscopy to determine their chemical composition and quality.

These tests were intended to demonstrate that, despite the biocompatible properties of titanium, the usage of titanium-based implants may be followed by migration of ions of the metal to the soft tissues surrounding interosseous dental implants.

2. Apparatus and Reagents

2.1 Apparatus

The determination of the concentration of Ti(II) and the other metals was performed with a VISTA-MPX spectrophotometer with inductively coupled plasma (VARIAN ICP). The parameters for ICP-OES determination have been compiled in Table 1.

Table 1.

A scanning electron microscope Model SU3500, by Hitachi, was applied in the research. The preparation of the samples for the scanning electron microscope photo consisted of the application of the sample on a double sided sticky table (tape coal). Photos were taken at voltages of 15 kV and 20 kV in a wide range of magnifications.

An MDS – 2000 microwave oven was used for mineralization conducted in Teflon bombs in a mixture of 65% HNO_3, at 60 psi and a power of 40. After mineralization, the samples were quantitatively transferred into 10 mL flasks, filled up with redistilled water, and the content of titanium and other metals was determined.

2.2 Chemicals and materials

The stock standard solution, 1000 $\mu g \cdot mL^{-1}$, was purchased from Merck (Darmstadt, Germany). The heavy metal ions were prepared by using nitric acid to dissolve corresponding pure metals and diluting to the required concentration (100 $ng \cdot mL^{-1}$) with ultrapure water. Nitric acid of an analytical reagent grade was purchased from Fluck (Buchs, Switzerland).

The research material consisted of mucosa segments, 4 mm in diameter. The implantation procedures were carried out with the use of the closed healing method. After 4-6 months of osseo-integration, implant exposure was conducted through excision of the mucosa covering the implant bearing surface in order to place the healing screw in the implant. The exposure

was performed with a scalpel to exclude the possibility of metal particles from mechanical damage entering the research material. Prior to implantation, a segment of the mucosa from the area of the planned implant bed was collected. This fragment as well as the segments of mucosa taken from the alveolar ridge during other surgical procedures constituted the control group and came from patients who did not have metal restorations in their oral cavities.

The clinical studies were conducted with the consent of the Bioethics Commission of Karol Marcinkowski University of Medical Sciences in Poznan.

The content of titanium ions in the investigated samples containing the segments of mucosa from above the dental implants was determined, after previous mineralization in a microwave oven MDS – 2000, on an inductively-coupled plasma emission spectrometer VISTA-MPX produced by VARIAN ICP. The mineralization was conducted in Teflon bombs in a mixture of 65% HNO_3 at 60 psi and a power of 80. After the mineralization, the samples were quantitatively transferred into 10 mL flasks, filled up with redistilled water and then, the content of titanium and other metals was determined by means of calibration-curve method. The parameters for ICP-OES determination have been compiled in Table 1.

The same technique and analytical procedure was applied to determine metal ions released from the implant to the solution of 1% lactic acid.

Preparation of samples for scanning electron microscopy pictures consisted of the application of the sample to the double-sided sticky table (carbon tape).

3. Results and discussion

3. 1. Microscopic analysis of the chemical composition of the surface of the implant systems

Numerous observations demonstrated that good bio-tolerance is inseparable from good corrosion resistance of an alloy. Thus, the composition of the implant alloy, both qualitative and quantitative, evolved toward constructing such structures which are resistant to bio-electrical and bio-mechanical corrosion [2].

In order to determine the qualitative composition of eight different implant systems, several pictures of their surface were made by means of the scanning electron microscope Model SU3500, by Hitachi. X-ray EDS (energy dispersive X-ray spectroscopy) microanalysis, used in the experiment, is a rapid technique, well-suited for small samples without their specification prior to preparation. An important aspect is that the sample remains unchanged after the analysis. Using scanning electron microscopy, one may determine the qualitative composition of the material of which dental implants are made. Table 2 shows the qualitative chemical composition of the surface of the implant systems under study as identified by SEM. We used BSE-3D (Backscattered Electron) detector under the following conditions: beam voltage 15 kV, pressure 70 Pa.

Table 2.

3. 2. Analysis of content of released metal ions in Astra and Dentium systems

This study concerned determination of the metal ions, especially the quantities in which they were released from dental implant systems that had not been used before (Astra and Dentium[1]) in a 1% solution of lactic acid. This solution was chosen due to the fact that it had

[1] As for the Dentium implant system, the research concentrated on fastening titanium screws being part of this system.

a pH value similar to that prevailing in the mouth. The study was undertaken to determine whether or not, despite the biocompatible properties of titanium, migration of ions of this metal was observed to the soft tissues surrounding the implant. Before performing the experiment, pictures of the implant surface were taken by means of a scanning-electron microscope. Fig. 1 and 2 show the SEM images and EDS spectra for Astra, while Fig. 3 and 4 – for Dentium system.

Fig.1.

Fig. 2

Fig. 3

Fig. 4

The results of the research on the release of metal ions to the environment similar to that which prevailed in the oral cavity are shown graphically in Fig. 5 and 6.

Fig. 5

Fig. 6

The research proved what some previous experiments showed; namely, the fact that the migration process of the metal ions from the implant to the surrounding tissues was significant. In the case of the Astra system subjected to a three-month long experiment, the following amounts of the labeled elements were detected as having undergone migration: Cr - 0.73 [μg/g], Fe - 176.8 [μg/g], Cu - 15.3 [μg/g], Ni - 64.0 [μg/g], Zn - 57.1 [μg/g], while in the case of the Dentium system: Fe - 53.5 [μg/g], Ti - 2.3 [μg/g]. Some of the above-mentioned elements may cause or contribute to allergic reactions of the skin.

3. 3. Analysis of the content of titanium ions and other metals in the mucosa

The main objective of the study was to demonstrate the extent to which migration of titanium and other metals ions to soft tissues occurred, before and after implantation of intraosseous dental implants. For each sample, after previous mineralization, the content of titanium and other metals was determined in dry weight in mg/g by means of ICP - OES. Figures 7a and 7b shows the content of the elements in the mucous membrane before and after implantation [μg/g].

Fig. 7a

Fig. 7b

The study showed an increased content of titanium and such metals as nickel and copper in the tissues of the mucous membrane covering the intraosseous implants compared with the initial state of the tissues (taken before and after the implantation of the same patient). It should be noted that the whole process took place in the absence of any contact with the implants, and other metal additions. The determined concentration of the metals was as follows: titanium from 1.7 to 13.1 μg/g, nickel 10.0 – 0.8 μg/g, aluminum 5.3 – 100.5 μg/g, zinc 16.1 – 118.8 μg/g, and copper 0.3 – 13.6 μg/g, respectively.

It should be noted that for each of the patients a different system was implanted. Table 3 shows the results obtained from the analysis of the metals released to the mucosa from the intraosseous implant systems DIO and Osteoplant.

Table 3

The reports appearing in the world literature of adverse effects of titanium on the human body clearly and definitely cannot rule out the immune mechanism being the result of contact

between titanium and body tissues. The presence of titanium ions from implants in the soft tissues of the oral cavity seems to be completely biologically indifferent, but probably such migration is not sufficient enough to cause negative reactions. Translocation of titanium ions may, however, be a potential factor causing specific reactions, the effects of which are hard to determine at the moment.

A comparison of the results of SEM analysis with the one of the analysis of the metals released to the mucosa from the intraosseous implants reveals a similarity in the occurrence of these metals in the case of the DIO system. Figures 8 - 10 show the SEM images and EDS spectra for the DIO system.

The analysis of the elements distribution in the Osteoplant system (enlargement 100x, second point) registered an increased content of calcium, potassium, sodium, magnesium, and titanium. For greater magnification (1600x, second point) a significant iron content was detected at the surface of the test system. When analyzing the results of SEM analysis of the metals released to the mucosa from intraosseous implants, an occurrence of similar types of metals in the Osteoplant system may be pointed out. Figures 11 - 12 show SEM images and spectra for the Osteoplant system.

Conclusions

Trace amounts of titanium and other metals, indicated in the mucous membrane covering the implants during the osseo-integration indicate the local bio-chemical processes occurring in the tissue surrounding the implants. Despite the covering of the titanium implant by oxide passive film, a corrosion of the implants occurs, thereby titanium and other metals ions are released into the surrounding tissues. We proved, by a combination of ICP-OES and SEM analysis, that indicated metals were released from the titanium implant. Based on the results of ICP-OES analysis we can conclude that in the mucosa after implantation there was a significant increase of aluminum and zinc content. Two samples showed increased content of nickel. Chromium seems to be least migrated into the human body from the titanium implants.

Our research has shown a higher content of titanium and metals such as nickel and copper in the tissues of the mucous membrane covering the intraosseous implants compared with pure tissues (taken from the same patient), i.e., the ones not in contact with implants and other metal additions. The determined concentration of the metals was as follows: titanium from 1.7 to 13.1 μg/g, nickel 10.0 – 0.8 μg/g, aluminum 5.3 – 100.5 μg/g, zinc 16.1 – 118.8 μg/g, and copper 0.3 – 13.6 μg/g, respectively.

It is worth noting that the producers of the implants do not mention the fact of the presence of other metals in titanium alloys, just stating that they produce implants of “class IV”. For example, producer of Biomet implant declares that the content of the alloys is the following: nitrogen – 0.05%, carbon – 0.10%, hydrogen – 0.015%, iron – 0.5%, oxygen – 0.4%, and titanium – 98.935%. As may be seen, none of the metals detected in our research (except of iron and titanium) is declared there.

The research on the release of metal ions to the environment similar to that prevailing in the mouth confirms that such a process actually takes place. In the case of the Astra system subjected to a three-month experiment, the following amounts of the selected elements migrated to the solution: Cr - 0.73 μg/g, Fe - 176.8 μg/g, Cu - 15.3 μg/g, Ni - 64.0 μg/g, Zn - 57.1 μg/g, while for the Dentium system the following values were obtained: 53.5 μg/g of Fe and 2.3 μg/g of Ti. The marked elements demonstrate the following properties. Chromium is involved in the absorption of glucose, but also stimulates the metabolism of carbohydrates. Its deficiency contributes to increased levels of cholesterol and blood sugar, constraining growth

and changes in the circulatory system. While in excess, this metal is toxic, causes bronchial asthma, damage to the liver, kidneys and perigraft cells, as well as allergic reactions [35, 36].

Iron determines the activity of hemoglobin in supplying oxygen from the lungs to the body. Its excess, due to corrosion of the implant, accumulates in tissues and spleen cells perigraft destroying lysosomes and hindering the diffusion of enzymes across cell membranes. Iron can catalyze reactions of free radical formation, resulting in atherosclerosis, liver cirrhosis, cancer and mutagenic changes. Nickel at low concentrations is treated as a relatively non-toxic element. Its deficiency may be the cause of anemia, or stunted growth. At higher concentrations it shows a carcinogenic effect, causes allergic reactions and inflammation of the perigraft tissues. Also, skin contact with nickel-containing materials can cause or sustain allergic reactions [5].

Recent reports appearing in the world literature describe adverse effects of titanium on the human body and do not allow to definitely rule out the immune mechanism being the result of contact between titanium and the body tissues. The presence of titanium ions in the soft tissues of the oral cavity appears to cast doubt on the total biological inertness of titanium implants, but probably is not sufficient enough to cause negative reactions. Translocation of titanium ions may, however, be a potential factor causing specific reactions, the effects of which are, however, difficult to determine [5].

Our research fits into a global trend of proving possible danger of some metals (such as mercury, gold, nickel, cobalt, tin and chromium), provoking allergies to large population of humans. For example, the research of Laine et al. [37] and Alanko et al. [38] showed that only 11.5% of the patients did not display a positive result for an allergy to any of the metals tested. Thus, we see our research as quite important for majority of the potential patients.

Compliance with Ethical Standards

This research was carried out thanks to the laboratory of Adam Mickiewicz University Foundation in Poznań, Poland, established within the project WND-POIG.05.01.00-00-058/2011 "Waste Cluster – raising the standards of waste management using new technologies". This project is co-financed by the European Union from the European Regional Development Fund.

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

Literature

[1] Lazzara, R.J. Dental implant system design and its potential impact on the establishment and sustainability of aesthetics. J. Impl. and Recon.Dentistry.1(2012) 1-7.

[2] Bauer, S. Schmuki, P. von der Marck, K. Park J. Engineering biocompatibile implant surfaces. Part I: Materials and surfaces. Progr. Mat. Sci.58(2013) 261-326. DOI: 10.1016/j.pmatsci.2012.09.001

[3] J. C. Wataha, Principles of biocompatibility for dental practitioners, Journal of Prosthetic Dentistry, 86 (2), 203–209 (2001). DOI:http://dx.doi.org/10.1067/mpr.2001.117056

[4] Brantley, W. A. Eliades, T. Orthodontic materials: scientific and clinical aspects. Georg. Thieme. Verlag. London. (2001) 307-324.

[5] L. Evrard (2011). Titanium: A New Allergen, Implant Dentistry - A Rapidly Evolving Practice, Prof. Ilser Turkyilmaz (Ed.), ISBN: 978-953-307-658-4, InTech, Available from: http://www.intechopen.com/books/implant-dentistry-a-rapidly-evolving-practice/titanium-a-new-allergen

[6] Steinemann S.G., Corrosion of surgical implants – in vivo and in vitro tests, In: Winter, G.D. Leray J.L., de Groot K., Evaluation of Biomaterials, John Wiley & Sons, New York,1980.

[7] Buser, D. Broggini, N. Wieland, M. Schenk, R.K. Denzer, A.J. Cochran, D.L. Enhanced bone apposition to a chemically modified SLA titanium surface. J. Dent. Res.83(2004) 529-553. http://dx.doi.org/10.1177/154405910408300704

[8] Zhao, G. Schwartz, Z. Wieland, M. Rupp, F. Geis-Gerstorfer, J. Cochran, D.L. High surface energy enhances cell response to titanium substrate microstructure. J. Biomed. Mater. Res. A74(2005) 49-58. PIMD 15924300 [PubMed]

[9] le Guehennec, L. Soueidan, A. Layrolle, P. Amouring, Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent. Mat. 23 (2007) 844-854. DOI: 10.1016/j.dental.2006.06.025

[10] Stejskal, V.D.M., Cederbrant K., Lindvall A., Forrsbeck M. MELISA – an in vitro tool for the study of metal allergy. Toxic in Vitro 8(1994) 991-1000. DOI: 10.1016/0887-2333(94)90233-X

[11] Prochazkova, J., Sterzl I., Kucerova H., Bartova J., Stejskal Vera DM. The beneficial effect of amalgam replacement on health in patients with autoimmunity. Neuroendocrinology Letters 25(2004) 211-218. EL250304A07 Copyright Neuroendocrinology Letters, http://www.nel.edu

[12] Pye, A.D. Lockhart, D.E.A. Dawson, M.P. Murry, C.A. Smith, A.J. A review of dental implants and infection. J. Hosp. Inf. 72(2009) 104-110. DOI: http://dx.doi.org/10.1016/j.jhin.2009.03.007

[13] Frisken, K.W. Danie, G.W. Lugowski, S. Jordan, G. A study of titanium release into body organs following the insertion of single threaded screw implants into the mandibles of sheep. Aust. Dent. J. 47(3) (2002) 214-21. PMID12405460 [PubMed]

[14] Ungersböck, A. Perren, S.M. Pohler, O. Comparison of the tissue reaction to implants made of a beta titanium alloy and pure titanium. Experimental study on rabbits. J. Mater. Sci. Mater. Med.5(1994) 788–792. DOI: 10.1007/BF00213136

[15] Tomizawa, Y. Hanawa, T. Corrosion of pure titanium sternal wire. Ann. Thorac. Surg. 84(2007) 1012–1014. PMID:17720423[PubMed].

[16] Müller, K. Thon, E.V. Hypersensititivity to titanium: clinical and laboratory ewidence. Neuroendocrinol. Lett.27(2006) 31–35. PMID:17261997 NEL270706A11, http://www.nel.edu

[17] Stejskal, V. Hudecek, R. Stejskal, J. Sterzl, I. Diagnosis and treatment of metal-induced side-effects. Neuroendocrinol. Lett. 27(2006) 7–16. PMID:17261999 [PubMed]

[18] Olmedo, D.G. Michanié, E. Olvi, L. Santini-Araujo, E. Cabrini, R.L. Malignant fibrous histiocytoma associated with coxofemoralarthrodesis. Tumori 93(5) (2007) 504-50.

[19] Pongnarisorn, N.J. Gemmell, E. Tan, A.E.S. Henry, P.J. Marshall, R.I. Seymour, G.J. Inflammation associated with implants with different surface types. Clin. Oral Implants. Res.18(1) (2007) 114-125. DOI: 10.1111/j.1600-0501.2006.01304.x

[20] Berglundh, T. Lindhe, J. Ericsson, I. Marinello, C.P. Liljenberg, B. Thomsen, P. The soft tissue barrier at implants and teeth. Clin. Oral Implants Res. 2(2) (1991) 81-90. DOI: 10.1034/j.1600-0501.1991.020206.x

[21] Berglundh, T. Lindhe, J. Marinello, C. Ericsson, I. Liljenberg, B. Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. Clin. Oral Implants Res. 3(1) (1992) 1-8. DOI: 10.1034/j.1600-0501.1992.030101.x

[22] Jorgenson, S. Mayer, M.H. Ellenbogen, R.G. Centeno, J.A. Johnson, F.B. Mullick, F.G. Manson PN. Detection of Titanium in Human Tissues after Craniofacial Surgery. Plastic & Reconstr. Surg. 99(4) (1997) 976-997.

[23] Okazaki, Y. Ito, Y. Kyo, K. Tateishi, T. Corrosion resistance and corrosion fatigue strength of new titanium alloys for medical implants without V and Al. Mat. Sci. Eng. A 213(1996) 138-147. DOI:10.1016/0921-5093(96)10247-1

[24] Semlitsh, M.F. Weber, H. Streicher, R.M. Schon, R. Joint replacement components made of hotforged and surface treated Ti-6Al-7Nb alloy. Biomat. 11(1992) 781-788.

[25] Bordji, K. Jouzeau, J.Y. Mainard, D. Payan, E. Netter, P. Rie, K.T. Stucku, T. Hage-Ali M. Cytocompatibility of Ti-6Al-4V and Ti-5Al-2.5Fe alloys according to three surface treatments using human fibroblasts and osteoblants. Biomat.17(1996) 929-940. DOI:10.1016/0142-9612(96)83289-3

[26] Kumazawa, R. Watari, F. Takashi, N. Effects of Ti ions and particles on neutrophil function and morphology, Biomat. 23(2002) 3757-3764. DOI:10.1016/S0142-9612(02)00115-1

[27] Scales, J.T. Black, J. Staining around titanium alloy prosthesis-an orthopedic enigma. J. Bone Joint Surg. 73B(1991) 534-536.

[28] Koike M., Fujii H., In vitro assessment of corrosive properties of titanium as a biomaterial, J. Oral Rehabil. 2001 Jun;28(6):540-8. DOI: 10.1046/j.1365-2842.2001.00690.x

[29] Koike M., Fujii H., The corrosion resistance of pure titanium in organic acids, Biomaterials. 2001 Nov;22(21):2931-6. DOI:10.1016/S0142-9612(01)00040-0

[30] Yamazoe M., Study of corrosion of combinations of titanium/Ti-6Al-4V implants and dental alloys., Dent Mater J. 2010 Oct;29(5):542-53. Epub 2010 Sep 1. http://doi.org/10.4012/dmj.2010-005

[31] Abey S., Mathew M.T., Lee D.J., Knoernschild K.L., Wimmer M.A., Sukotjo C., Electrochemical behavior of titanium in artificial saliva: influence of pH. J Oral Implantol. 2014 Feb;40(1):3-10. DOI: 10.1563/AAID-JOI-D-11-00054. 2011 Nov 21.

[32] L. P. Faverani, J. F. Fogac¸, T. Machado, E. A. Silva, V. Adelino, R. Bara, W. G. Assunc¸ J Bio Tribo Corros (2015) 1:20 DOI 10.1007/s40735-015-0020-1

[33] Venable Ch.S., Stuck W.G., Beach A.: The Effects on Bone of the Presence of Metals; Based Upon Electrolysis. Ann. Surg. 1937, 105 (6), 917-938

[34] E. Frank, H. Zitter, Metallische Implantate in der Knochenchirurgie. Springer Verlag, Wien - New York 1977.

[35] Taher N.M., Al Jabab A.S.: Galvanic corrosion behavior of implant suprastructure dental alloys. Dent. Mater. 2003, 19, 54–59. DOI:10.1016/S0109-5641(02)00008-8

[36] Muris J., Feilzer A.J.: Micro analysis of metals in dental restorations as part of a diagnostic approach in metal allergies. Neuroendocrinol. Lett. 2006, 27, 49–52. PMID:16804513 NEL270706A04

[37] Laine J., Kalimo K. & Happonen RP. Contact allergy to dental restorative materials in patients with oral lichenoid lesions. Contact Dermatitis. 1997;36:141-146. DOI: 10.1111/j.1600-0536.1997.tb00396.x

[38] Alanko K., Kanerva L., Jolanki R., Kannas L. & Estlander T. Oral mucosal diseases investigated by patch testing with a dental screening series. Contact Dermatitis, 1996; 34 (4):263-267. PMID: 8730164

Table 1. The operating parameters of ICP-OES determination

Method parameters	Value
RF (power) [kW]	1.20
Plasma flow [L min^{-1}]	15.0
Nebulizer flow [L min^{-1}]	0.9
Viewing height [mm]	12
Pump rate	15
Rinse time [s]	10
Auxiliary flow [L min^{-1}]	1.50
Replicate read time [s]	5.0
Instrument stabilization [s]	15
Sample uptake delay [s]	30
Element – Emission line [nm]	Ti – 336.122 Cr – 267.716 Cu – 327.395 Ni – 231.604 Zn – 213.857

Table 2. Qualitative chemical composition of the surface of the implant systems determined by means of SEM method

	DIO	Astra	Sky	Dentium	Osteoplant	Neoss	Biomet
Ti	+	+	+	+	+	+	+
C	+	+	+	+	+	+	+
O	+	+	+	+	+	+	+
N	+	+	+	+	+	+	+
Ca	+	+	+	+	+	+	+
Al	+	+	+	+	+	+	+
Si	+	+	+	+	+	+	+
Na	+	+	+	+	+	+	+
K	+	+	+	+	+	+	+
Cl	+	+	+	+	+	+	+
P	+	+	+	+	-	+	+
Mg	+	+	+	-	+	+	-
Cr	-	-	+	+	+	+	+
Fe	-	-	+	-	+	+	+
F	-	-	+	-	-	+	+
Zn	+	+	-	-	-	+	-
Ni	-	-	+	-	-	+	+
Cu	-	-	+	-	-	+	-
W	-	-	+	-	-	-	-
V	-	-	-	+	-	-	-
Mn	-	-	-	-	+	-	-
Sc	+	-	-	-	-	-	-
Au	-	-	+	-	-	-	-
Ag	-	-	-	+	-	-	-
Ge	-	-	+	-	-	-	-
Sn	-	-	-	+	-	-	-
Br	-	-	-	-	+	-	-
Ba	-	-	-	-	-	+	-
I	-	-	-	-	-	+	-

Table 3. Results of analysis of metals released to the mucosa from intraosseous implant systems DIO and Osteoplant

Patient / type of implanted system	**Content [µg / g] of metals released to the mucosa from intra-osseous implant systems DIO and Osteoplant**					
	Zn [µg/g]	Ni [µg/g]	Cu [µg/g]	Ti [µg/g]	Cr [µg/g]	Al [µg/g]
Patient 1 / DIO	29.7	15.1	13.6	13.1	< LD	< LD
Patient 2 / Osteoplant	16.1	0.8	3.0	1.7	< LD	< LD
Patient 3 / DIO	18.5	< LD	7.7	2.0	< LD	< LD
Patient 4 / DIO	23.2	< LD	7.9	1.2	0.23	< LD
Patient 5 / DIO	35.8	< LD	24.4	26.8	1.37	5.3
Patient 6 / DIO	14.3	< LD	2.1	0.9	< LD	6.0
Patient 7/ DIO	19.8	< LD	6.5	12.5	< LD	41.1
Patient 8 / Osteoplant	102.8	< LD	9.1	6.8	1.09	60.2
Patient 9 / Osteoplant	118.8	< LD	72.1	5.3	0.75	7.6
Patient 10 / DIO	53.2	< LD	29.2	11.7	< LD	105.0

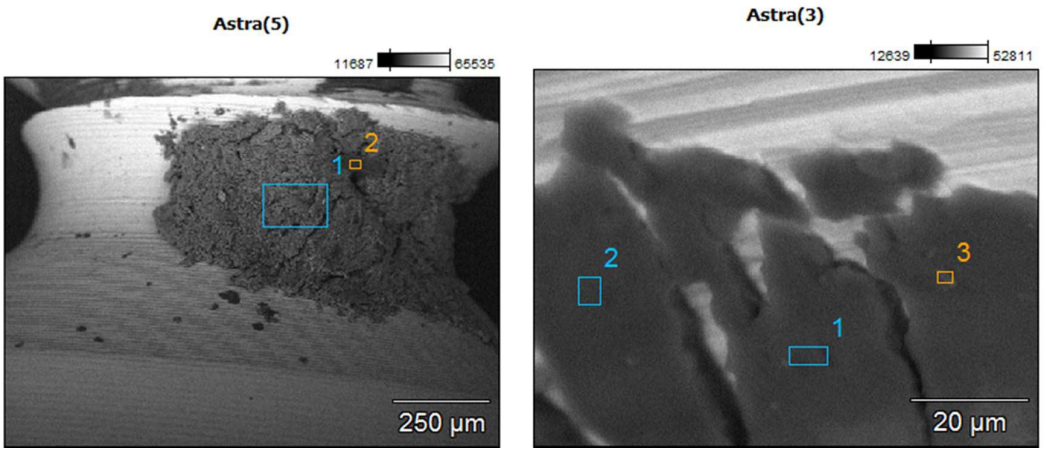
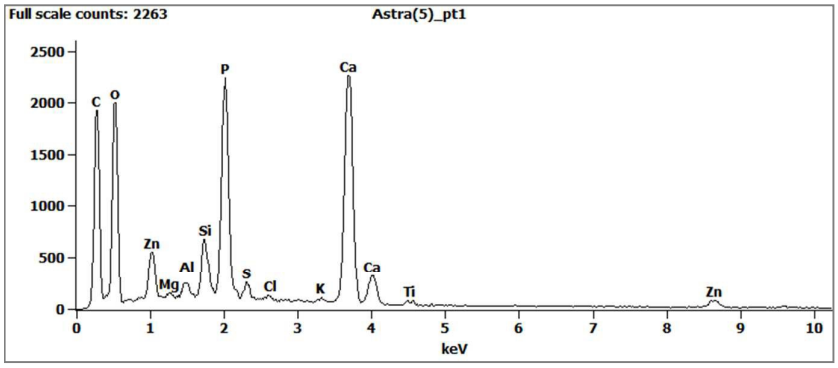


Fig.1. SEM image of Astra system surface. For the pointed areas, elemental analysis was performed



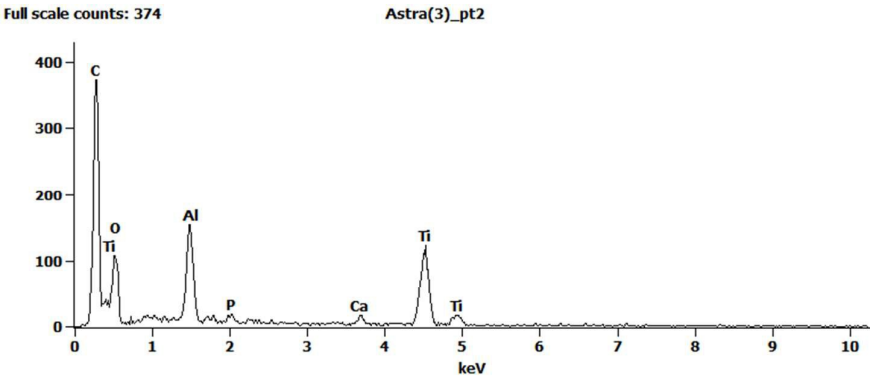


Fig. 2. EDS spectrum of point analysis of surface of Astra system

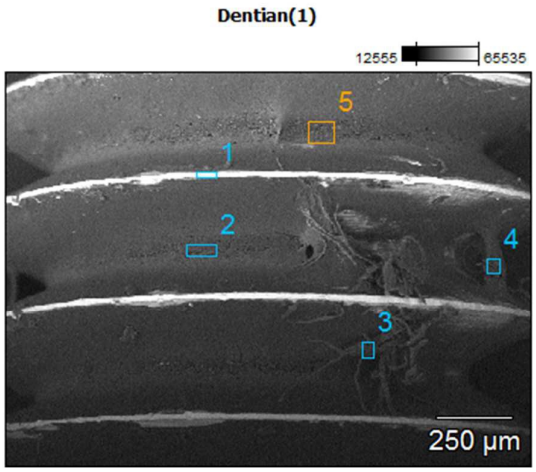


Fig. 3. SEM image of Dentium system surface. For the pointed areas, elemental analysis was performed

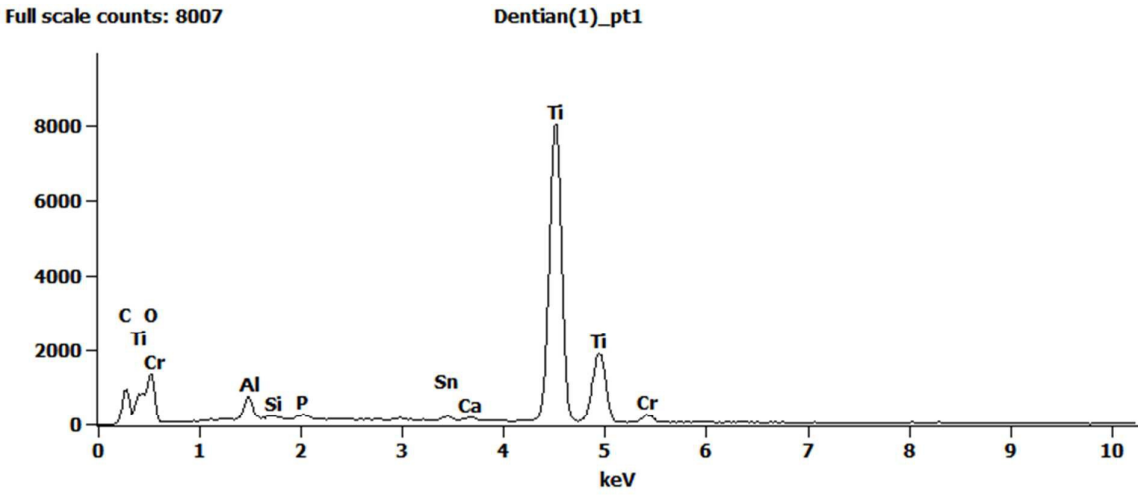


Fig. 4. EDS spectrum of point analysis of surface of Dentium system

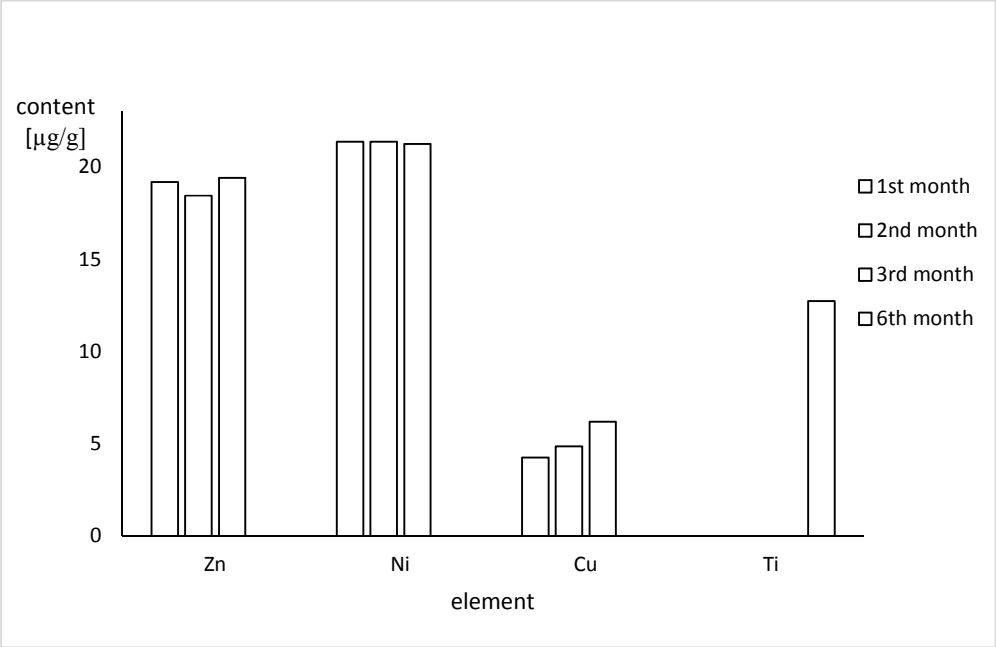


Fig. 5. Content of elements released from the implant in Astra system [µg/g]

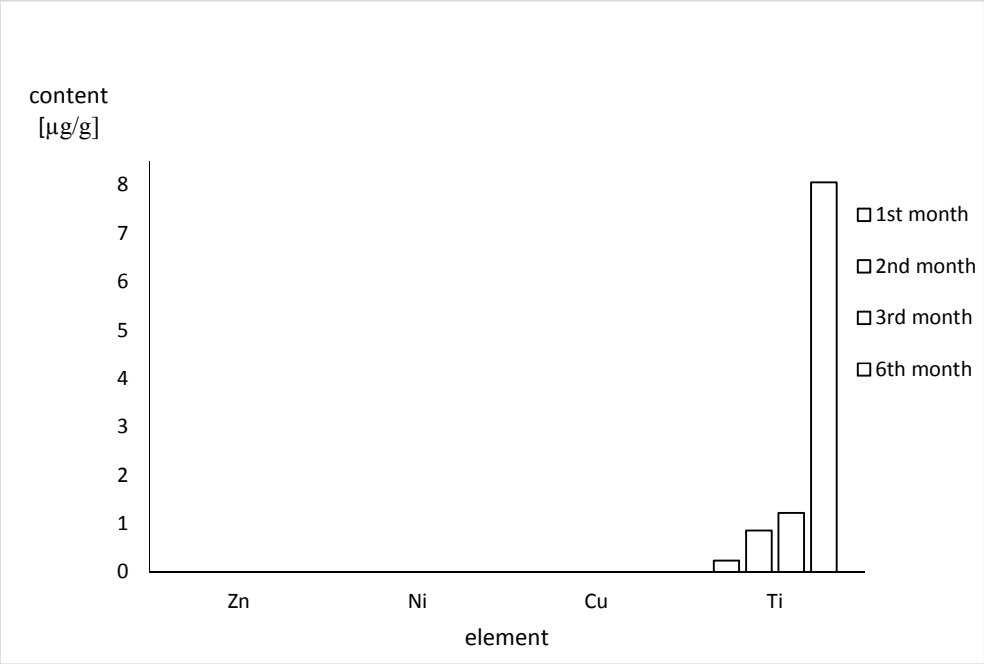


Fig. 6. Content of elements released from the implant in Dentium system [µg/g]

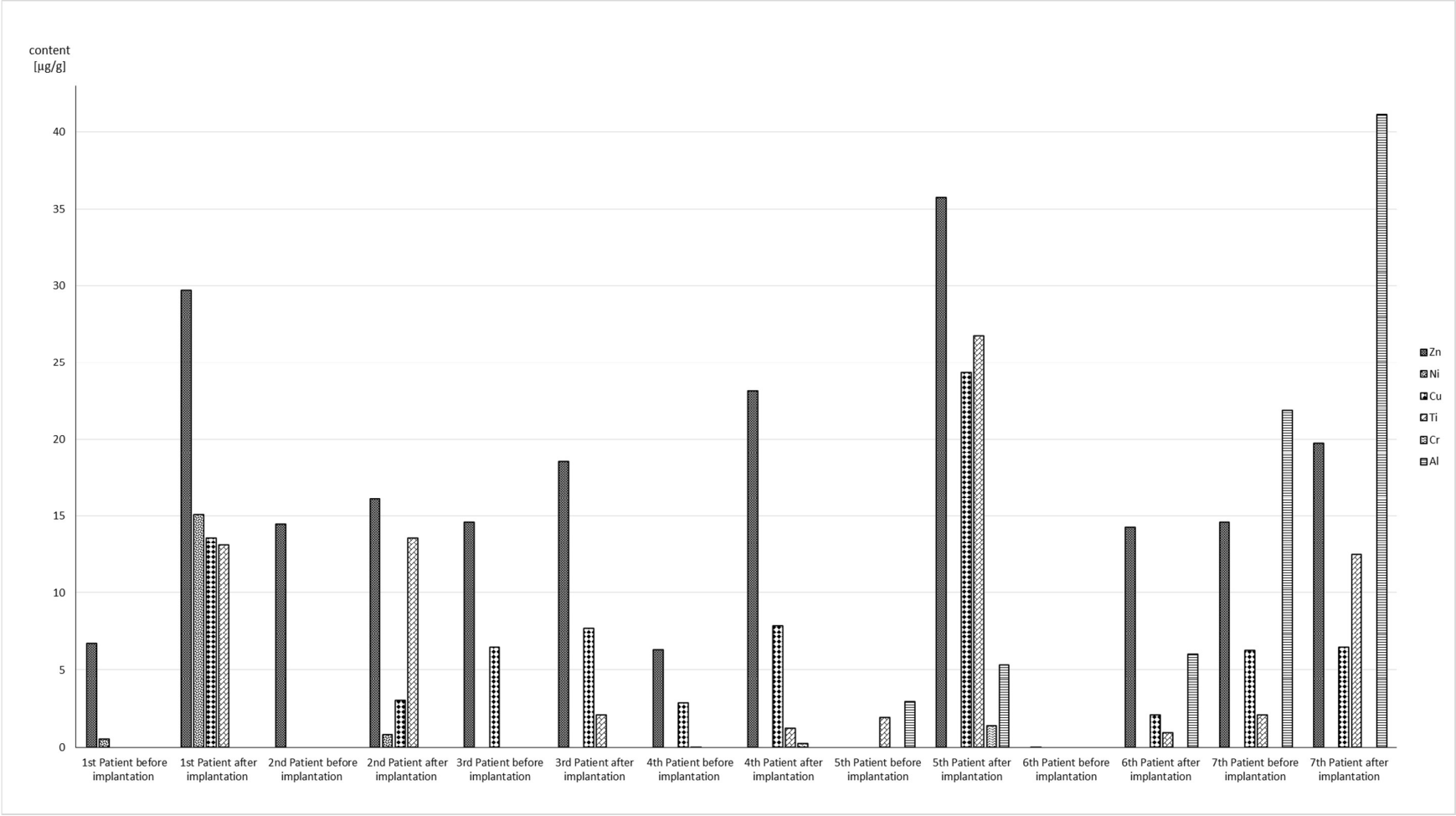


Fig. 7a. Content of elements in the mucosa before and after implantation [µg/g], patients 1-7

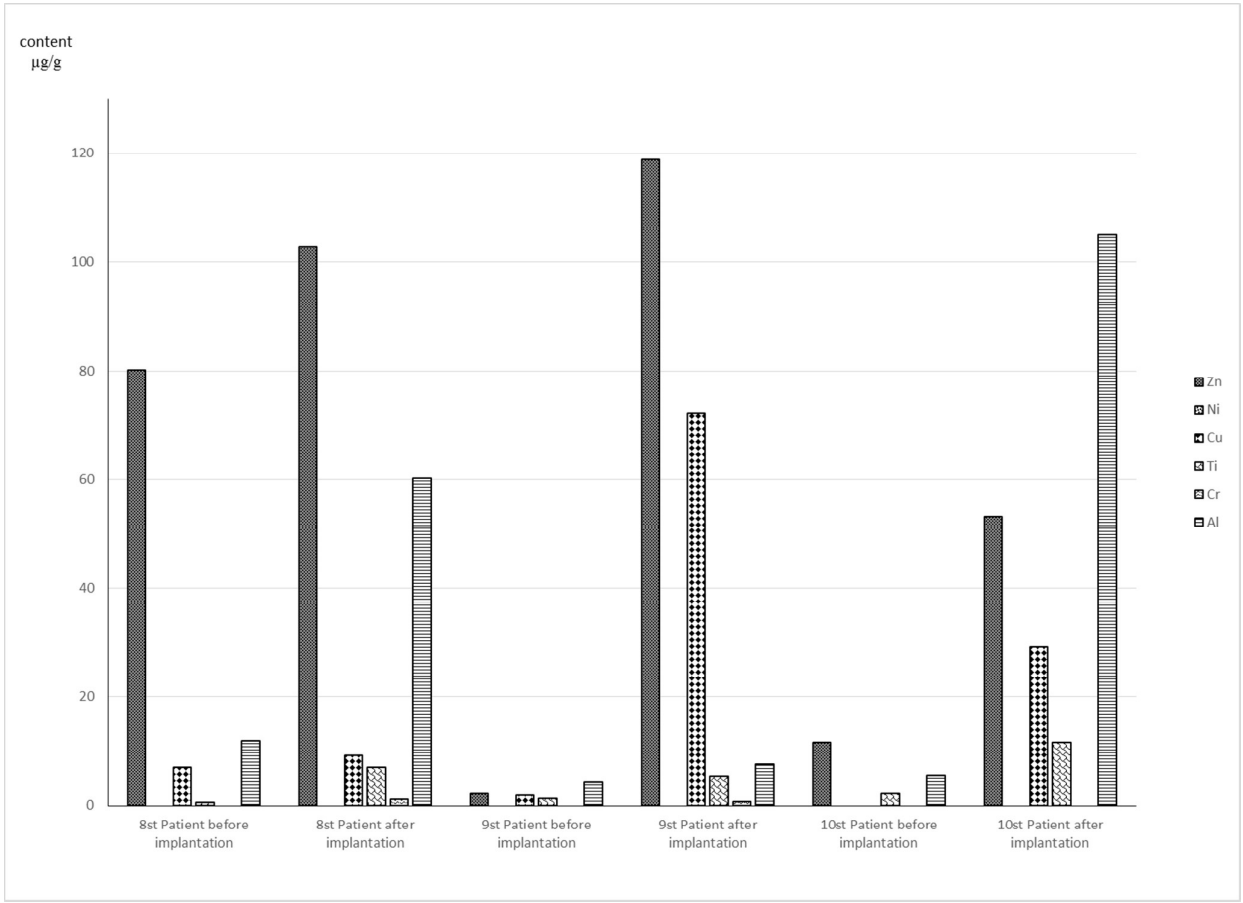


Fig. 7b. Content of elements in the mucosa before and after implantation [µg/g], patients 8-10

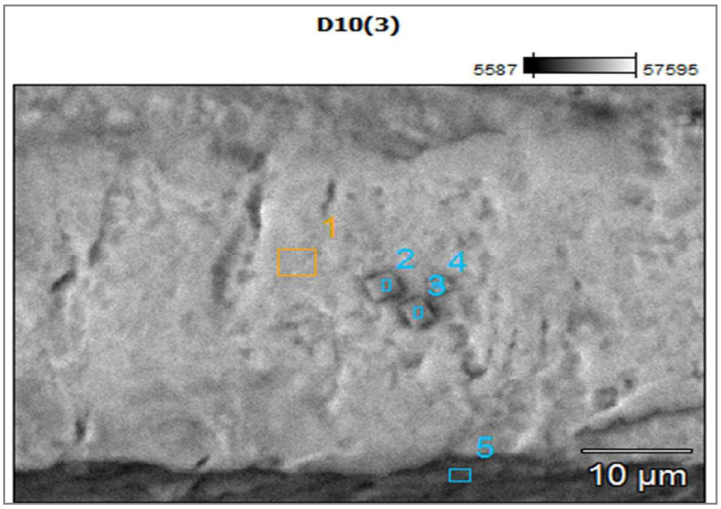


Fig. 8. SEM image of the surface of DIO (3) system, enlarged 1500x. For the pointed areas, elemental analysis was performed

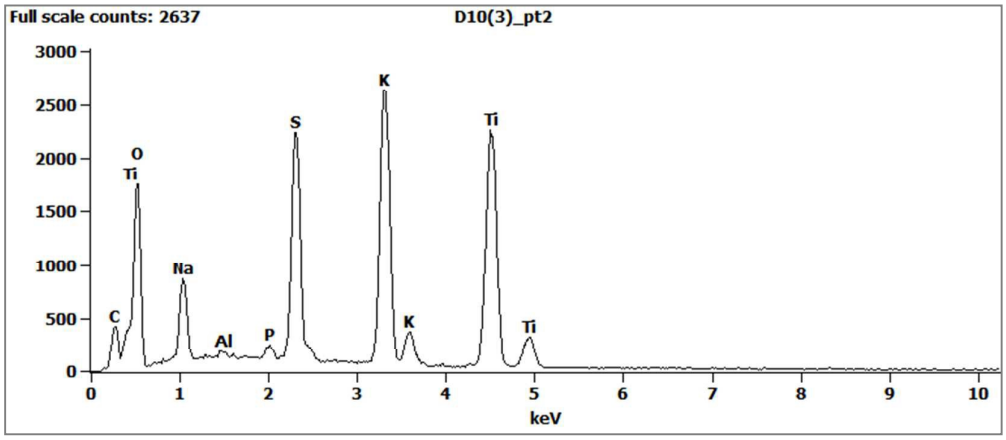


Fig. 2. EDS spectrum of point analysis of the surface of DIO system (3rd area)

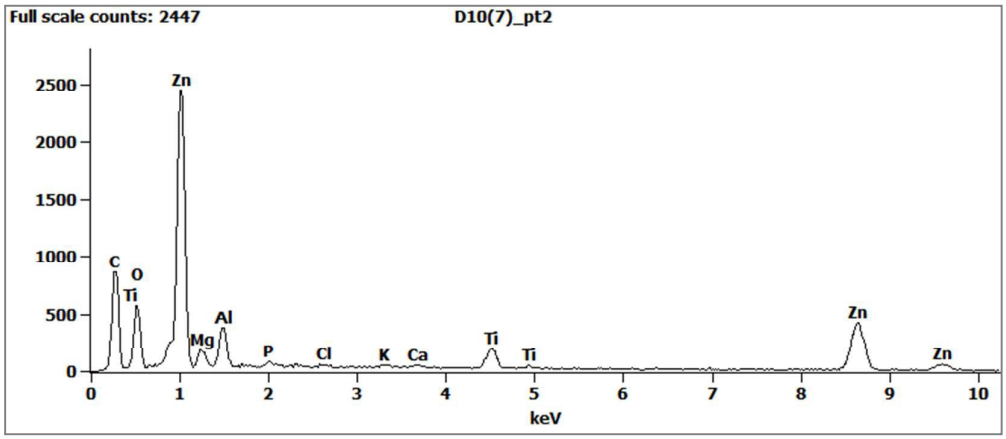


Fig. 10. EDS spectrum of point analysis of the surface of DIO system (5th area)

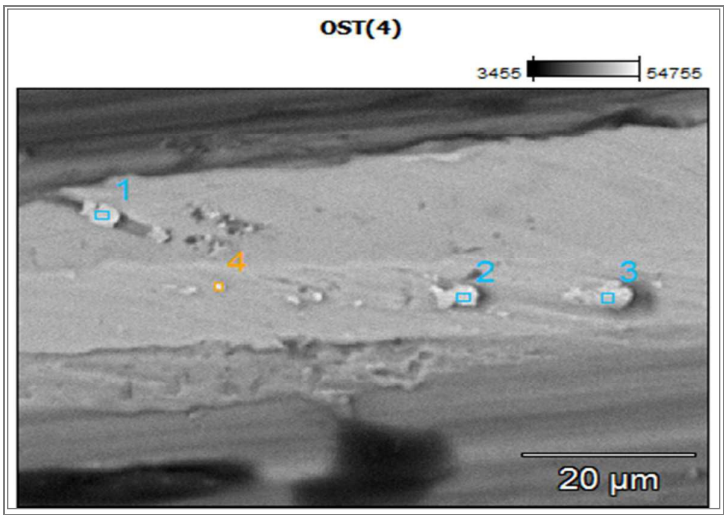


Fig. 11. SEM image of the surface of OSTEOPLANT (4) system, enlarged 1600x. For the pointed areas, elemental analysis was performed

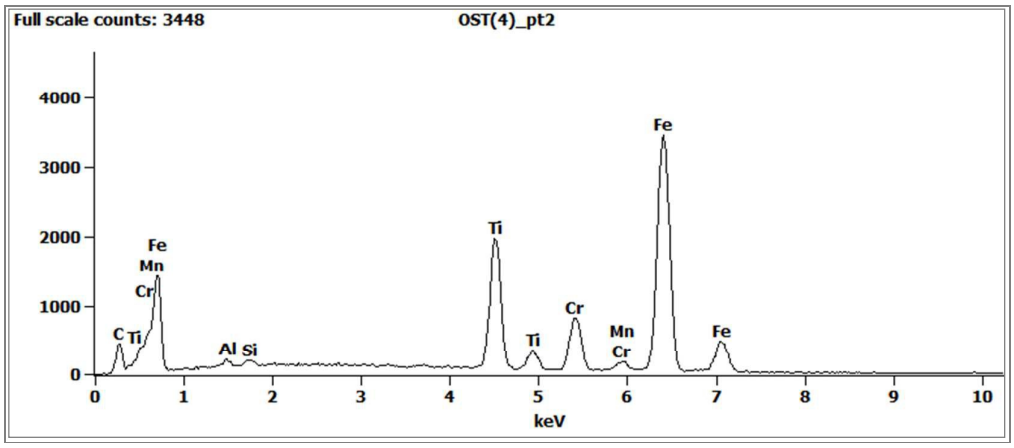


Fig. 12. EDS spectrum of point analysis of the surface of OSTEOPLANT system (4)