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Analytical methodology

Usefulness of laser ablation ICP-MS for analysis of metallic particles released to oral mucosa after insertion of dental implants

Adam Sajnóg[a], Anetta Hanć[a], Ryszard Koczorowski[b], Krzysztof Makuch[b], Danuta Barałkiewicz[a,*]

[a] Department of Trace Element Analysis by Spectroscopy Method, Faculty of Chemistry, Adam Mickiewicz University in Poznan, Umultowska 89b, 61-614 Poznan, Poland
[b] Clinic of Geriatric Dentistry, Karol Marcinkowski University of Medical Sciences in Poznań, Bukowska 70, 60-812 Poznan, Poland

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ABSTRACT

Despite the fact that titanium is considered highly biocompatible, its presence in the oral cavity (an environment of frequently changing pH and temperature) may result in the release of titanium from intraosseous implants into the oral mucosa, causing a range of reactions from the human body. Fragments of oral mucosa collected from patients after dental implant insertion were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The study revealed an elevated content of elements (Ti, Al, V) which are components of the metal implants and temporary cover screws. Dynamic ablation of the tissue surface was used in order to obtain maps of the content and distribution of analyzed elements. The material consisted of 30 oral mucosa tissue fragments collected 3–5 months after implantation and 10 samples collected before implantation (control group). The application of optical microscope allowed for indication and confirmation of the location of metal particles prior to LA-ICP-MS analysis. The so-obtained map permitted location of regions containing metal particles. LA-ICP-MS analysis revealed groups of samples with similar properties of metal particles, thus confirming that those metal particles were the main source of the elevated content of metals (Ti, Al, V) in the tissue after implantation. A calibration strategy based on matrix matched solid standards with powdered egg white proteins as matrix material was applied with ^{34}S as an internal standard. The accuracy of the analytical method was verified by ablating pellets of certified reference material ERM-BB422 Fish muscle.

1. Introduction

An intensive development of analytical techniques makes it possible to obtain an increasing amount of information from the examined object. Essentially, in order to determine the content of elements, especially at trace level, a solid sample is first mineralized and then transformed into a solution. This procedure results in the loss of information about the spatial distribution and form of elements in the sample, providing only an average content in the entire mass of the sample. In addition, samples with a low mass and a small size are not representative and the data obtained after the mineralization does not have a high informational value in the interpretation of the examined object. To ensure metrological correctness, the sample should be collected in a representative way, since the number of samples taken for analysis affects its homogeneity. The minimum weight of a sample for mineralization should be similar to the weight recommended by the manufacturer of certified reference material (CRM) with a matrix similar to the matrix of the sample. The minimal portions of CRMs for elemental analysis vary depending on the particular material and range from 100 mg (ERM BCR-668 Mussel tissue), through 200 mg (ERM BB-184 Bovine muscle) to 500 mg (NIST SRM 1845 Whole egg powder).

The introduction of laser ablation (LA) with detection in a mass spectrometer with inductively coupled plasma (ICP-MS) allows the content of the elements on the surface of the solid sample to be recognized without the need for sample mineralization. LA-ICP-MS was chosen because examination of the spatial distribution of chemical elements is possible in samples with small dimensions, of the order of several mm and a weight of a few milligrams. The method is characterized by the multielemental analysis of the surface or depth profile of the solid sample, minimum treatment of the sample before analysis, micro-destructive effect on the sample and good values of precision and limit of detection [1,2]. A particularly useful feature is its bioimaging ability, i.e. the representation of data in the form of two-dimensional maps of content of the elements with a spatial resolution of up to 10 μm [3,4]. Bioimaging is increasingly being used in the analysis of organs and tissues, including clinical samples, in order to diagnose an illness, mechanism of transport and effects of drugs or the effect of the interaction of implants and prosthesis with the surrounding tissues. A

* Corresponding author.
E-mail address: danutaba@amu.edu.pl (D. Barałkiewicz).

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significantly large proportion of LA-ICP-MS research on soft tissues originated from human organs concerns the brain or its fragments as well as other soft tissues [5–12]. In order to present the results of LA-ICP-MS in quantitative units a calibration procedure should be applied which will allow the evaluation and comparison of measured values with the literature data and data obtained with other analytical techniques. Due to a lack of calibration standards and CRM dedicated for analysis of tissues by LA-ICP-MS, alternative calibration strategies must be developed and applied. Many approaches have been successfully developed for soft tissue analysis and they may generally be divided into three groups: matrix matched solid standards (e.g. homogenized tissue, pressed pellets of powdered material), non-matrix matched solid standards (based on gelatin, agarose, printed patterns) and liquid standards, including isotope dilution aqueous standards [13–15]. LA-ICP-MS is useful in assessing the influence of metal implants on the surrounding tissues. It has been shown that after insertion of the implant the migration of elements, which are components of the implant, takes place. [16–18].

Dental implants are used as a substitute for missing teeth, also in the state of edentulism. The most commonly used material for the production of implants is titanium and its alloys. Titanium is considered to be a metal with good biocompatibility, good mechanical properties and high resistance to corrosion due to the protective passivation layer of titanium oxide [19]. However, as a result of changing conditions of pH, temperature, and the influence of a combination of body fluids and mechanical forces during use, the implants are gradually subjected to electrochemical corrosion and ion release to the surrounding tissues [20,21]. Besides Ti other metals and alloys are used to produce implants, for example Al, V, Cr and Ni which can eventually result in a particularly serious outcome since these elements may act adversely on human health. Constant research is carried out on the composition, production process and microstructure of metal implants so as to obtain a material with the desired durability, rigidity, lightness, resistance to abrasion and biocompatibility. Ions may accumulate in the adjacent tissues causing inflammation and allergic reactions, which can even lead to rejection of the implant. Moreover, the aforementioned conditions may contribute to the formation of nano- and microparticles of metal or metal oxide, which continue to undergo electrochemical dissolution or can even be transported through the circulatory and lymphatic system to further organs [22–24]. However, the literature generally lacks papers with quantitative data obtained by elemental imaging techniques regarding the interaction of soft tissues and metal objects, particularly medical implants.

Our previous work was focused on the developing and validating the method of quantitative imaging of the distribution of Ti, Al and V by LA-ICP-MS in oral mucosa after implantation procedure [26]. The results of previous analyses suggested the possibility of occurring of metallic particles on the surface of analyzed tissues. Therefore, the main goals of the present study were as follows: (1) extending the applicability of the previously developed procedure by improving the laser ablation system parameters and applying the optical microscope, (2) apply the extended procedure to an analysis of 30 oral mucosa samples collected in two different clinics. Special emphasis has been brought on the issue of the characterization of solid particles originating from surgery procedure of insertion of the implant and their impact on the content of the analyzed elements in tissue samples.

2. Materials and methods

2.1. Samples

The objects of the study were fragments of the oral mucosa obtained from patients undergoing treatment with dental implants. The implantation procedures were performed in two independent dental clinics in Poland. Samples were selected from patients who did not undergo complex dental treatment or show signs of inflammation of the oral cavity. Before implantation the portion of the mucous membrane covering the alveolar ridge was collected and served as a control group. After placing the implant with a closing screw a closed healing method was applied. On completion of the osseointegration process, lasting 3–5 months, the implant was unveiled by cutting and a tissue specimen of the mucosa surrounding the implant was taken which served as a test sample. The intraosseous implant was made of grade IV pure Ti, while the temporary closing screw was made of Ti6Al4V alloy or pure Ti, depending on the applied implant system. The composition of Ti6Al4V is ca. 90% Ti, 6% Al, 4% V, 0.25% Fe, 0.2% O [25]. Fragments of mucosa were rinsed with deionized water in order to remove excessive blood and any residues after treatment and drilling. The samples were placed between two PET bases in order to avoid deformation and stored in plastic containers at −20 °C. Samples of the control group and those collected after implantation were treated in the same way. Directly before LA-ICP-MS analysis samples were dried in a laboratory oven with mechanical convection in 25 °C for ca. 60 min (SLW 32 SDT, Pol-Eko, Poland) and placed on a PET base with a drop of pharmacologically pure gelatin (Gelatin, European Pharmacopeia grade, VWR Chemicals) serving as an adhesive agent. The volume of the gelatin droplet was adjusted to the specific mucosa sample in the range of 2–10 µL so as to ensure that gelatin would not protrude beyond sample's contour. The sample collection and preparation steps are presented schematically in Fig. 1. The aim of the study was to investigate the oral mucosa surface (direct contact of tissue with the implant) so that no preablation was conducted to achieve the intended purpose of the current research. The following quantities of tissue samples were analyzed by LA-ICP-MS: 10 control samples and 30 samples after implantation.

2.2. Apparatus and conditions

An LA system (LSX-500, CETAC Technologies, USA) with a quadrupole ICP-MS (Elan DRC II, Perkin-Elmer Sciex, Canada) was used to analyze in-situ oral mucosa tissue samples. Prior to measurements the instrumentation was optimized by setting the nebulizer gas flow, RF generator power and ion lens voltage and ablating the standard reference glass material NIST SRM 610 so as to obtain maximum signal intensity for $^{24}Mg^+$, $^{115}In^+$, $^{238}U^+$ and to set the ratio of oxide $^{232}Th^{16}O^+/^{232}Th^+ < 0.2\%$ and doubly charged ions $^{42}Ca^{2+}/^{42}Ca^+ < 0.5\%$. The following isotopes were selected for analysis: ^{49}Ti, ^{27}Al, ^{51}V and ^{34}S which served as the internal standard. Selection of internal standard was carried out according to the procedure described in the paper by Sajnóg et al. [26]. The optimization of laser conditions was performed by ablating a pellet of matrix-matched solid standard using the single variable method. The parameters for optimization were: energy of laser beam, spot size, shot frequency and scanning speed. Factors influencing the choice of laser parameters were the highest intensity of the signal of an internal standard taking into account the lowest value of relative standard deviation (RSD). The parameters were set so as to obtain a compromise between the sufficient intensity of analytical signals and the spatial resolution of images generated from acquired analytical signals. The parameters used in the current research are presented in Table 1. The images of distribution of elements were generated from data acquired after creating a rectangular line scan pattern composed of parallel lines of the same length on the surface of the sample. The width of the ablation line was 50 µm and the distance between the centers of the lines was 50 µm. Number and length of scan lines were adjusted to the dimensions of the sample under investigation. LA was performed line after line starting from the left edge of the sample with a short pause at the end of each line. The software used was Microsoft Excel 2010 for data treatment and OriginLab Pro 2016 for making 2D contour plots. Photographs of tissues were made using an optical stereoscopic microscope and with microscope integrated with LA system. The device makes it possible to preview a sample in real-time, dynamically change the zoom and save the image in a computer. The prolonged wash out of aerosol rich in