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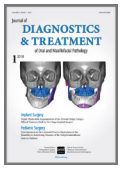

Detection of Titanium Particles in Soft Tissues Adjacent to the Fixators in Patients With Facial Fractures and Bone Defects*

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ABOUT ARTICLE

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ABSTRACT

Background.
Open reposition and rigid internal fixation are the main methods of treatment for traumatic injuries of the facial skull and an important stage of bone-plastic, reconstructive, and orthognathic surgery. In contemporary maxillofacial surgery, fixators, implants, and endoprostheses made of titanium or its alloys are widely used due to the high corrosion resistance and biocompatibility. However, recent studies have shown that none of the metal implants used in maxillofacial surgery, orthopedics or traumatology is completely inert. Moreover, they always interact with the surrounding biological environment. Thus, a number of studies have revealed the release of titanium to the adjacent soft tissues.
Material and Methods.
Titanium fixators (plates and screws) removed in 12 patients in late terms after osteosynthesis, as well as biopsies of the periosteum and fibrous capsule adjacent to the fixation elements made of titanium were investigated. Microscopic fluorescence spectroscopic analysis (M4 TORNADO micro-ray fluorescence spectrometer; Bruker, Bremen, Germany) was used to determine the elemental composition of the removed soft tissue fragments. Scanning electron microscopy (microscope model JSM-6060; JEOL, Japan) was used to study structural changes on the surface of titanium plates and screws. The obtained results were analized with the use of Spirman correlation coefficient, calculated by the IBM SPSS Statistics v.23 software.
Results.
X-ray fluorescence analysis revealed the inclusion of titanium in all investigated samples with an average content of titanium 48.14% ± 31.1% in metal deposition areas. For samples removed in patients with traumatic facial fractures after metallosteosynthesis, the average content of titanium was 55.6%, and for reconstructive surgeries – 37.72%. The acquired maps of the element deposition showed no topographic inhomogenity of titanium particles distribution. The main distribution patterns were the following: 1) areas of clearly outlined intensive titanium inclusions (90.9-800 μm), and 2) diffuse titanium inclusions which were poorly demarcated. Electronic microscopy of the investigated fixators revealed deformation of the thread, bending of screws, deformation and surface defects of the plates caused by mechanical damage, including microcracks, sharp edges, scratches, dimples.

Introduction

Open reduction and rigid internal fixation are the main methods of treatment for traumatic injuries of the facial bones and an important stage in bone-plastic, reconstructive and orthognathic surgery. A large number of available fixators differ in shape, size and design features. The most common are metal plates and screws, which have the adequate stiffness and strength to provide stabile fixation of the bone fragments under cyclic functional load [1].

Stainless steel [1], titanium, and its alloys, zirconium alloys, tantalum are used for fixators manufacturing. According to modern concepts, these materials, should be biocompatible (from the chemical, physico-mechanical and biological points of view) to avoid toxic and carcinogenic effect, as well as any kind of immune response [1]. However, studies of recent years have shown that none of the metal implants used in maxillofacial surgery, orthopedics or traumatology is completely inert. Moreover, they always interact

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with the surrounding biological environment [2, 3]. The release of metal from plates and screws into the living tissues after the implantation and the resulting pathological changes of varying severity have been reported for most alloys used to date [4-7]. The release of metal from the fixator results from the corrosion, friction and micro-destruction during the interaction of the 'fixator-bone' system elements under the functional load or mechanical damage to plates and screws at installation or removal [2, 7].

It has been proved that stainless steel, which was widely used for the manufacture of fixators in the second half of the past century, undergo significant biodegradation and cause local tissue reactions [8-11]. The constituent metals, including chromium, nickel, molybdenum and iron showed a certain degree of toxicity [8, 12-14]. Meachim and Winter reported that the high content of corrosion products around stainless steel implants was associated with chronic inflammatory reaction. Therefore, in contemporary maxillofacial surgery, the most widely used are fixators, implants, endoprostheses of titanium and its alloys [7, 12, 15-20] due to the fact that along with good mechanical properties they have high corrosion resistance and biocompatibility exceeding the similar characteristics of medical steel [7, 15-17, 19, 21-24].

High corrosion resistance and biocompatibility of titanium implants is determined by the formation of a passivating surface layer of titanium oxides [25, 26].

Nevertheless, the biocorrosion of titanium fixators in long terms following the implantation was detected both by light and electron microscopy in a series of studies [2, 11, 19, 20, 27-31]. Ferguson *et al* [33] reported the ionization and release of the metal from the surface of titanium implants into surrounding soft tissues. This process is often accompanied by the changing of the peri-implant color of soft tissue into stable greyish [7], although the impregnation of small metal particles may be present and visible at the microscopic level even if there is no macroscopic change of the tissue color [33]. Larger metal grit can get into the tissues through existing surface defects that arise during the manufacture of the fixator as well as due to corrosion, surface contamination or mechanical damage while installing, removing or operating [34].

The main mechanisms behind the release of metal into the tissue are mechanical wear and corrosion. The processes of the release of metallic micro and nano particles as well as metal ions are accelerated when the protective oxide layer becomes thinner due to the plate bending, microcracking, damage to the surface of the plate or screw with a drill, screwdriver or other surgical instruments [25, 26]. According to A. Rosenberg (1993) [35], pigmentation of tissues due to metallosis was more pronounced around the curved sections of the plates. Friction in the plate-screw and plate-bone systems is another important factor involved in the degradationof the fixator surface and the occurence of small metallic inclusions in the tissues. However, the analysis of literature points to the lack of consensus as to the mechanisms of the metal release into the tissue, as well as the degree of titanium miniplates surface degradation in the long-term presence inside the human body [12]. Biological effects caused by the release of titanium into the tissues also remain poorly studied, and the results of the related research are often controversial.

It has been established that metal implants and products of their degradation can cause both local and general reactions of varying severity in the human body [10, 33, 36]. A number of publications suggest that titanium, which is believed to be a bio-inert material, has the potential to cause chronic inflammation and some immunological responses [3, 7, 9-11, 14, 19, 26, 27, 33]. Although clinical trials have not provided convincing evidence of significant damage caused by the continued preservation of titanium plates in the human body, titanium particles in the tissues are associated with the activation of monocytes and macrophages, the release of mediators of bone resorption, fibroblast stimulation, affected bone healing, hypersensitivity reactions, and impaired immune response [37]. Titanium can be "attacked" by several different types of immune cells, namely macrophages, histiocytes, giant cells of foreign bodies, lymphocytes and granulocytes [7, 11, 38] releasing active forms of oxygen and contributing to further degradation of the implant surface, which is usually very slow. A significant increase in titanium content in such internal organs as lungs, spleen, liver and kidneys following the experimental installation of titanium implants to the long bones and mandible was also reported [39, 40].

Intracellular location of titanium particles may be caused by phagocytosis [41], but in most cases they are extracellularly located and surrounded by fibrous connective tissue [2, 7] with no or moderate manifestations of a chronic inflammatory reaction [41].

It should be noted that titanium alloys used in maxillofacial surgery include vanadium and aluminum, which are significantly more toxic than titanium. Ions of vanadium affect lipid metabolism, have a cytotoxic effect on tissues and cause the destruction of some enzymes. The ions of aluminum suppress synthesis of ATP, therefore the high content can significantly reduce the metabolic activity of bone tissue and slow down mineralization. Aluminium also suppresses erythropoiesis and affects the central nervous system. The cellular toxicity caused by aluminum is associated with Alzheimer's disease, parkinsonism and osteomalacia [44]. Some studies reported the presence of aluminum both on the surface of titanium plates [28] and in soft tissues adjacent to them [2, 43]. However, the cumulative effects of small quantity of titanium alloy corrosion products still need the further investigations [45].

The tissue response to corrosion and release of metal particles into the surrounding tissue are the main arguments in favor of removing the metal miniplates after fracture healing [1, 26, 43]. According to the literature, the frequency of plates removal in patients after osteosynthesis is from 3 to 18% and more. In 22% plates are removed in absence of any complications, at patients' requests [17, 45, 46]. At the same time, the removal of fixators can present significant technical difficulties. It creates discomfort for the patient associated with the need for an additional surgery [15-18] the risks of which may exceed the positive effect, since scientific studies did not reveal a reliable relationship between the intensity of metallosis and manifestations of inflammation [35, 41, 44].

In addition, the severity of the metallosis is variable in different patients, and the factors affecting it remain underinvestigated. Obviously, the optimization of strategy for the removal of fixators in the remote postoperative period and prevention of negative effects associated with their installation requires an in-depth study of the mechanisms of the fixator interaction with biological tissues and understanding the processes which determine the release of metal particles from their surface into the surrounding biological environment.

The aim of the study was to investigate the microstructural changes on the surface of fixation elements (titanium plates and screws), and to determine the content and distribution of titanium and other chemical elements in adjacent soft tissues, as well as factors influencing these parameters in the long-term period following osteosynthesis of the facial bones.

Materials and Methods

Materials of the study included titanium fixators (plates and screws), removed in 12 patients in the long terms following osteosynthesis, as well as biopsy samples of the periosteum and fibrous capsule adjacent to the fixing titanium elements. All patients were treated in the Center of Maxillofacial Surgery and Stomatology in Kyiv Regional Hospital and gave their consents to participate in the study. The expertise of the research materials was conducted according to the approval (#106, November 07, 2017) of Bioethics Commission of Bogomolets National Medical University.

The average age of patients was 30 years, the ratio of men and women in the group was 2:1. All patients underwent the osteosynthesis of the facial bones (8 patients) or reconstructive surgeries on the jaws (4 patients) with the use of titanium fixators. The following types of fixators were used: I-Plant (Ukraine), Stryker (Kalamazoo, Michigan, USA), and Conmet (Moscow, Russia). All the fixators were made of medical titanium (Grade 4). The lenght of the period from installation to the removal of the fixator was from 5 months to 3 years (an average of 11.6 ± 11 months). The reasons for removing the fixators were: exposure of fixation elements (33.3%), removal of the fixator during the regular stages of reconstructive interventions in multi-stage surgical treatment (33.3%), patients' complaints of pain and discomfort in the fixator area (25%) and patient's requests (8.3%). Surgeries were performed according to standard protocols by use of intra-oral access in 91.6% of cases (in one patient an external access was used to remove the reconstructive plate). Information on the local status and patients' general health, the use of medicines, bad habits, working and everyday life conditions, peculiarities of primary surgical intervention, the course of the postoperative period, the clinical and radiological findings of treatment were transferred to the patient's database to analyse the factors related to the intensity of surface degradation and ion exchange between fixators and surrounding tissues.

When removing the fixator, surrounding soft tissues and the bone surface were carefully examined to detect macroscopic signs of metalosis and inflammatory reactions. The attention was paid to the stability of the fixator and the degree of the fixation elements integration with the surrounding bone. The periosteum or fibrous capsule adjacent to the fixation elements were removed and fixed in 10% formaldehyde solution. To determine the elemental composition of the removed soft tissue fragments in accordance with standard analytical techniques, a micro-X-ray fluorescence spectral analysis was carried out by micro-X-ray fluorescence spectrometer (model M4 TORNADO) manufactured by Bruker (Bremen, Germany). The objects of the study were placed in the working chamber of the spectrometer where pressure of 20 mbar was created by means of vacuum pump. The sample was translated into the focal plane using autofocus. The objects of the study (soft tissue biopsy) were exposed to the X-ray beam. Atoms passed into an excited status then emitted fluorescent radiation, which is unique for each element, its intensity was recorded by the detector. The source of X-ray radiation in the spectrometer was a microfocus X-ray tube with operating parameters as follows: voltage of 50 kV and current of 500 μA.

Scanning electron microscopy (SEM) by raster electron microscope JSM-6060 (JEOL, Japan), micron marker 100 micrometer (μm)-500μm, was used for detailed study of structural changes on the surface of titanium plates and screws. The removed fixators were carefully washed with 10% formaldehyde solution to remove the residual biological tissues, then they were degreased, washed in 96% alcohol, and dried in vacuo. Electron microscopy was carried out in different fields of view at magnification of 1:30 and acceleration voltage of 30 kilovolts (kV).

The obtained results were analized with the use of Spirman correlation coefficient, calculated by the IBM SPSS Statistics v.23 software.

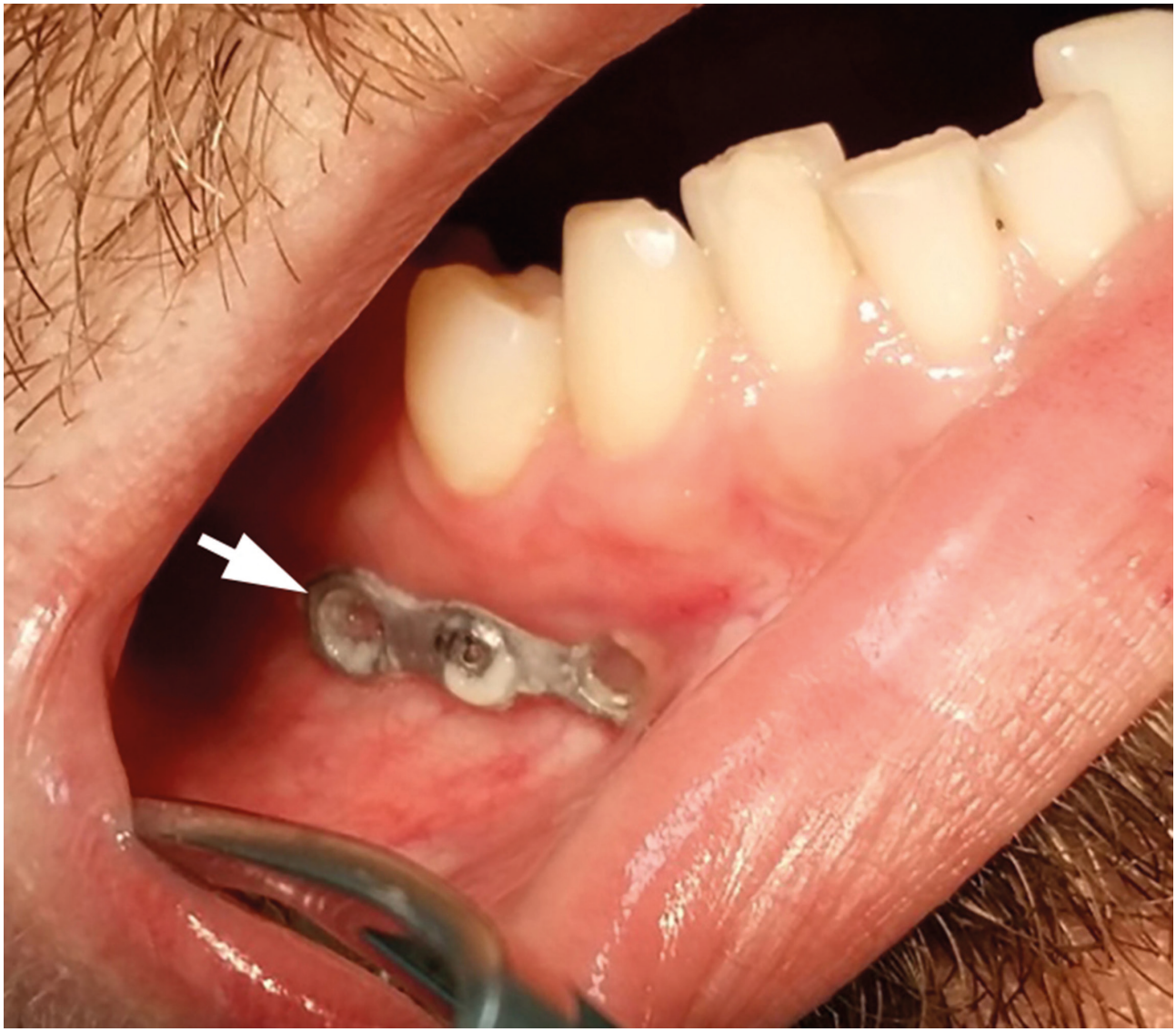


FIGURE 1. A 11.5-month follow-up photograph after mandibular fracture shows exposure of titanium miniplate (*arrow*) in the oral cavity without significant signs of chronic inflammation.

Results

At the removal of the fixation elements, macroscopic signs of chronic inflammation in adjacent soft tissues were noted in 1 (8.3%) patient. Exposure of fixators was noted in 4 (33.3%) patients (Fig 1). Local grey coloring was seen in 8 (66.6%) patients, predominantly in the area of the installed screws. In most observations, the loosening of at least one of the fixing screws was noted.

In all the cases, electronmicroscopy of fixation elements (plates and screws), which were removed in late terms after the installation, revealed signs of surface damage, including macrostructural ones such as deformation of the thread or bending of the screws (Fig 2), various deformations and surface defects of the plates (Fig 3), microcracking, sharp edges, and metal scratches, tongues and splinters. According to our data, the degradation of the surface of titanium fixators resulting from corrosion can hardly ever be determined. In some cases, on the surface of the fixators, there were small dimples which resembled the shells of corrosion, but their true nature was difficult to establish.

The study of soft tissues by X-ray fluorescence analysis of the scanning plane revealed the spectra of the following elements of the periodic table: phosphorus (P), sulfur (S), calcium (Ca), titanium (Ti), chromium (Cr), iron (ferrum) (Fe), nickel (Ni), copper (cuprum) (Cu), zinc (Zn), strontium (Sr), rhodium (Rh) (Table 1; Figs 4, 5). The applied method allowed not only detecting the presence of metals in the tissues, but also studying the features of the distribution. Thus, the presence of sites with an increased content of certain chemical elements in some cases was conditioned by the relief of the plate, contours of its holes, the turns of thread of the fixing screws (Figs 6, 7).

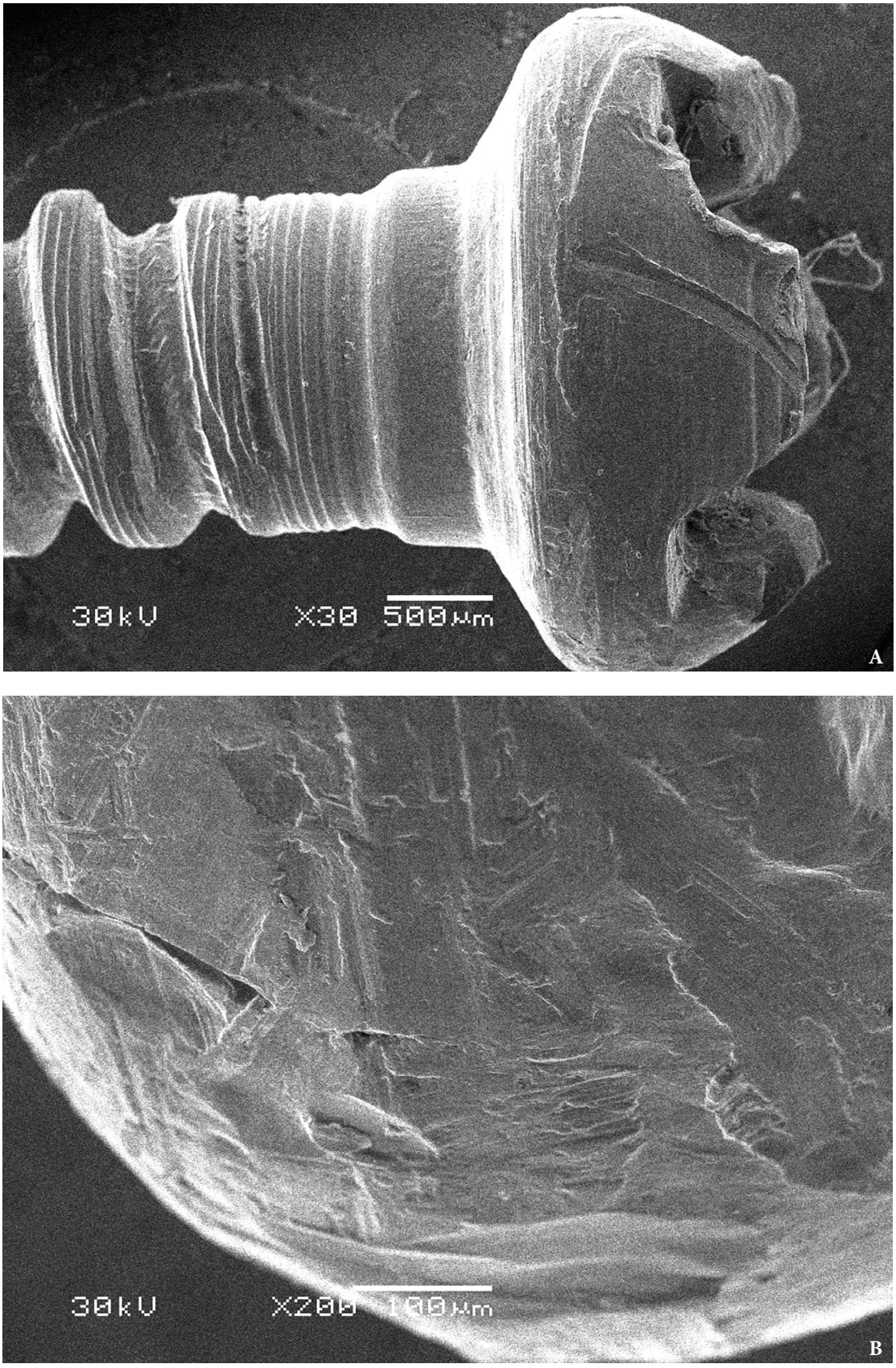


FIGURE 2. (**A**, **B**) SEM surface of the removed titanium screw in different maginifications (**A**: magnification, x 30; scale bar, 500 µm; voltage, 30 kV) (**B**: magnification, × 200; scale bar, 100 µm; voltage, 30 kV). There is seen deformation of screw threads and screw hinge, its bend, numerous defects of the surface, including microcracks, sharp edges, metal scratches, tonges and splinters.

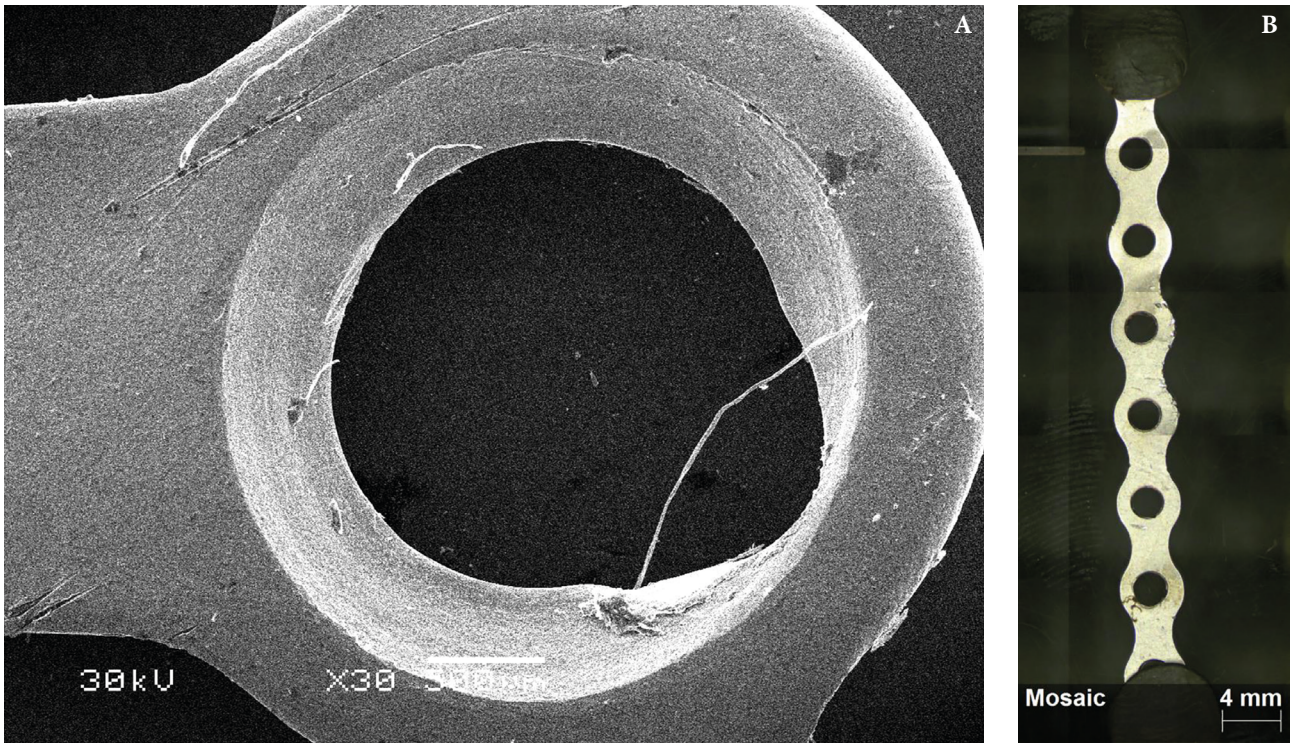


FIGURE 3. Appearance of the surface of the removed titanium miniplate at SEM (**A**: magnification, × 30; scale bar, 500 μm) and at optical magnification, × 10 (**B**; scale bar, 4 mm). The deformation of the screw hole, scratches, surface defects, microcracks, sharp edges, metal tonges, and splinters are seen.

TABLE 1. The obtained spectrum and concentration of chemical elements in the investigated areas. Mass percent (%)

Spectrum	P	S	Ca	Ti	Fe	Ni	Zn	Sr	Rh
Point 2	1.35	3.34	0.46	88.22	5.39	0.06	0.12	1.06	0.00
Point 1	0.40	0.68	0.17	97.29	1.29	0.04	0.01	0.10	0.00
Mean value	0.88	2.01	0.32	92.76	3.34	0.05	0.07	0.58	0.00
Sigma	0.67	1.88	0.20	6.42	2.90	0.01	0.08	0.68	0.00
Sigma mean	0.47	1.33	0.14	4.54	2.05	0.01	0.05	0.48	0.00

P – phosphorus; *S* – sulfur; *Ca* – calcium; *Ti* – titanium; *Cr* – chromium; *Fe* – ferrum (iron); *Ni* – nickel; *Cu* – cuprum (copper); *Zn* – zinc; *Sr* – strontium; *Rh* – rhodium

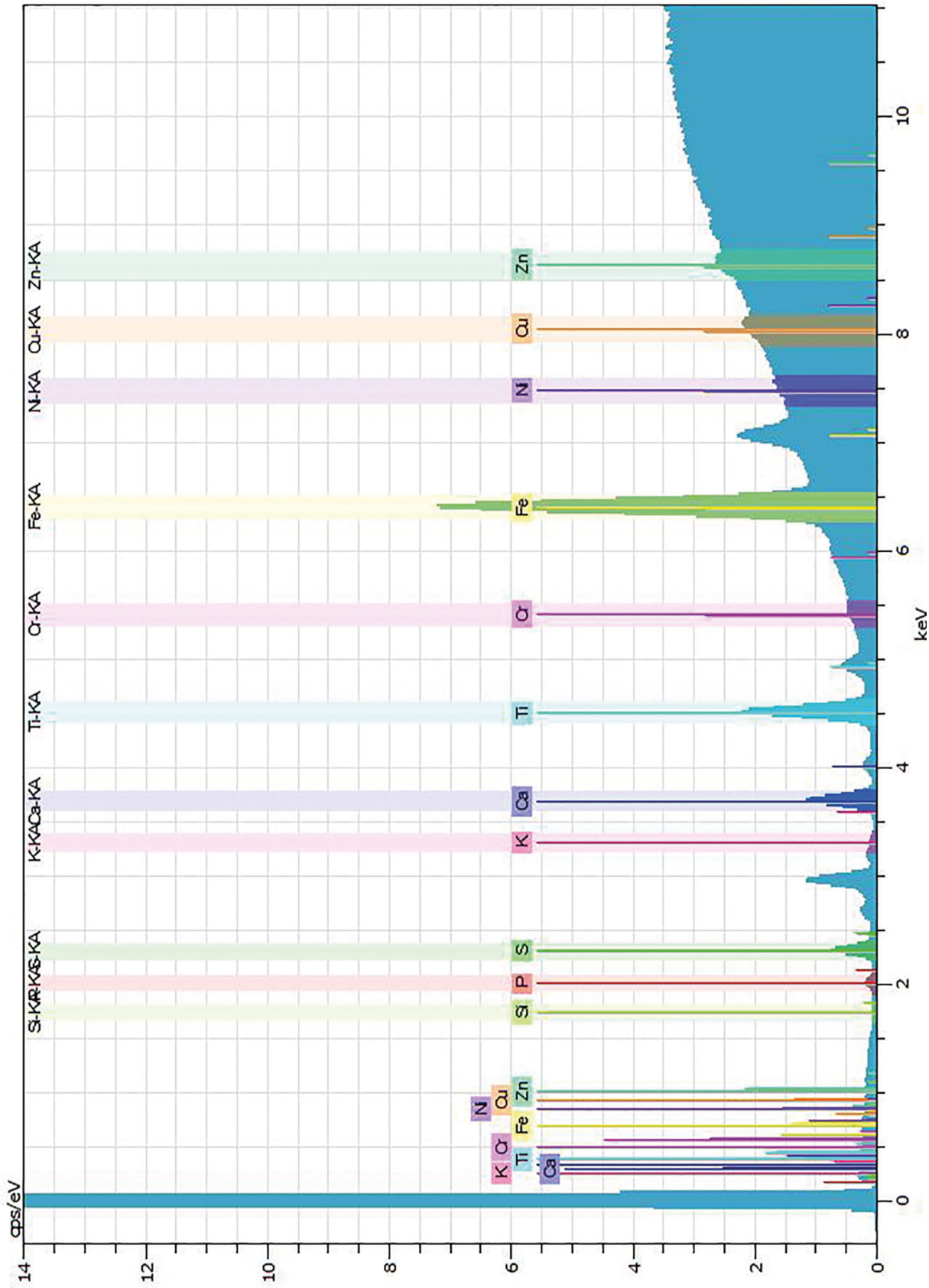


FIGURE 4. The obtained spectrum and concentration of chemical elements in the investigated areas (percentages by mass).

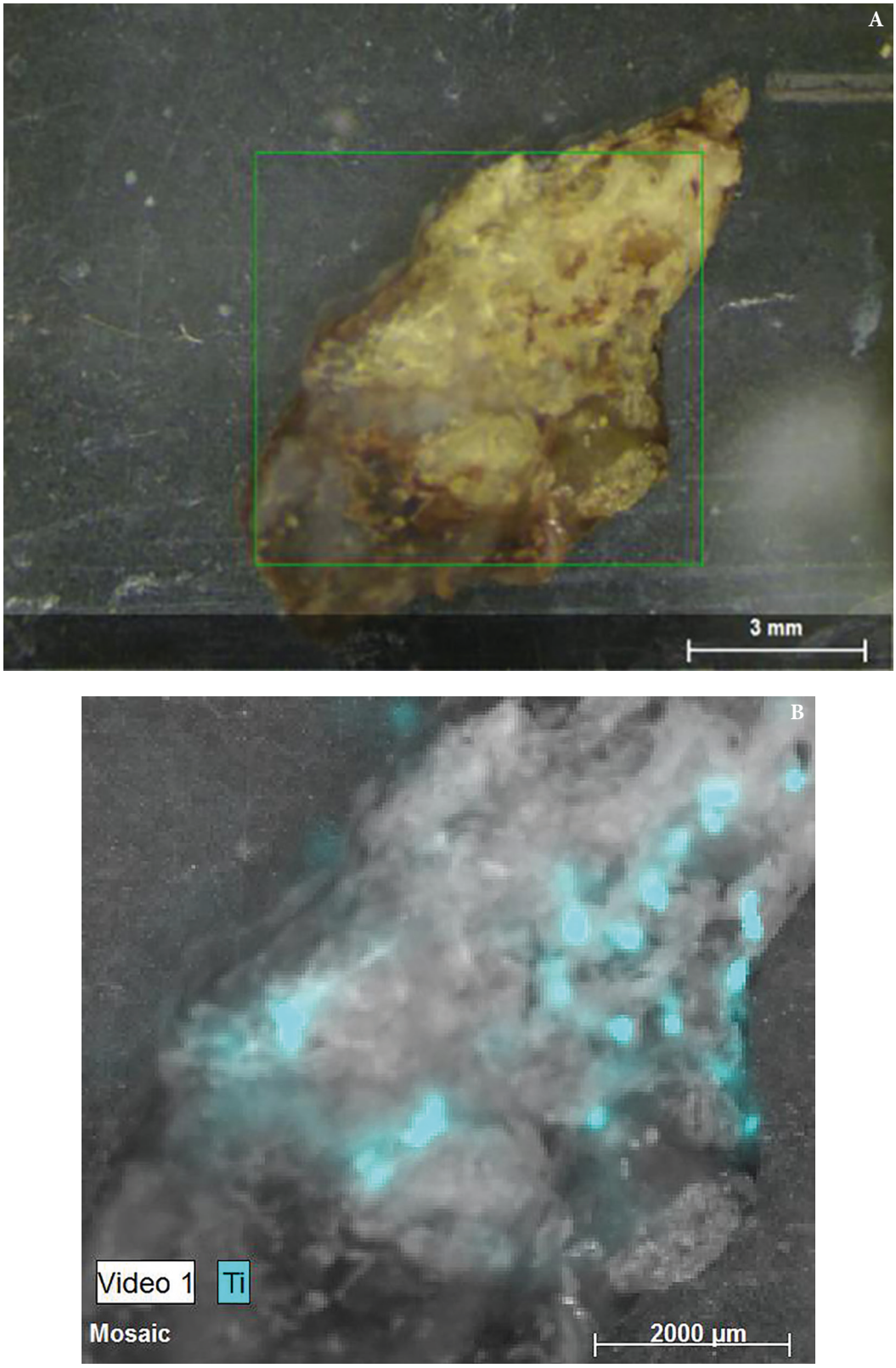


FIGURE 5. Analysis of the study of scanning area of biopsysample of soft tissue. (**A**) Area of scanning (scale bar, 3 mm.) (**B**) Distribution map P, S, Ca, Ti, Fe, Zn (scale bar, 2000 µm.)
(Fig 5 continued on next page.)

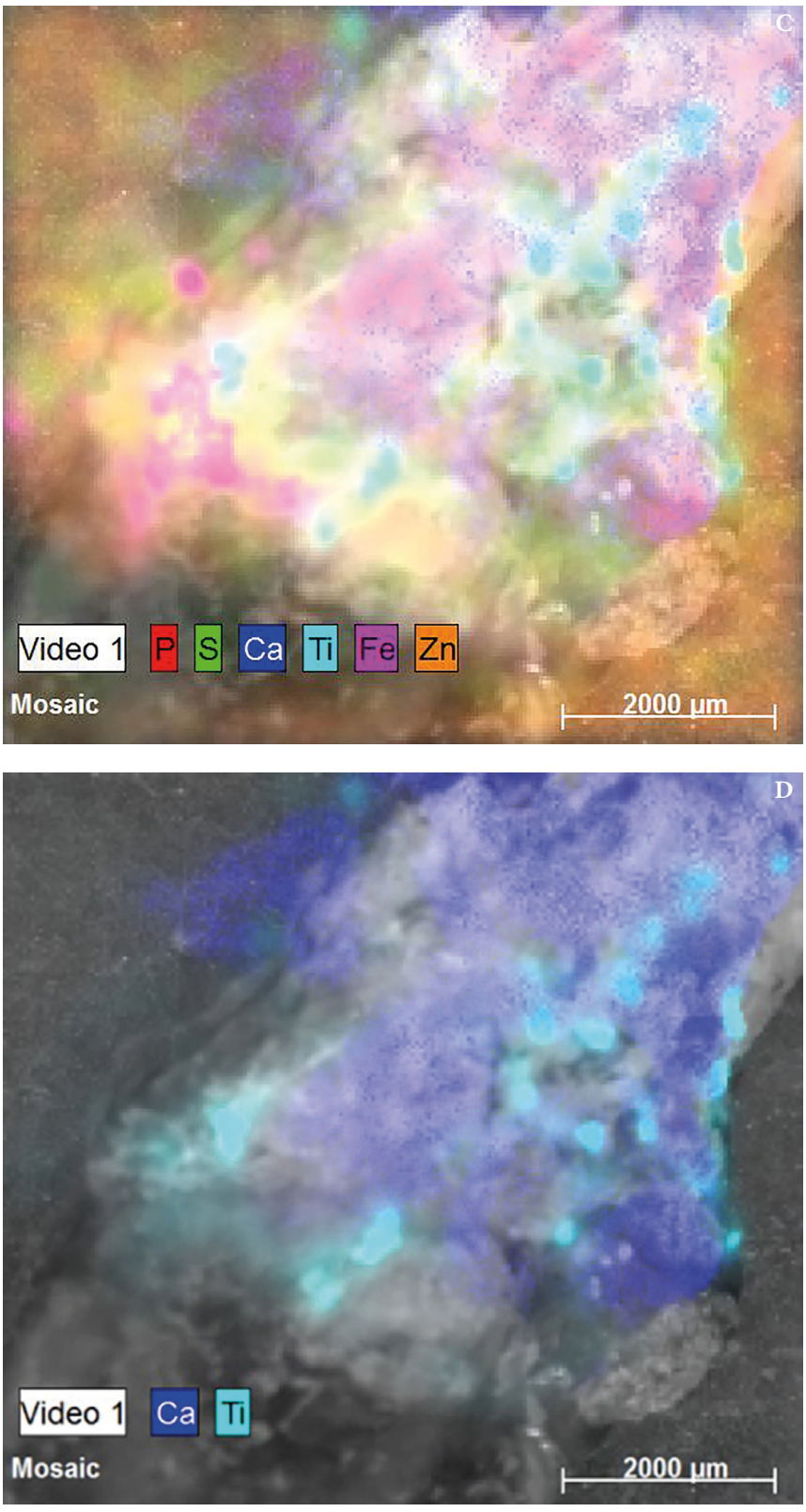


FIGURE 5. (cont'd). (**C**) Distribution map Ti on the scan area (other elements are hidden). (**D**) Map of Ca, Ti distribution on the scan area (scale bar, 2000 µm.)

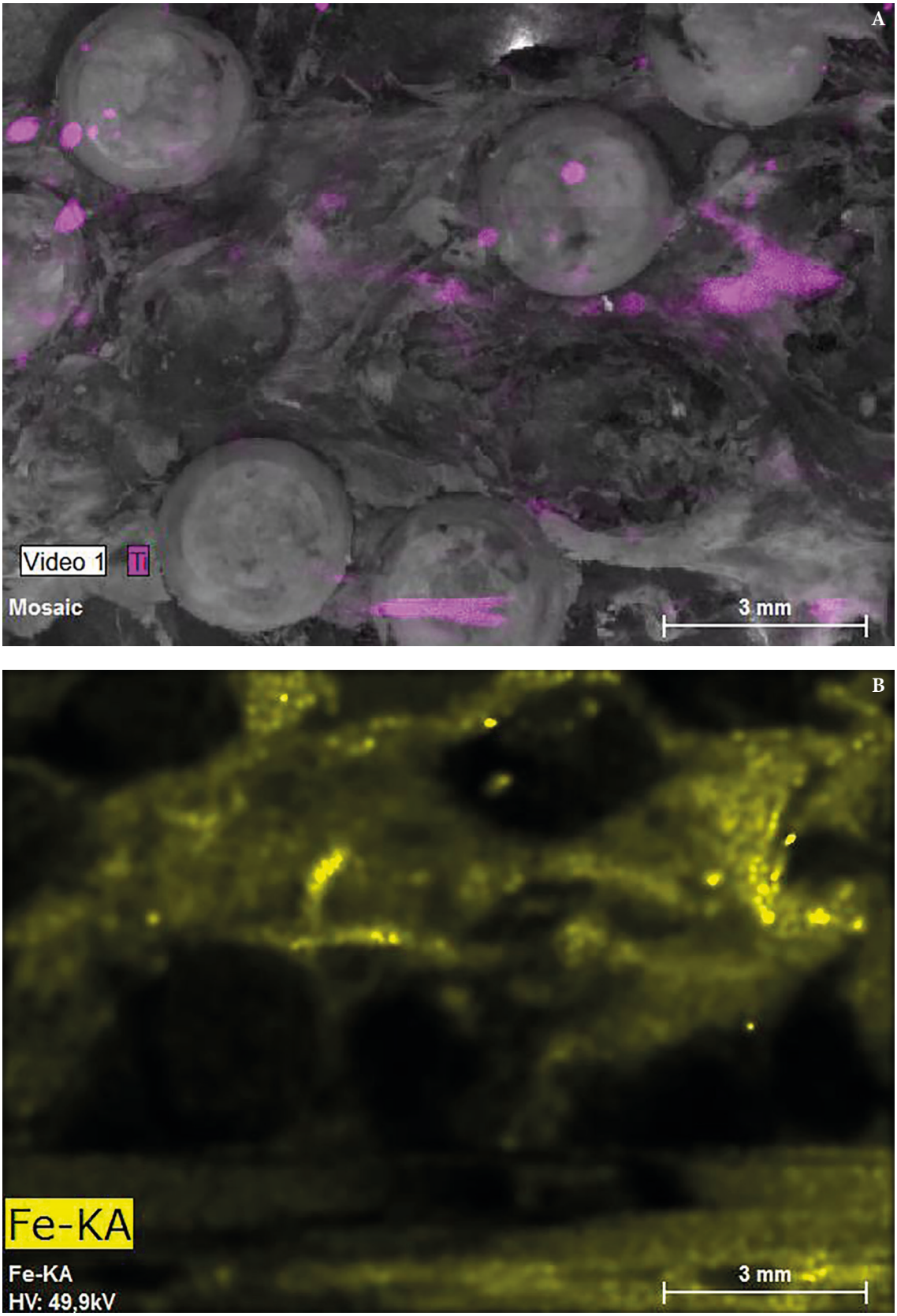
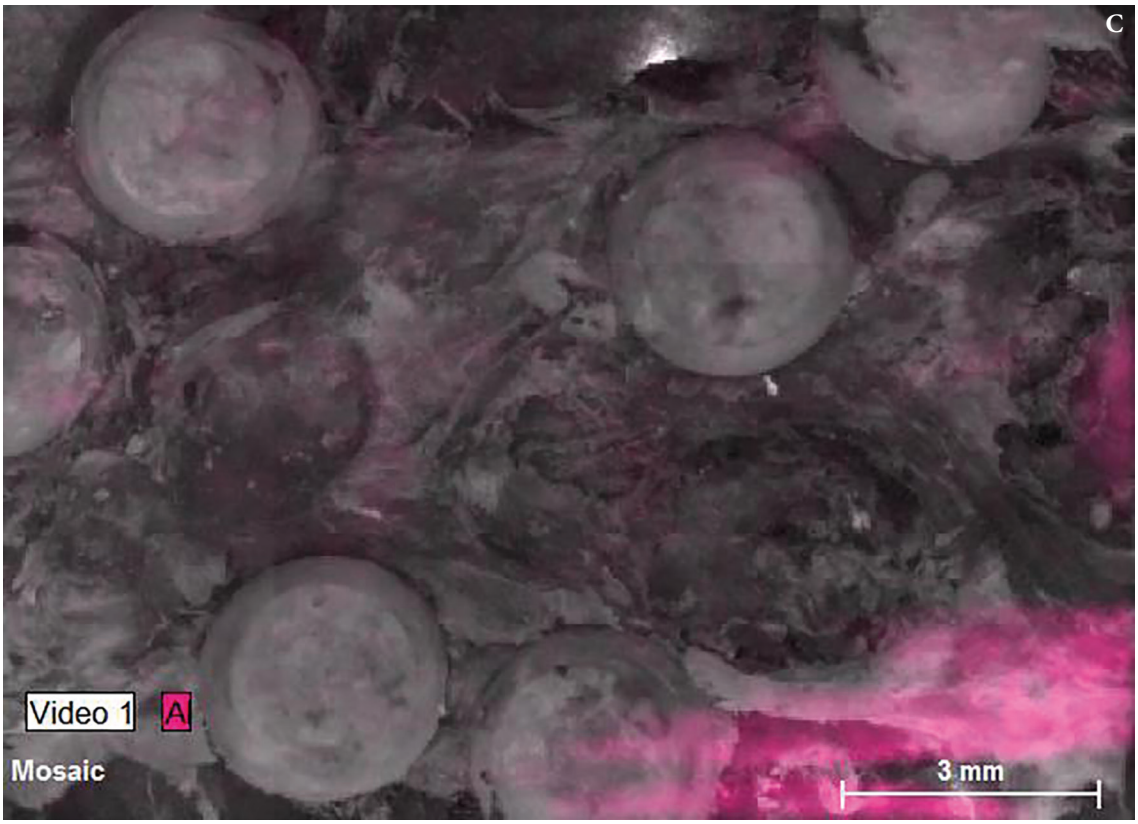


FIGURE 6. Ti (**A**), Fe (**B**), Al (**C**), and Ca (**D**) distribution maps on the scanning area of soft tissue adjacent to the removed titanium grid (two types of titanium distribution are noted: the first one is represented by clearly outlined intense inclusions sized 100 -800 μm and more and high titanium content (up to 90%); around these particles and in areas close to the fixator, there are poorly outlined diffuse inclusions of titanium (second type) with lower percentage content (**A**). The detected iron could be either of biological origin or it could get into the tissues from the surface of the surgical instruments used to install the fixators (**B**) (scale bar, 3 mm.) **(Fig 6 continued on next page.)**



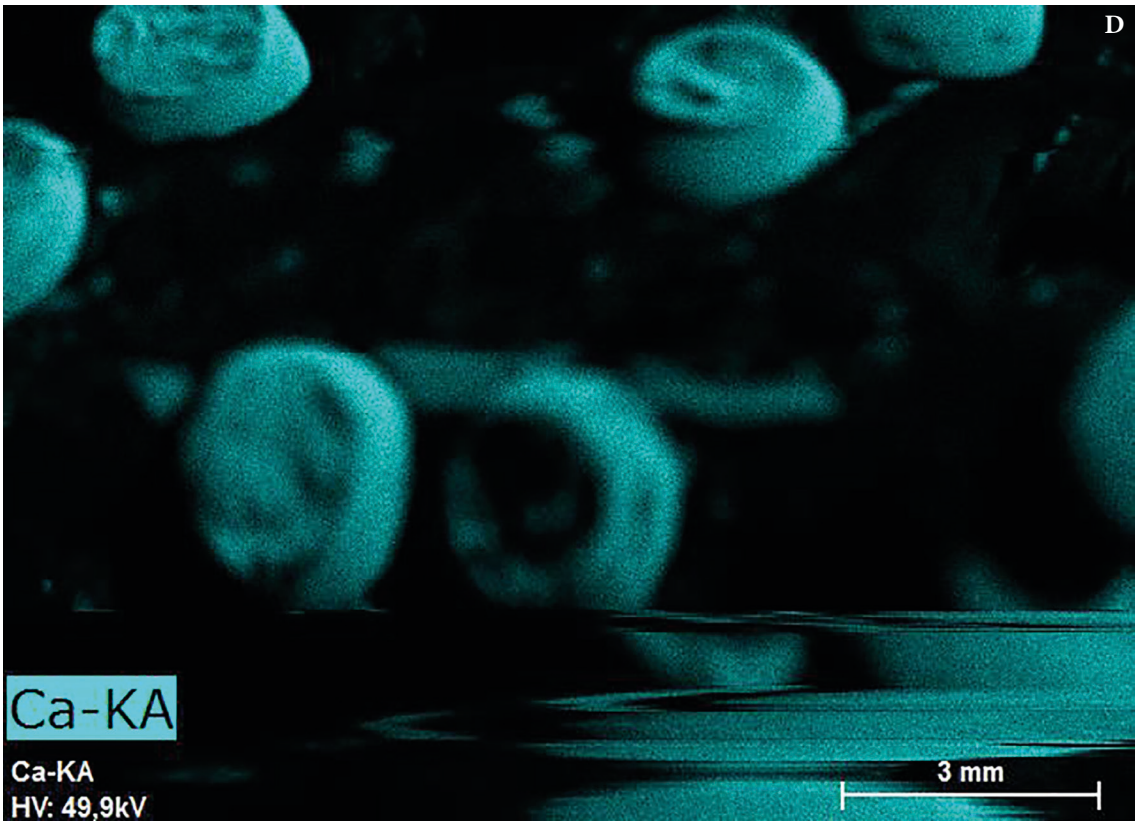


FIGURE 6. (cont'd). Al was detected in very small quantities and it was topographically linked with the sites of titanium deposition (**C**). The revealed Ca was unevenly distributed, its increased content was seen in areas of periosteal osteogenesis, including in the areas of free holes (**D**) (scale bar, 3 mm).

The received maps showed a quite uniform distribution of P, S, Cu and Zn which are normally present in large quantities in soft tissues. Ca was present in all the specimens studied, but the distribution parttens were nonuniform. An increase in its content was seen in sections of periosteal osteogenesis around the plate, including the area of its free openings (Fig 6D). Moderate amount of Fe was noted in all samples. The detected iron could be either of biological origin, due to its presence in hemoglobin, or it could get into the tissues from the surface of the surgical instruments used to install the fixators. Thus, in 41.6% of cases in some local sites along with high Fe deposits there were found Cr and Ni, which are constituents of medical steel (Fig 7). Small amount of Sr was seen in all observations, which is generally characteristic of this geographic area. In 3 (25%) cases, insignificant amount of Al (4.57 ± 5.13%) was detected which was topographically linked with areas of titanium deposition.

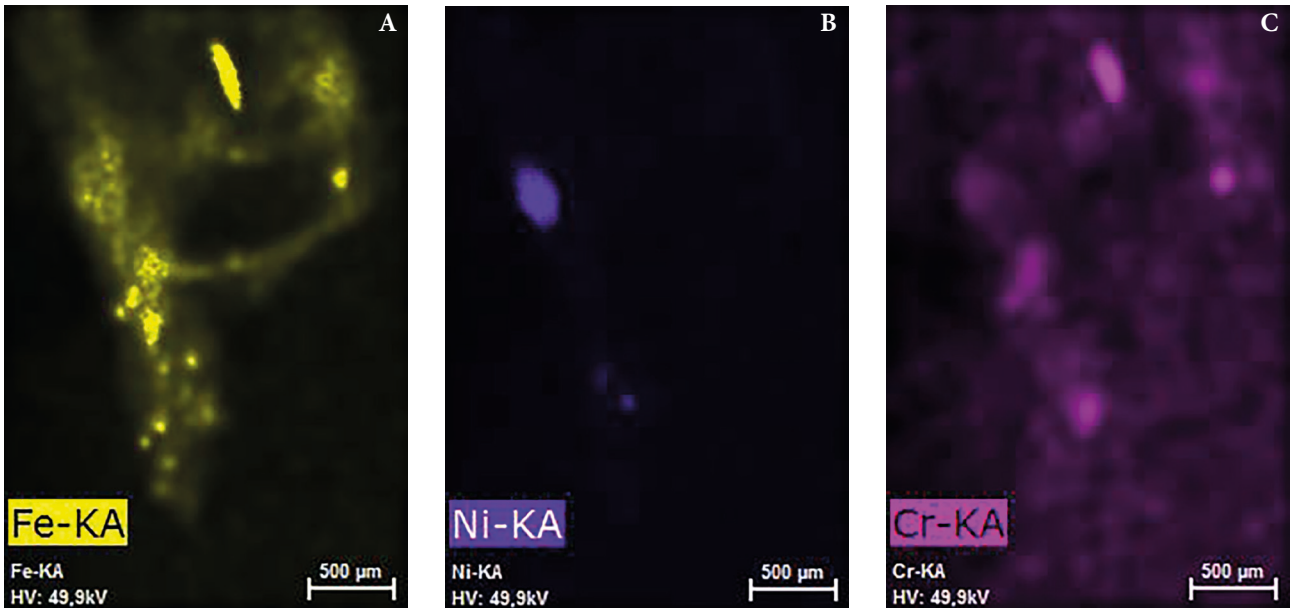


FIGURE 7. Fe, Ni, Cr distribution maps (**A-C**) in the area of scanning (scale bar, 500 µm) of soft tissues adjacent to the removed titanium screw. In some local sites along with high Fe deposits, there is seen Cr and Ni, which are constituents of medical steel.

The presence of Rh in the spectrum can be explained by the material of M4 TORNADO tube emitting continuous radiation and bremsstrahlung which affects the spectral background of the excited spectrum inclusion.

The average content of titanium of 48.14 ± 31.1% at sites of local deposition was detected in all the studied samples (Fig 8). Samples removed following osteosynthesis in patients with traumatic fractures of the facial skull bones, showed the average content of titanium at the sites of metal deposition being 55.6 ± 29.4%, whereas in the samples removed in patients with reconstructive and restorative interventions, where fixators had been less loaded, it was less (37.72 ± 30.2%). As can be seen from the above, although titanium alloys are considered bioinert and the ones which do not actually interact with the internal environment, the data suggest that titanium plates and screws on the surgical site eventually undergo active transformations resulting from physical and chemical processes. The latter can proceed more intensively if the fixation elements are exposed to a significant stress and deformation.

The acquired maps of the element distribution show uneven distribution of titanium with two types of titanium inclusions and different character of their distribution revealed in all cases. The first type is represented by clearly outlined intensive inclusion with dimensions of 100-800 µm and more and high titanium content (up to 90%). The presence of such inclusions can be explained by the separation of large debrises of fixing elements during the procedures through the contact between the moving drill and the hole of the plate or between the screw thread, plate and bone. Around these particles and in tissues adjacent to the fixators, there were also detected poorly demarcated, diffuse inclusions of titanium (second type) with a lower percentage of this chemical element, which probably resulted from surface corrosive degradation of the fixator.

The analysis of the obtained data revealed no significant impact of the titanium content in tissues on the development of inflammatory complications and exposure of the plate ($r = 0.465$, $p > 0.05$). The correlation between the content of titanium and the duration of the period while the plate remained in the human body ($r = 0.38$, $p > 0.05$), between the content of titanium and the type of plate ($r = 0.237$, $p > 0.05$) also turned out to be unsignificant.

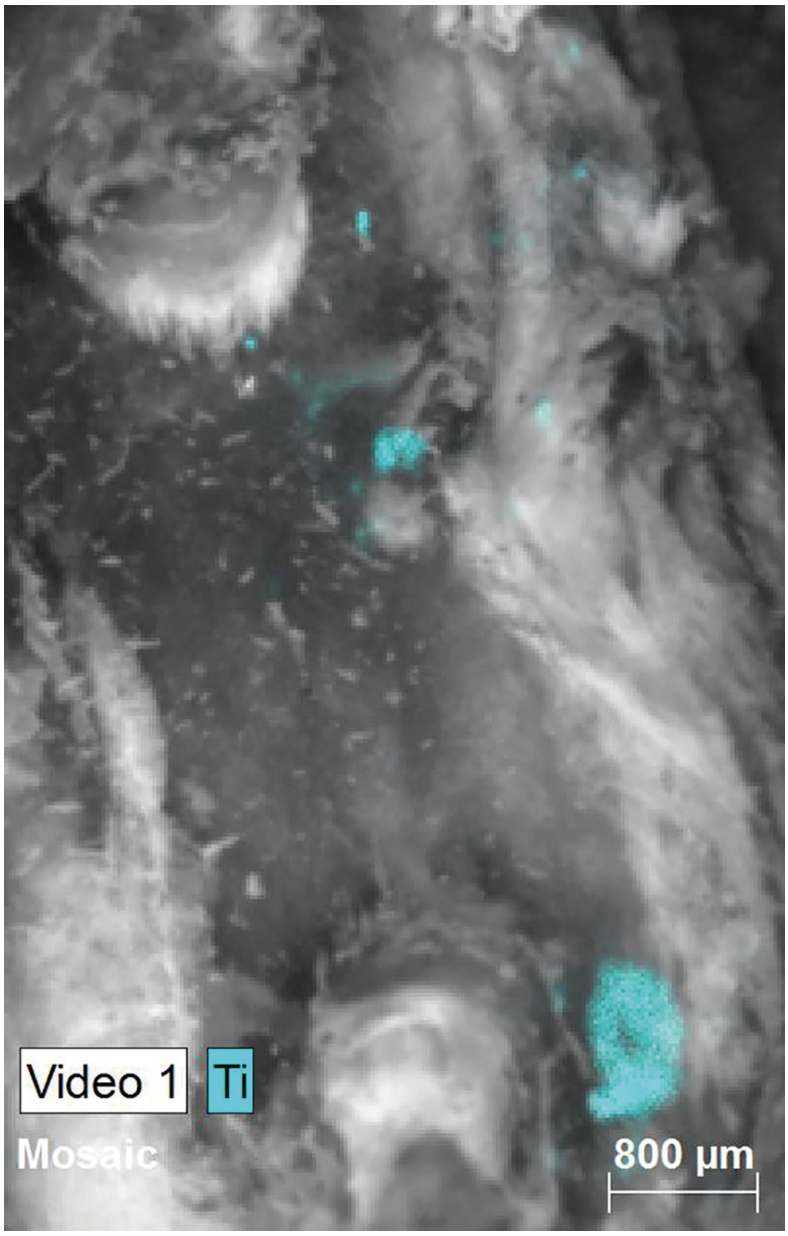


FIGURE 8. Ti distribution map. In the scan area of the removed fragment of the periosteum adjacent to the plate (magnification, × 10; scale bar, 800 µm), there are clearly limited intensive inclusions sized 100-800 µm with titanium content (*blue color*) of 92.76%.

Discussion

The study of mechanisms behind the interaction of titanium fixators with biological tissues during their prolonged presence in a human body is very important for defining strategy and indications for removing fixation elements, and for long-term prognosis of surgical interventions.

Numerous studies indicate that metal particles and ions can be released from the surface of the plates into the surrounding tissues, under the influence of mechanical, chemical and biological factors. According to our data, titanium inclusions in soft tissues adjacent to the fixation elements were found in all 100% of samples in terms of more than 5 months. Jonas *et al* [12], and Theologie-Lygidakis *et al* [47] reported somewhat lower figures: according to their research, titanium inclusions were detected in 20-68% of investigated biopsy samples by means of light, transient electron microscopy, X-ray microanalysis, or electron diffraction. Sections of biopsy specimens used by the authors were of different thicknesses following preliminary preparation. We associate discrepancies in the results obtained with the technical limitations of the research methods used by the authors [7, 11, 31, 38], in comparison with which X-ray fluorescence analysis has greater accuracy and informativity.

When analyzing the distribution of metals in samples of biological tissues, we also found two types of titanium inclusions that had different characteristics. More often, titanium was represented as intense, clearly otlined

inclusions (particles) sized from 100 to 800 μm and more. The content of titanium in these areas averaged 48%. In addition, around these particles and in areas adjacent to the plate, the diffusion of titanium inclusion was poorly outlined.

Based on light and electron microscopy findings, several authors also reported the presence of 2 types of titanium particles in soft tissues following a long-term implantation of miniplates: 1) colloidal particles located in histiocytes, fibroblasts or intercellular space, and 2) larger metal fragments [2, 7, 10, 11, 20, 27, 31, 38].

According to the researchers, larger particles of titanium [7, 28], resulted from the mechanical damage during the installation of fixation elements, including damage to the surface by surgical instruments, by a drill to form holes for fixing screws, titanium chipping while tightening these screws, and friction that occurs between the fixation elements under functional load conditions, especially due to insufficient stability of the 'fixator-bone' system [7, 28, 36]. Such a mechanism for the formation of large titanium particles is indirectly confirmed by the deposits of iron, chromium and nickel (which are the constituents of the medical steel used for manufacturing of surgical instruments) close to the large titanium inclusions in 41.6% of observations.

According to the authors, small colloidal particles of titanium are of different origin. It is believed that they are likely to arise as a result of the titanium biocorrosion [7, 19, 26, 38].

The mechanism of osteosynthesis devices corrosion is complex and probably includes four main components: depassivating, fretting, galvanic component and exposure to local factors of surrounding biological environment [48, 49]. Titanium plates and screws exhibit high corrosion resistance in the presence of a surface oxide layer that is chemically inert. The loss of this layer under the exposure to mechanical, chemical and biological factors (depassivation) results in a partial dissolution and degradation of the titanium surface that occurs intensively in the presence of reactive oxygen forms and electrolytic (electro-chemical) processes [12, 25]. The protective oxide layer on the surface of the plates quickly restores, except for the conditions when the 'fixator-bone' system is not sufficiently stabile and its elements are exposed to constant friction during repeated masticatory and non-masticatory movements.

To understand which of the mechanisms for the release of titanium into tissues is more important, it is significant to study the surface of the fixators removed at different terms following osteosynthesis. When conducting this study on the surface of all removed fixators we found such signs of mechanical damage as scratches, microcracks, surface defects, dimples, sharp edges, metal tongues and splinters that may have occurred during the manufacturing, installation, operation, and removal of the fixator. The above defects are a likely source of titanium fragments in the tissue adjacent to the fixator. In the case of infection or exposure of the plate, they act as retention points for the fixation of microorganisms and the formation of biofilms responsible for the development of chronic inflammatory processes and they are the main reason for the removal of fixators [21, 34]. No defects that could be uniquely qualified as signs of corrosive degradation were seen on the surface of the fixators. In few observations, we noted minor single surface defects of rounded shape similar to the corrosion shells occurring on the surface of steel structures. Such defects could have occurred during manufacturing of plates as evidenced by the studies conducted by Acero *et al* (1990) [50], Torgersen and Gjerdet (1994) [51].

Langford [34] reported similar findings resulted from the analysis of surface changes of removed plates and screws during an observation period of up to 13 years following osteosynthesis of the facial bones. He notes that surgical procedures and defects in the production of titanium miniplates were likely to be the main source of metal particle release into the tissue. In his study, no evidence was found to confirm that titanium miniplates installed for osteosynthesis of the facial bones should be routinely removed due to corrosion [34].

Interestingly, the signs of diffusion of small colloidal titanium inclusions associated with the corrosion process were observed mainly around large particles (fragments) of the metal. Probably the particles arising from mechanical damage and defects in the surface of the plate deprived of protective oxide layer are the main source of corrosive release of metal ions into the tissue due to increased surface area, depassivation, and capability of triggering cellular and tissue responses. The degradation in this regard may occur more intensively than the destruction of the fixator surface contacting with biological tissues. In favor of this hypothesis, French (1984) [8] shows that the formation of metal particles significantly increases the surface area available for the oxidation and release of ions into biological tissues. Jonas observed the initial signs of surface degradation of titanium alloys in the areas of fixator damage and believed that damage caused by the procedure for plate installing was the starting point for biocorrosion. Similar results were reported in other studies [15, 35, 41]. According to French [8], even in stainless steel fixators, reliable signs of corrosion were noted only on a small area of the screw-plate contact. The researcher did not find the link between the severity of corrosion and the duration of period when the fixator remained in the human body. In his opinion, it is indicative of the fact that the most intensive processes of corrosion proceed immediately following the installation the fixator, then they slow down and almost stop.

Our study found no significant correlations between the content of titanium in the tissues and the time the plate remained in the human body, either. In addition, the release of metal particles did not seem to depend on the manufacturer or the type of plate used.

The biological significance of the biodegradation

of metal fixators with the release of metal particles into tissues and the related potential risks are the subjects of discussion. According to Rae (1986) [52], metal particles of 1 to 10 μm are capable of activating monocytes and macrophages *in vitro*, and they also stimulate the release of mediators of bone resorption, prostaglandin E2 and interleukin-1, directly stimulate fibroblasts, and increase the synthesis of collagen. This determines their potential capability of causing inflammatory reactions. In addition, according to a number of researchers, interleukin-1, a potent bone resorbing agent, may be responsible for the loosening and loss of screws in the absence of infectious suppurative and inflammatory complications.

However, numerous studies of biopsy samples of soft tissue adjacent to the fixator, in the overwhelming majority of cases, revealed only minimal or poorly marked signs of chronic inflammation with minor lymphocyte-macrophage infiltration, less often with granular formation and small focal areas of necrosis. Such a tissue response was seen only in the presence of metallic inclusions in the tissues and was topographically related to them. At the same time, French [8] reported about a time-related decrease in the severity of the inflammatory tissue response in cases the fixator remained in the body for a prolonged period. The response did not depend on the degree of metallosis.

We did not find a significant correlation between the content of titanium in the tissues and the clinical manifestations of inflammation in the area adjacent to the fixator, either. Such manifestations were mainly conditioned by the occurrence of an infection, exposure of the plate and biomechanical characteristics of the system (instability, loosening, and loss of screws).

Literature review shows that to date, there is no convincing clinical evidence of titanium fixators contribution in the occurrence of inflammatory reactions in the surrounding soft tissues due to corrosion and the release of metal particles, the capability of aggravating the remote prognosis of surgical interventions and causing harm to the patient's health. Hypothetically, corrosion and mechanical damage to titanium fixators made of alloys containing vanadium and aluminum (metals whose toxicity is proved), can lead to their release into the tissue. The effect of these toxic components was studied only in isolated studies, which did not confirm the crucial role of aluminum and vanadium in the occurrence of inflammatory reactions in tissues adjacent to the titanium miniplates. According to our data, a very little amount of aluminum (4.57 ± 5.13%) was detected in the tissues adjacent to the fixator only in 25% of cases. There was no evidence of the presence of vanadium in biopsy samples.

Another potential risk is associated with the capability of titanium depositing not only in the tissues adjacent to the fixator, but also in tissues and organs distant from the site of osteosynthesis. Onodera *et al* (1993) [53] identified titanium particles in the submandibular lymph nodes of the patient 2 years after the reconstructive plate was installed on the mandible. Bessho *et al* (1993) [19] showed that titanium released from the miniplates can enter the vascular system and spread from the implantation site to distant organs. Biological effects of titanium in this case are practically uninvestigated.

The insight into the uncertainty about the long-term side effects of metal plates was provided in the recommendation of the Strasbourg Osteosynthesis Research Group (S.O.R.G.) in 1991, which concluded that the removal of non-functional titanium miniplates is desirable, provided that the procedure does not cause a significant risk to the patient. However, based on a survey of a significant number of maxillofacial surgeons, Matthew and Frame (1999) [41] found that miniplates and screws are not routinely removed. The decision to remove miniplastes in the maxillofacial area is taken in the presence of complications, certain clinical symptoms or at the patient's insistence [34]. In these conditions, the role of measures aimed at reducing the penetration of metal particles in the tissue and the associated negative effects significantly increases.

So, our research suggests that titanium fixators interact with the surrounding biological environment. This is accompanied by the release of metal particles into adjacent tissues, which was observed in all investigated samples. The main mechanisms involved into release of titanium into the adjacent biological tissues are corrosion and mechanical damage to the surface of the fixator by surgical instruments, drills, etc. during its installation, the contact of the plate and the thread of the fixing screws when they are screwed in, the friction of the elements in fixator-bone system, especially with insufficient stability of osteosynthesis, loosening of screws, plastic deformation of plates. According to our data, the degradation of the surface of titanium fixators due to corrosion can hardly ever be determined. Biocorrosion occurs mainly around small particles (debrises) of titanium and in areas of mechanical damage to the surface deprived of a protective oxide layer therefore more exposed to the chemical and biological factors of the environment. The main way to reduce titanium penetration into surrounding tissues is to minimize mechanical damage to the plate during its installation and operation. This implies, in particular, to follow the manufacturer's protocol for fixator installation, to avoid plate bending in the wound and the contact of the plate with the drill while making holes in the bone, to use titanium or ceramic drills, to install screws perpendicular to the surface of the plate, not at an angle to it, to employ surgical techniques and fixators that ensure the functional stability of the 'fixator-bone' system and minimize friction between its elements, to avoid conditions under which the plate is exposed to plastic deformation and destruction at the micro and macro levels in the process of functioning. Given the potential risk of the release of toxic impurities from titanium alloys into tissues, it is reasonable to search and develop new materials and alloys with improved biological and mechanical properties.

Conclusions

1 Following osteosynthesis and reconstructive interventions on the facial bones, titanium miniplates and screws interact with the surrounding biological environment which results in the release of metal particles into the adjacent tissues observed in all 100% of the studied biopsy samples wthin the time periods from 5 months to 3 years.

2. The main mechanisms involved in titanium release into surrounding tissues are corrosion and mechanical damage to the surface of the fixator by surgical instruments during its installation, the contact of the plate and the thread of the fixing screws when they are screwed in, and the friction of the elements in 'fixator-bone' system under functional load. In this case, the biocorrosion is of lesser importance and it occurs predominantly around tiny particles (debrises) of titanium and on sites of mechanical damage to the surface of the fixator, which loose the protective oxide layer and became exposed to the chemical and biological influences of the environment.
3. The distribution of metals in samples of biological tissues was characterized by the presence of two types of titanium inclusions that had different characteristics. More often, titanium was detected as intense, clearly outlined inclusions sized from 100 to 800 μm, with a high content of titanium (an average of 48.1 ± 31%) resulted from mechanical damage to the fixation elements during their installation. In addition, around these particles and in areas adjacent to the plate, there were also detected poorly outlined diffuse inclusions of titanium, where its content was lower.
4. There was no significant correlation between the content of titanium in tissues and the time the plate remained in the human body ($r = 0.38$, $p > 0.05$), between the content of titanium and the development of inflammatory complications or exposure of the plate ($r = 0.465$, $p > 0.05$), between the titanium content and the type of plate used ($r = 0.237$, $p > 0.05$).
5. The main approach to reduce titanium penetration into surrounding tissues is to minimize mechanical damage to the plate during its installation and functioning. This implies, in particular, to follow the protocol for fixator installation, to avoid plate bending in the wound and the contact of the plate with the drill while making holes in the bone, to install screws perpendicular to the surface of the plate, not at an angle to it, toemploy surgical techniques and fixators that ensure the functional stability of the 'fixator-bone' system and minimize friction between its elements, to avoid conditions under which the plate is exposed to plastic deformation and destruction at the micro and macro levels, and to use alloys with improved biological and mechanical properties.

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Conflict of Interests

The authors declare that they have no conflict of interest.

Role of Author

The authors are equaly contributed to that article.

Ethical Approval

The study protocol (#106, November 07, 2017) was approved by the Bioethics Commission of the Bogomolets National Medical University, Kyiv, Ukraine.

Patient Consent

Not required.

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