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FEATURES OF USING ORTHOPEDIC IMPLANTS ON AN EXPERIMENTAL MODEL OF SENSITIZATION TO NICKEL (NI)

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Summary

Introduction. The theme of metal hypersensitivity (MHS) reactions is among the most controversial and complex issues in orthopedic and trauma practice. MHS diagnoses are diagnostically challenging because they are exclusionary diagnoses. It is currently uncertain which biomarkers can reliably predict a potential pathological response to implants.
The aim of this research is to investigate hypersensitivity reactions to orthopedic implants containing nickel (Ni).
Materials and methods. This research was conducted on female Wistar rats in accordance with the standards of bioethical principles. To obtain conclusive results, the animals were divided into two experimental groups: with prior sensitization to Freund's adjuvant containing Ni and without it. Nickel plates with a total surface area of 24 mm2 were implanted in the rats under general anesthesia. Five months after the intervention, the animals were removed from the experiment, and histological examination of the obtained samples was conducted. Extracted implants were examined using electron microscopy to assess the implant surface. The local elemental composition of the implants was analyzed using an energy-dispersive spectrometer.
Results. Under the experimental conditions, it was demonstrated that after 5 months following the operation, a dense connective tissue capsule with an inflammatory infiltrate in the capsule lumen had formed around the implants in the rats. This suggests a possible manifestation of hypersensitivity reactions to implants containing Ni. Electron microscopy of the surface of the extracted implants revealed corrosion phenomena in all the samples. The degree of corrosion was more pronounced in the group of animals with prior sensitization to Ni, and distant particles of Ni were detected, which can be characterized as the beginning of implant degradation.
Conclusions. A connective tissue capsule forms around the implants, and it was found to be 34.8 % denser in animals sensitized to Ni prior to implantation, which may indicate tissue reactions with signs of hypersensitivity. Further research will provide a deeper understanding of the fundamental inflammatory and immunological reactions to metals present in implants. This, in turn, will facilitate the identification of clinically useful applications necessary for the development of diagnostic or prognostic tests for patients with metal implants.

Key words: **implant, nickel, experiment, immune response, sensitization, metal hypersensitivity reaction, animal model, biocompatibility, rats**

INTRODUCTION

The theme of metal hypersensitivity (MHS) reactions is one of the most controversial and complex issues in orthopedic and trauma practice. Currently, there is a lack of reliable diagnostic or screening tools to assess the full spectrum of patient reactions to metal implants. Most metals present in implants are immunologically active and can lead to allergic reactions [1-3]. Hypersensitivity reactions are a function of the

adaptive immune system. According to the classification of Gell and Coomb's, they are divided into Type I, Type II (antibody-mediated), Type III, and Type IV (cell-mediated, delayed) reactions [4].

The majority of MHSs in orthopedic implants are of Type IV, delayed hypersensitivity reactions. Considering the high percentage of metal hypersensitivity (MHS) reactions, especially to nickel (Ni), in the general population, and the presence of this specific substance in standard implants [5-9], it can be critically important to assess a patient's hypersensitivity before surgical intervention to prevent reactions. General manifestations of MHS reactions can include one or multiple symptoms, such as contact dermatitis, vasculitis, urticaria, erythema, as well as postoperative wound healing disruptions, pseudo-infection (an imitation of infection), swelling, synovitis, pain, stiffness, or limited mobility, and aseptic implant instability. Therefore, MHS reactions to implants present diagnostic and therapeutic challenges [6, 10-11].

The absence of general treatment recommendations leads to an individualized approach to treatment, potentially resulting in inconsistency, which, in turn, complicates the development of guidelines [11].

Metal hypersensitivity (MHS) reactions are diagnostically challenging because they are exclusionary diagnoses. It is currently uncertain which biomarkers can reliably predict a potential pathological reaction to implants [12]. In any case, before testing for a reaction to a metal-containing implant, more common factors, including infection, implant instability, implant wear, and/or fracture, should be ruled out [11].

Currently, there are a limited number of approaches to predict or diagnose adverse reactions to metals. The most common diagnostic tests include patch testing and lymphocyte transformation testing; however, there is no clear guidance on how to use these tests in clinical settings.

The number of patients with total joint arthroplasty with positive tests for MHS reactions has exponentially increased over the past 20 years [13]. As the number of joint replacements and the use of orthopedic implants continue to rise, it is expected that the number of complications related to implant reactions will also increase [14, 15]. According to estimates, approximately 11 million people will be living with total hip or knee joint replacements by 2030 [16]. Therefore, evaluating MHS reactions in orthopedic implants has become an increasingly relevant issue in modern implantology.

The existing gaps and shortcomings underscore the need for new, clinically significant diagnostic and prognostic tests before implantation to determine the likelihood of an allergic response caused by the implant and to adequately assess the full spectrum of possible reactions after implantation.

THE AIM OF THE STUDY

The aim of this research is to investigate hypersensitivity reactions to orthopedic implants containing nickel (Ni).

MATERIALS AND METHODS

Experiments on animals were conducted in the vivarium of the O. V. Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine in accordance with the Law of Ukraine «On the Protection of Animals from Cruelty» (No. 3447-IV, dated February 21, 2006), adhering to the requirements of the European Parliament and Council (2010) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) [17-19].

The research was conducted on 25 female Wistar rats, weighing between 150-170 grams, following the standards of bioethical principles. The animals were divided into two experimental groups:

Group 1 – Control (n=6) – without prior sensitization to Ni and with the implantation of a Ni-containing implant.

Group 2 – Experimental (n=13) – with prior sensitization to Ni and the implantation of a Ni-containing implant.

Sensitization to Ni was induced using a modification of the described method [20]. To achieve this, animals were intraperitoneally (i/p) injected with 50 µl of a 10 µmol/ml solution of NiSO4 (CAS 10101-97-0, Sigma-Aldrich, USA) in incomplete Freund's adjuvant (Sigma-Aldrich, USA) for the initial immunization. Booster doses of Ni were administered to the rats via intradermal (i/d) injections of 50 µl of a 2 µmol/ml NiSO4 solution in complete Freund's adjuvant (Sigma-Aldrich, USA) using 28G1/2 needles to boost the immune response at 2 and 4 weeks. Skin tests for Ni were conducted on the animals 3 days later, following the methodology described [21, 22]. For this, 0.01-0.02 ml of the Ni solution in physiological saline (0.9 % NaCl solution) was introduced into the ear pinna, using a 30G needle, while an equivalent volume of 0.9 % NaCl solution was used as a control. The animals were observed for 10-15 minutes for their reaction to the administered solution.

Ten days after the third immunization, sensitization to Ni was assessed using an «ear test». To do this, rats were intradermally injected with 50 µl of a 1 µmol solution of NiSO4 into the ear pinna using a 30G needle. For the control, 50 µl of a 0.9 % NaCl solution was injected into the ear pinna of the other ear. After 48 hours following the injections, delayed hypersensitivity reactions were determined by measuring the increase in ear thickness

compared to the value after the injection of the 0.9 % NaCl solution. Measurements were taken using a micrometer.

After the last injection of Freund's adjuvant in rats sensitized to Ni, surgical intervention was performed to implant Ni-containing plates.

The implants used for the study were generously provided by the LLC «ORTOSINTEZ» (Kyiv, Ukraine). All samples had previously undergone spectroscopy to assess the local elemental composition in the study material. (See Figure 1 and Table 1).

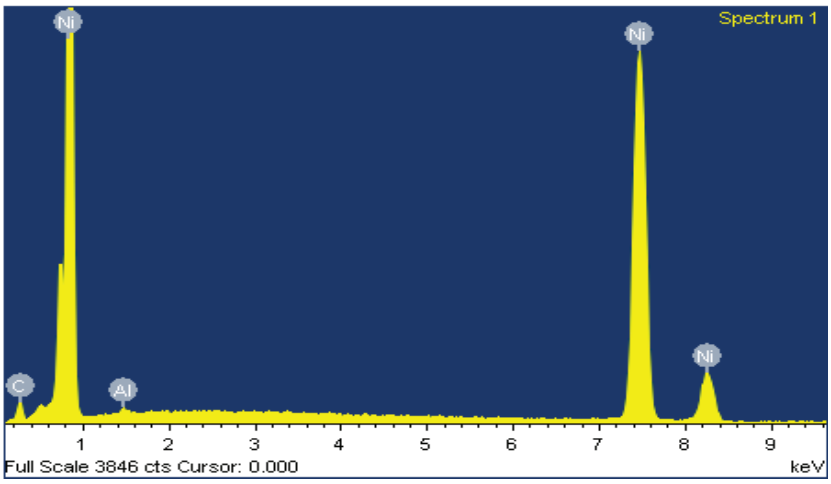


Fig. 1. Spectroscopy data regarding the elemental content in the studied implants

Table 1

Elemental composition of the used implants

Elements	Al	C	Ni
Percentage of elements	0,40 %	0,70 %	98,90 %

The surgical procedures were performed under aseptic and antiseptic conditions, with general anesthesia induced by intraperitoneal injection of sodium thiopental at a dose of 50 mg/kg of body weight. Using a posterior midline approach along the vertebral line, the skin was incised, and a subfascial pocket was formed in the interscapular area. A Ni-containing plate measuring 6.0 x 4.0 x 1.0 mm was implanted into the pocket. After the surgical procedure, the wound was closed in layers with Prolene 3-0 sutures (Johnson & Johnson, USA) and treated with the antiseptic agent Povidone-iodine (Betadine, Hungary). To prevent bacterial infections, the animals were administered the antibiotic Ceftriaxone (Arterium, Ukraine) at a dose of 20 mg/kg of body weight via intraperitoneal injection.

The animals were kept in separate cages measuring 60x40x50 cm, and they were observed for 5 months. During this period, a general assessment of their health and behavior, body weight measurements, and skin examinations were conducted monthly, with special attention given to the postoperative scar and the skin above the implant.

After 5 months following implantation, the rats were euthanized by administering a lethal dose of sodium thiopental (150-200 mg/kg) via intraperitoneal injection.

After the removal of encapsulated implants for histological analysis, the capsule samples were fixed in a 10 % neutral formalin solution. Dehydration was carried out in isopropanol, and the material was embedded in Leica Surgipath Paraplast Regular paraffin (Leica, USA) for tissue sectioning. Sections with a thickness of 4 μm were prepared from the paraffin blocks. The obtained microslides were stained with hematoxylin and eosin to examine the morphological features of the capsule and with Sirius Red with picric acid to detect collagen [23]. Microphotographs were captured using a digital camera Olympus C3040ZOOM and an Olympus BX51 microscope (Olympus, Japan). Morphometric analysis was conducted using ImageJ software.

The surface condition of the extracted implants was examined using scanning electron microscopy (SEM) with a Tescan Mira 3 LMU scanning electron microscope (Tescan, Czech Republic) equipped with an energy-dispersive spectrometer Oxford Instruments X-Max 80 (Oxford Instruments, United Kingdom). The samples were investigated in secondary electron (SE) and backscattered electron (BSE) detection modes. The local elemental composition of the implants was analyzed using an energy-dispersive spectrometer Oxford Instruments X-Max 80 (Oxford Instruments, United Kingdom).

Statistical data analysis was conducted using StatPlus 7.0 software. For statistical data processing, one-way analysis of variance (ANOVA) with Bonferroni correction and correlation analysis (Spearman's rank correlation coefficient or rank-biserial correlation coefficient) were employed. The results are presented as the mean value and standard error (M±m) with consideration of variance (d). Differences between groups were considered statistically significant at $p<0.05$.

RESULTS

The overall response to metal implants consists of both local and systemic components [24, 25]. Immunologic cell responses can be either innate (requiring no prior exposure) or adaptive (acquired only after encountering an antigen). Both cellular responses are associated with side effects that, in turn, are related to metal implants. Such responses can be observed histologically, but this analysis is possible only when tissue biopsy or implant removal is performed.

In the conducted experimental study, all groups of animals exhibited swelling and redness upon the subcutaneous injection of a Ni solution into the ear pinna area. However, a similar reaction was observed with the injection of NaCl 0.9 %. Therefore, it was concluded that such a test is not sensitive for evaluating the reaction to Ni. Five months after the implantation of Ni-containing plates, no local or generalized skin lesions were observed.

Five months after implantation, encapsulated implants were found in the animals. Subsequently, the surrounding tissues were dissected to conduct histological analysis. According to the data presented in Table 2, the cavity volume and capsule diameter, based on linear morphometry results, were similar. However, visually, the quantity of inflammatory infiltrate delineating the implant from the capsule wall was greater in group 2 (see Figure 2).

Table 2

Capsule sizes around implants in the control and experimental groups 5 months after surgery

Group	Average capsule diameter, mm	Average diameter of the capsule cavity, mm	Ratio of average capsule diameters to cavity
Group 1	10,6±1,57	6,97±1,23	1,58±0,10
Group 2	10,2±0,70	7,03±0,79	1,54±0,11
p	0,79	0,97	0,82

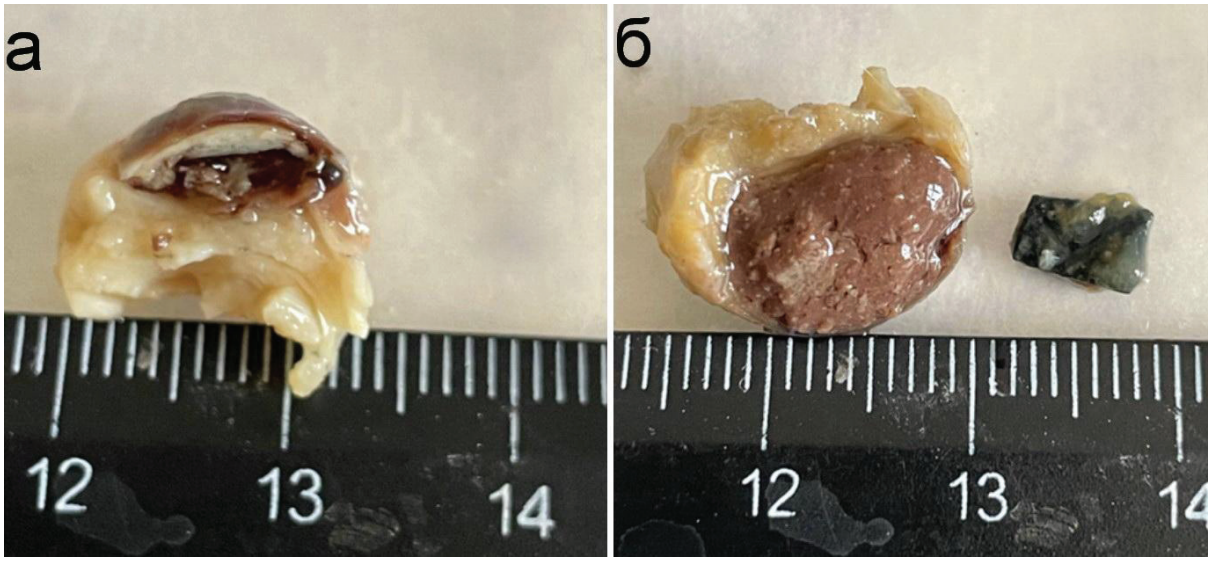
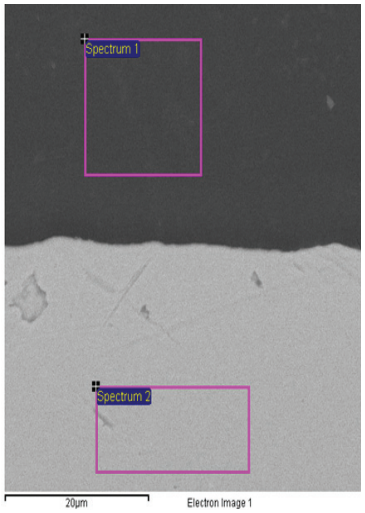


Fig.2. Inflammatory response to the implant. Group 2 (b) exhibits a larger inflammatory infiltrate compared to Group 1 (a). Macroscopic preparation

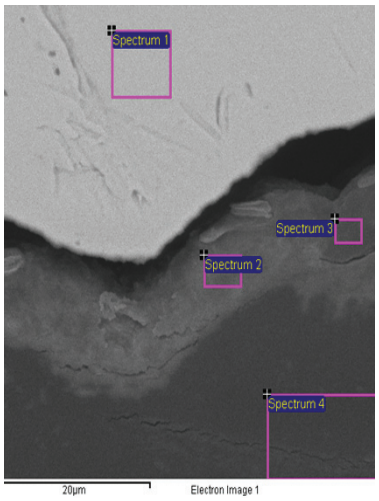
To conduct the research using electron microscopy, the surfaces of the removed metal plates were first mechanically secured using an epoxy compound to preserve the surface layer. After the compound's polymerization, the sample was cross-sectioned. Subsequently, the cross-section was ground and polished to reveal the boundary between the metal and the surface layer and identify the elemental composition of individual layers.

The oxidized surface of the implants was covered with an organic film. The presence of macroelements on the oxidized surface was identified using energy-dispersive spectroscopy, and the results are presented in Figure 3.



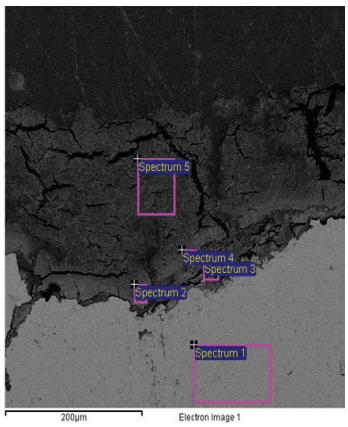
a

Spectrum	C	O	Ni
Spectrum 1	84.54	15.46	0.00
Spectrum 2	19.37	-	80.63



b

Spectrum	C	N	O	Si	P	S	Cl	Ca	Ni
Spectrum 1	17.36	-	-	-	-	-	-	-	82.64
Spectrum 2	57.58	-	18.63	-	3.31	4.00	-	0.92	15.56
Spectrum 3	76.05	3.25	16.09	-	-	-	0.30	-	4.32
Spectrum 4	82.02	-	17.25	0.20	-	-	0.28	-	0.24



c

Spectrum	C	N	O	Si	Ni
Spectrum 1	17.71	-	-	-	82.29
Spectrum 2	41.41	6.03	26.81	1.86	23.89
Spectrum 3	54.96		24.13	2.54	18.37
Spectrum 4	43.14	4.30	26.77	1.06	24.72
Spectrum 5	62.90	13.83	20.35	1.36	1.56

Fig. 3. Scanning electron microscopy of implant surfaces and the content of elements extracted after 5 months from laboratory animal tissues. Legend: a surface of intact nickel implants, b implants extracted from animals in Group 1, c implants extracted from animals in Group 2. SEM, × 20 and 200 µm

The intact nickel plate sample is shown in (Figure 3a), and the Spectrum 2 region corresponds to the metal area (Ni). In the second sample, the implant was extracted from animals in Group 1 (Figure 3b) – the Spectrum 1 region corresponds to the metal (Ni), and the Spectrum 2 and 3 regions show the elemental composition of the layer formed on the metal surface. According to the elemental composition, it may consist of Ni, oxygen (O), phosphorus

(P), sulfur (S), and silicon (Si), indicating the presence of corrosion in this sample.

In the third sample, which was an implant extracted from animals in Group 2 (Figure 3c), the Spectrum 1 region corresponds to the metal (Ni), while all the other Spectrums correspond to different parts of the layer formed on the metal surface. The elemental composition of these regions (the presence of oxygen (O), nitrogen (N), and nickel (Ni)) is present in higher percentage values, indicating a more pronounced corrosion of the metal surface. The Spectrum 5 region shows the absence of metal (Ni), which can be characterized as the beginning of degradation. The presence of silicon (Si) in the composition can be explained by the fragility of the «coating», which led to the inclusion of abrasive particles during grinding.

In the histological examination, a significant variability in the morphometric features of the capsule wall that formed around the implants was observed. The main morphological characteristics of these tissues included the presence of fibrous connective tissue with numerous collagen fibers, angiogenesis, and infiltration of macrophages and neutrophils (Figure 4).

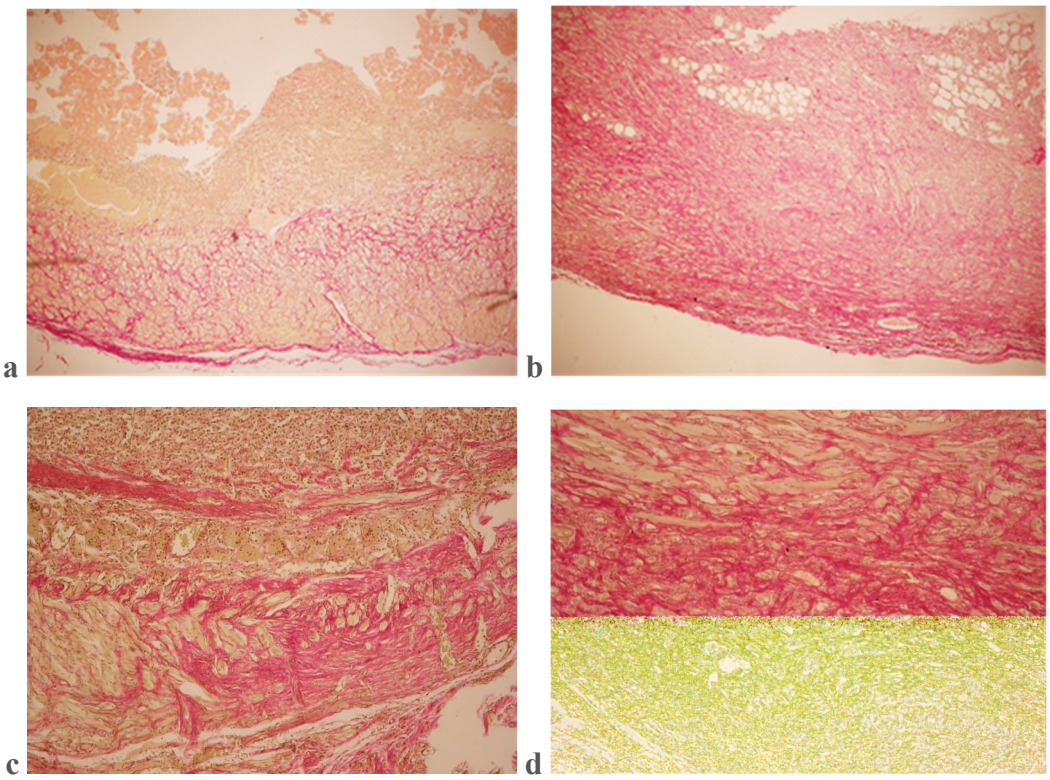


Fig. 4. Capsule wall around the implant. The density of connective tissue in Group 1 (a, c) is lower than in Group 2 (b, d). Sirius red, picric acid. Light microscopy, ×40 (a, b) and ×100 (c, d).

Two dominant histological variants of tissue reactions to the implant were identified: a) implant encapsulation with moderate macrophage infiltration, b) encapsulation with intense mixed infiltration of macrophages and neutrophils. In Group 2, foci of collagenogenesis already occurred within the inflammatory infiltrate, which were not structurally associated with the fibrous capsule wall. Cellular debris was detected within the infiltrate, and in Group 2, additional focal or multiple hemosiderin clusters and macrophages phagocytizing this oxidation product were identified (hemosiderin granules were found in the cytoplasm of macrophages). This was a distinct histological feature of Group 2 compared to Group 1. At the same time, Group 2 exhibited a tendency towards more intensive infiltration by large macrophages.

According to the presented data in Figure 5, there was no statistically significant difference in the thickness of the capsule around the implants between Group 1 and Group 2 ($p=0.71$).

At the same time, in the animals of Group 2, the specific density of collagen fibers was significantly greater by 34.8 % ($p<0.05$). Analysis of the morphometric data of the capsule walls showed significantly higher variability in the values for the first ($d1=65.5$ vs. $d2=172.6$) and the second parameter ($d1=45.7$ vs. $d2=318.7$). The variance of these two parameters was greater in Group 2, indicating a different characteristic of tissue reactions. The Spearman's correlation coefficient ($Rho=0.42$, $p=0.14$) did not show a correlation between capsule thickness and the specific density of collagen in the capsule.

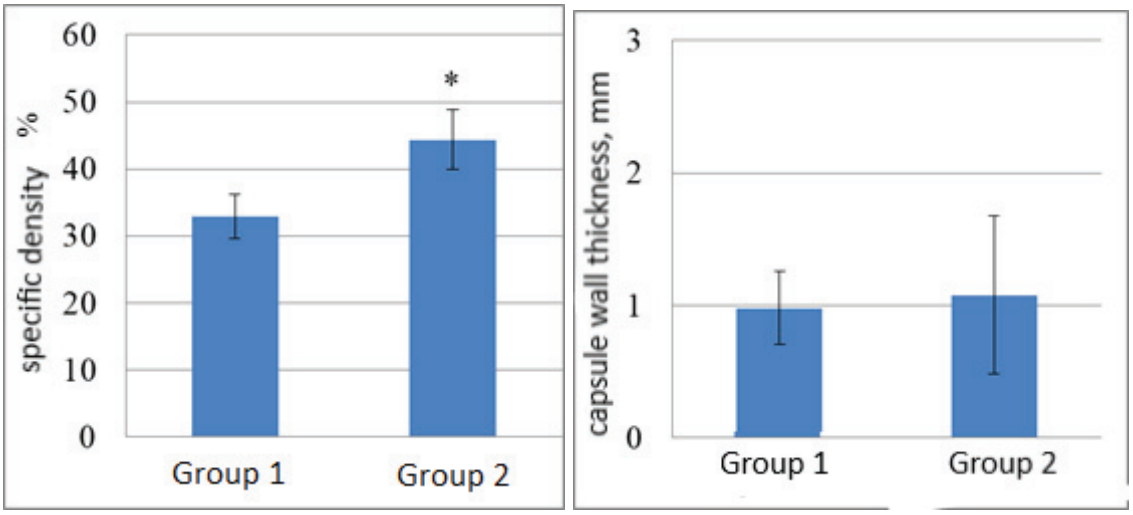


Fig. 5. Results of morphometry of the connective tissue capsule wall. The indices are presented as the mean and standard error of the mean. Group comparisons at each observation time were performed using one-way ANOVA. A significant increase in the specific density of collagen in Group 2. * $p<0.05$.

DISCUSSION

The conducted research revealed that a dense connective tissue capsule with an inflammatory infiltrate formed around the Ni implants. This infiltrate contained macrophages, neutrophils, and lymphocytes within the capsule lumen, indicating the development of proinflammatory cellular reactions to the implant. This could be cautiously interpreted as a manifestation of a hypersensitivity reaction.

Ni is a well-known contact allergen, and despite its low content in metallic implants, tissue reactions to these implants are well-documented. Host responses to metallic implants involve both innate and adaptive immune mechanisms and are described as responses to foreign bodies. Initial reactions involve inflammatory infiltration, including the appearance of leukocytes, angiogenesis, and extracellular matrix remodeling with subsequent formation of a fibrous capsule. Although Ni allergy is common, establishing a causal relationship between true allergic reactions and adverse outcomes of metallic implants is an ongoing area of research.

Ni is not a direct allergen but, as a hapten, it can induce oxidative modifications of protein molecules, which can then lead to immune responses. Clearly, this doesn't happen immediately, and these biochemical changes around the implant occur over a certain period. Typically, immediately following implantation, the adsorption of host proteins on the implant's surface initiates a response to the foreign body. This triggers a coordinated cascade of inflammatory and cellular mechanisms that are critical for the acceptance of the implant [26].

Immediately following tissue damage, the intrinsic pathway of blood clotting is activated, initiating thrombus formation. Platelet activation through interaction with collagen and fibrin leads to platelet degranulation [27]. The early recruitment of neutrophils in the first few days further amplifies the inflammatory pathways. Neutrophils at the wound site are eventually displaced by macrophages, which differentiate in situ, primarily from monocytes recruited from circulation. Tissue injury in the host quickly accompanies coagulation and complement activation, as well as protein adsorption, including fibrinogen, fibronectin, vitronectin, and globulins, on the implant's surface. This heterogeneous mixture of stress response proteins and extracellular matrix components forms a matrix on the implant and around it [28]. Early events in response to the implant and, as a result, deviations from typical programs, can have significant consequences in the long term, including osteolysis, necrosis, pseudotumor formation, tissue granulation, and the contraction of the fibrous capsule [29].

In the initial 3 days following implantation, neutrophils are mobilized to the implant site; however, their *in situ* lifespan is short. In the subsequent phase, between 2-10 days, granulation tissue forms by deposition of the extracellular matrix by fibroblasts and neovascularization by proliferating endothelium [30]. The production of collagen types I and III by fibroblasts is crucial for the stability of the extracellular matrix in healing wounds. Glycosaminoglycans, elastic fibers, and other glycoproteins produced by fibroblasts modulate the mechanical and structural properties of the developing fibrous matrix [31].

The wound healing processes can be complicated by metallic particles formed as a result of wear or corrosion, including metal ions. These particles can have various effects. They may damage cells and be engulfed by

macrophages, which can lead to inflammation and tissue-destructive reactions of varying degrees.

Macrophages are the primary cells that play a crucial role in organizing fibrogenesis, angiogenesis, and tissue remodeling [32]. At the site of implantation, macrophages can be activated by metal residues and damage-associated molecular patterns (DAMPs), released after tissue injury and cell death, leading to the production of proinflammatory cytokines, chemokines, and other low-molecular-weight mediators of inflammation [33].

Phagocytosis by macrophages of metal particles with a diameter of less than 10 μm is a key mechanism through which implants can trigger inflammatory reactions [34-35]. Phagocytosed metal particles are subjected to endocytosis and transported to lysosomes, where the acidic microenvironment of these vesicles promotes particle corrosion, stimulating the further release of metal ions [36-37]. Since metal particles are resistant to complete degradation by lysosomes, cell death is a common endpoint for macrophages responding to metal particles, amplifying the inflammatory signal. Large metal particles can induce the fusion of macrophages, leading to the formation of foreign body giant cells (FBGCs) to sequester undigested particles [38]. This process plays a central role in granuloma formation and the continuation of implant-related inflammatory reactions [39].

The production of IL-1α, IL-1β, and TGFβ by macrophages in response to metal debris enhances the recruitment of neutrophils [40]. This rapid response by neutrophils can be characterized as an acute, highly localized stress program that involves the release of proteases, lysozymes, and reactive radicals in the form of neutrophil extracellular traps (NETs). This process promotes opsonization, clearance, and removal at the site of implantation [41-42]. Some of these mechanisms engage neutrophils in the initial production of metal particles through the generation of oxidants, pro-inflammatory chemokines, and cytokines [43]. The persistent and prolonged accumulation of neutrophils following a reaction to a foreign body may serve as an indicator of maladaptive responses to a metal implant, potentially predicting adverse events, including septic forms of implant rejection [44].

Histological data demonstrate the co-localization of lymphocytes with macrophages and giant cells in fibrous tissues surrounding prostheses and other metal implants, especially in the context of dysfunctional implants [45].

Similar results were obtained in our experimental research. The formation of a dense capsule around the implants was observed, but there was no close contact between the Ni surface and the capsule because the metal was separated from the capsule by the inflammatory infiltrate. Furthermore, a more intense inflammatory infiltration was observed around the implants with prior sensitization using Freund's complete adjuvant.

It's interesting that in such samples, additional collagen formation occurred within the inflammatory infiltrate, which can be considered as more intensive capsule formation around the implant. In our opinion, special attention should be paid to the connection between the clusters of hemosiderin in the tissue around the Ni implant and the higher collagen density in the capsule, as well as the appearance of collagenogenesis foci in the inflammatory infiltrate. The abundant accumulation of hemosiderin may indicate the occurrence of hemorrhages and subsequent hemolysis after «complete sensitization» with $NiSO_4$.

Scanning electron microscopy (SEM) revealed oxidation of the implant surface and the presence of organic coating. In all samples, a change in the relief of the nickel plates was observed, corresponding to corrosion. Group 2 showed a more pronounced manifestation of corrosion.

The future prospects of the research involve studying the immunophenotypic characteristics of cellular infiltrates and their reactions to the implant. It is anticipated that T-cells require a specific interaction between their T-cell receptor (TCR) and an antigenic peptide embedded in major histocompatibility complex (MHC) proteins located on the surface of antigen-presenting cells.

The studies by Clayton G. et al. proposed that metal ions act as haptens, forming coordination intramolecular complexes with MHC proteins and antigenic peptides [46]. Other reports suggest that metal ions can catalyze the cross-linking of the TCR/MHC complex [47]. The response of CD4+ T-helper cells to metal implants depends on the composition of the implant metal and often correlates with metal concentrations in the blood. The increased presence of T-cells in the tissues surrounding malfunctioning implants is observed, although their appearance does not necessarily indicate a maladaptive response [48].

The number of circulating T cells in peripheral blood is not associated with adverse local tissue reactions caused by implants [47], although this count typically decreases after implantation surgery [49].

CD4+ T cells from blood or collected from tissues demonstrate signs of sensitivity to metal antigens, including proliferation, expansion, and phenotype markers associated with activation [50]. The involvement of CD4+ T cells in responses to metal implants implies that the soluble cytokines produced by these cells are essential mediators of their contribution to the overall coordination of immune responses to metals.

Overall, CD4+ T cells may represent a crucial checkpoint in determining the durable effector responses to metal implants, from inflammation and allergic hypersensitivity to indirect tolerance [51, 52].

It remains unclear to what extent CD8+ T cells are involved in the response to metal implants. CD8+ cells responding to Ni haptens can be found in patients with Ni contact hypersensitivity.

The specific response of B-lymphocytes (B cells) to metal implants and metal fragments is unknown. The number of B cells appears to remain stable in individuals with metal implants [53]. B cells are permanent residents in tissues adjacent to implants [54]. The most significant role of B cells may lie in producing antibodies against metal haptens in mediating immediate hypersensitivity reactions of types I, II, and III, in contrast to the described dermal hypersensitivity focused on T cells [55].

Thus, further research is needed to understand the basic cellular and molecular mechanisms that may detect signals predicting the malfunction of a metal implant, as maladaptive responses leading to failure or adverse events can mimic normal responses. Additionally, the cellular, tissue, and temporal contexts of responses that can distinguish maladaptive responses from normal ones are necessary. A more comprehensive understanding of the fundamental inflammatory and immunological biology of metal reactions will facilitate the identification of clinically useful purposes needed to develop diagnostic or prognostic tests for patients with metal implants.

CONCLUSIONS

1. Histological examination revealed that a connective tissue capsule formed around the implants. The capsule had a 34.8 % higher collagen density in animals sensitized to Ni before implantation, indicating tissue reactions with signs of hypersensitivity.

2. Implant corrosion was observed in every retrieved sample, with more pronounced corrosion in the group of animals sensitized to Ni before implantation, leading to the appearance of distant Ni particles, which can be characterized as the beginning of implant degradation.

3. Further research will provide a deeper understanding of the fundamental inflammatory and immunological reactions to metals present in implants. This, in turn, will facilitate the identification of clinically useful applications necessary for the development of diagnostic or prognostic tests for patients with metal implants.

FUNDING AND CONFLICT OF INTEREST

The authors declare no conflict of interest.

The study was performed as a fragment of the complex scientific projects of the Scientific Department of mini-invasive surgery (State Institution of Science «Research and Practical Center of Preventive and Clinical Medicine» State Administrative Department) «Surgical treatment optimization based on multimodal fast recovery program using nanobiosencoric technologies and anaesthesiological providing» (state registration number 0122U000233; term: 2022-2024).

COMPLIANCE WITH ETHICAL REQUIREMENTS

Experiments on animals were conducted in the vivarium of the O. V. Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine in accordance with the Law of Ukraine «On the Protection of Animals from Cruelty» (No. 3447-IV, dated February 21, 2006), adhering to the requirements of the European Parliament and Council (2010) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Authors' contributions: According to the order of the authorship.

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Резюме

ОСОБЛИВОСТІ ВИКОРИСТАННЯ ОРТОПЕДИЧНИХ ІМПЛАНТАТІВ НА ЕКСПЕРИМЕНТАЛЬНІЙ МОДЕЛІ СЕНСИБІЛІЗАЦІЇ ДО НІКЕЛЮ (NI)

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Вступ. Тема реакції гіперчутливості до металів (РГМ) входить у перелік найбільш суперечливих та складних в ортопедо-травматологічній практиці. РГМ є діагностично складними, оскільки вони є діагнозом виключення. Достеменно невідомо, які біомаркери можуть достовірно передбачити потенційну патологічну реакцію на імплантати.
Мета даної роботи – дослідження реакції гіперчутливості на ортопедичні імплантати, що містять у своєму складі нікель.
Матеріали та методи. Дослідження проведено на щурах-самках лінії Wistar, відповідно до стандартів біоетичних принципів. Для отримання доказових результатів, тварин було розподілено на дві експериментальні групи: з попередньою сенсибілізацією ад'ювантом Фрейнда до Ni та без неї. Щурам під загальною анестезією були імплантовані нікелеві пластини загальною площею 24 мм^2. Через 5 місяців після втручання тварин виводили з експерименту, було проведено гістологічне дослідження отриманих зразків. Вилучені імпланти досліджували методом електронної мікроскопії для оцінки стану поверхні імплант. Локальний елементний склад імплантів аналізували за допомогою енергодисперсійного спектрометра.
Результати. За даних експериментальних умов було показано, що через 5 місяців після операції, у щурів навколо імплантів сформувалась щільна сполучнотканинна капсула з запальним інфільтратом у просвіті капсули. Це свідчить про можливий прояв реакції гіперчутливості на імпланти, що містять у своєму складі Ni. Електронна мікроскопія поверхні вилучених імплантів дозволила виявити явища корозії в усіх вилучених зразках. Ступінь корозії був більш вираженим у групі тварин з попередню сенсибілізацією до Ni, були виявлені віддалені часточки (Ni), що можна охарактеризувати, як початок руйнації імпланту.
Висновки. Навколо імплантатів формується сполучнотканинна капсула, яка за щільністю колагену на 34,8 % була більшою у піддослідних тварин до імплантації сенсибілізованих нікелем, що можуть відповідати проявам тканинних реакції з ознаками гіперчутливості. Подальше дослідження дасть глибше розуміння фундаментальної запальної та імунологічної реакцій на метали, що входять до складу імплантів. Це в свою чергу полегшить ідентифікацію клінічно корисних призначень, необхідних для розробки діагностичних або прогностичних тестів для пацієнтів з металевими імплантатами.

Ключові слова: **імплант, нікель, експеримент, імунна відповідь, сенсибілізація, реакція гіперчутливості до металів, моделювання на тваринах, біосумісність, щури**