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МИКРОСКОПИЧЕСКИЙ И ЭЛЕМЕНТНЫЙ АНАЛИЗ КОСТНОЙ ТКАНИ ЧЕЛЮСТИ ПРИ ТРАВМЕ

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Резюме. Поскольку костная ткань челюсти подвергается травматическому воздействию на этапе внедрения металлического дентального имплантата, актуальной остается проблема развития воспалительных осложнений, приводящих к срыву остеоинтеграции. Представляют интерес иммунологические механизмы развития воспалительного процесса при эмиссии наноразмерных металлосодержащих частиц, а также механизмы его стихания после удаления металлического объекта из костной ткани. В работе проведен микроскопический и элементный анализ сегмента костной ткани нижней челюсти крысы линии Wistar после искусственной травматизации. В ходе эксперимента моделировали процесс нахождения металлического инородного тела в костном ложе. Для этого в соединительнотканное соединение нижней челюсти крысы вводили инсулиновую иглу, с последующим ее извлечением через семь дней. Микроскопический анализ костной ткани проводили методом растровой электронной микроскопии с помощью прибора Tesscan Vega 4 с системой EDX Oxford Xplore 30. По данным электронной микроскопии шлифа челюсти крысы в области лунок нижних резцов при малом увеличении в прямой проекции определяются: поверхность кортикального слоя альвеол зубов, хрящевое и соединительнотканное соединение челюстей с разрывом, расслоение хрящевого слоя. В области хрящевого и соединительнотканного соединения альвеолярных отростков челюстей при большем увеличении в прямой проекции находятся плотноструктурные кристаллические включения, очаги некротизации. Элементный состав костной ткани был получен методом атомно-эмиссионной спектроскопии (прибор – эмиссионный спектрометр iCAP 6300 Duo). В исследуемом образце количественное соотношение кальция и фосфора составило 1,68, что незначительно превышает оптимальное, равное 1,67. Изменение данного соотношения в сторону увеличения говорит о

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снижении уровня фосфора в костной ткани, что можно интерпретировать как локальный остеопороз. Кроме того, обнаружены элементы: Bi, Ga, Pb, Ti, Zn в количестве 0,03-0,06 массовых процентов. Перечень этих элементов соответствует химическому составу инсулиновой иглы, что свидетельствует о проникновении металлических частиц в ткани костного ложа. Эмиссия наноразмерных частиц и их последующее объединение до микро- и субмикронных размеров, их персистенция, а также биокоррозия в зонах активного костеобразования могут являться пусковым механизмом для развития асептического воспалительного процесса. Этот эффект обусловлен как прямым повреждающим фактором, так и опосредованным воздействием, через специфичные сигнальные молекулы, вырабатываемые в ответ на повреждение тканей.

Ключевые слова: периимплантит, имплантация, наноразмерные частицы, воспаление, РЭМ-микроскопия, ремоделирование кости

MICROSCOPIC AND ELEMENTAL ANALYSIS OF JAW BONE TISSUE IN INJURY

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Abstract. Since the jawbone tissue is injured at the stage of metal dental implant introduction, the problem of inflammatory complication development leading to a breakdown in osseointegration remains relevant. Of interest are the immunological mechanisms of inflammatory process development during the emission of metal nanoparticles, as well as the mechanisms of its subsidence after the removal of a metal object. Microscopic and elemental analysis of the bone tissue of the Wistar rat lower jaw after artificial traumatization was carried out. During the experiment, the situation of presence of a metal foreign body in the bone bed was simulated. An insulin needle was injected into the connective tissue of the lower jaw, followed by its removal after seven days. Microscopic analysis of bone tissue was performed using a Tesscan Vega 4 scanning electron microscope. The teeth alveoli cortical layer surface, connection of the jaws with a gap, stratification of the cartilaginous layer were determined at low magnification in direct projection. Using higher magnification in the direct projection there are visible dense-structural crystalline inclusions, foci of necrosis in the area of junctions of the jaws alveolar processes. The elemental composition of bone tissue was obtained by atomic emission spectroscopy by iCAP 6300 Duo. In the test sample, the quantitative ratio of calcium and phosphorus was 1.68, which slightly exceeds the optimal value of 1.67. An upward change in this ratio indicates a decrease in phosphorus level, which can be interpreted as local osteoporosis. In addition, the following elements were found: Bi, Ga, Pb, Ti, Zn in the amount of 0.03-0.06 mass percent. The list of these elements corresponds to the chemical composition of an insulin needle, indicating the penetration of metal particles into bone bed tissues. The emission of nanoparticles and their subsequent association to micro- and submicron sizes, their persistence, as well as biocorrosion in areas of active bone formation can be a trigger for the development of an aseptic inflammatory process. This effect is due to both a direct damaging factor and an indirect effect through specific signal molecules produced in response to tissue damage.

Keywords: periimplantitis, dental implantation, nanoparticles, inflammation, scanning electron microscope, bone remodeling

Introduction

Traumatization of tissue structures in the body, in particular bone tissue, is always accompanied by the development of an inflammatory process. The study of post-traumatic bone changes is an important step in assessing, among other things, the immunological mechanisms of inflammation. In this case, the nature of the mechanical action plays an important role.

Dental implantation is a surgical intervention that involves the formation of a mechanical bone defect, followed by the introduction of a metal implant into the injured bone. Currently the causes of chronic inflammatory complications in the long-term functioning of dental implants are being actively studied. [4]. One of the reasons for the development of such complications is probably the release of nanoparticles of the oxide layer of the dental implant in the tissue of the bone bed [2, 7]. The accumulation and conglomeration of such particles, as well as their biocorrosion, can probably be a personalized cause of the development of mucositis and peri-implantitis, which is associated with the inability of the cells of the immune system to timely utilize these particles, preventing their conglomeration, and their early death.

It is of interest to study the immunological aspect of bone repair after removal of a metal foreign body against the background of an inflammatory process. Particularly interesting are the immunological mechanisms of the development of the inflammatory process during the emission of nanoparticles, as well as the mechanisms of its remitting after the removal of a metal object from the bone tissue.

The purpose of the study was to study electron microscopy and elemental composition of the jaw bone tissue after traumatization with a metal implant in order to identify the causes of the development of a persistent inflammatory process, as well as the likelihood of participation of nanoparticles in triggering the immunological mechanisms of implant rejection.

Materials and methods

The first stage of the research work was carried out on the basis of the Institute of Immunology and Physiology of Ural Branch, the Russian Academy of Sciences, Yekaterinburg. There was simulated the situation of presence a metal foreign body in the bone bed to assess the state of the bone tissue after a mechanical injury. To do this, an insulin needle 12 mm long was inserted into the connective tissue joint of the lower jaw of the Wistar rat, followed by its removal after seven days. A month after the removal of the foreign body, rats were taken out of the experiment to isolate the studied bone segment. All painful procedures were performed in accordance with the WMA Declaration of Helsinki on animal use in biomedical research.

Further, the study of the bone tissue sample was carried out in the laboratory of the Center for Collective Use of the Institute of High Temperature Electrochemistry of Ural Branch, the Russian Academy of Sciences, Yekaterinburg. Microscopic analysis was performed by scanning electron microscopy (SEM microscopy) using a Tesscan Vega 4 scanning microscope with an EDX Oxford Xplore 30 system. The bones were preliminarily poured into Struers Epofix epoxy resin, then a slice was prepared on an Allied metprep 4 grinding and polishing machine. The elemental composition of bone tissue was obtained by atomic emission spectroscopy (instrument – emission spectrometer iCAP 6300 Duo, Thermo Scientific, UK). Range of determined elements: Li – N, Na – S, K – Se, Rb – Mo, Ru – I, Cs – Nd, Sm – Bi, Th, U, Pu.

Results and discussion

Figures 1-3 show the data of SEM microscopy of a segment of the bone tissue of the lower jaw of a rat in different projections and scales.

In the studied segment of bone tissue, 67 chemical elements were determined by atomic emission spectroscopy. The highest mass fraction belongs to the following elements: calcium (22.13%), phosphorus (13.14%), magnesium (0.69%), sodium (0.58%), sulfur (0.16%) because these are the basic elements of any mineralized bone tissue, including jaw bone tissue [6]. The skeletal system actively responds to the influence of various environmental and endogenous factors. It is known that the state of the body's immune defense also affects the morphogenesis of the skeletal system. Calcium and phosphorus are the main elements of any bone tissue, the ratio of which is an indicator of the quality of mineralization. For bones, the optimal ratio of calcium and phosphorus is 1.67, which indicates a normal degree of bone mineralization. In the studied sample of bone tissue, this ratio is 1.68. Even such a slight change in the Ca/P balance indicates the occurrence of pathological processes leading to the impossibility of normal repair. An upward change in this ratio indicates a decrease in the level of phosphorus in the bone tissue, which can be interpreted as local osteoporosis. This state of the peri-implant area prevents normal bone tissue remodeling and provokes the development of bone tissue resorption processes.

In addition, elemental analysis showed the presence of foreign elements in the bone tissue, the amount of which corresponds to the range from 0.01% to 0.06%. Basically, these are elements that form simple substances, metals: bismuth, gallium,

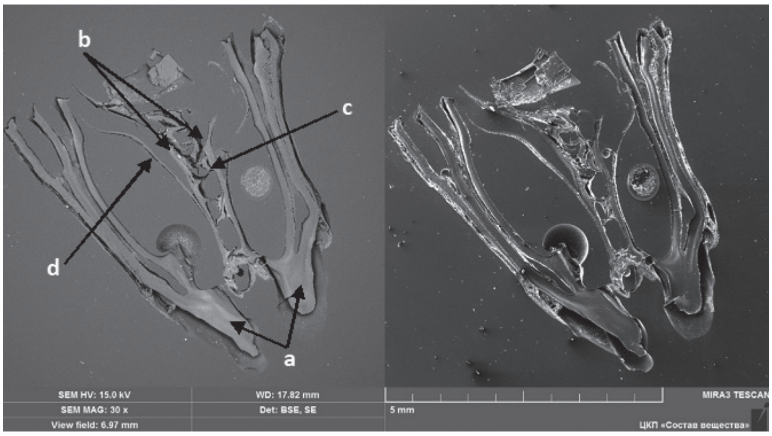

Figure 1. SEM microscopy of a slice of a rat jaw in the area of the holes of the lower incisors at low magnification in direct projection seven days after the needle was inserted into the connective tissue joint of the jaws

Note. There are determined the surface of the cortical layer of the alveoli of the teeth (a), cartilaginous connection (b) and connective tissue connection (c) of the jaws with a gap, stratification of the cartilaginous layer (d).

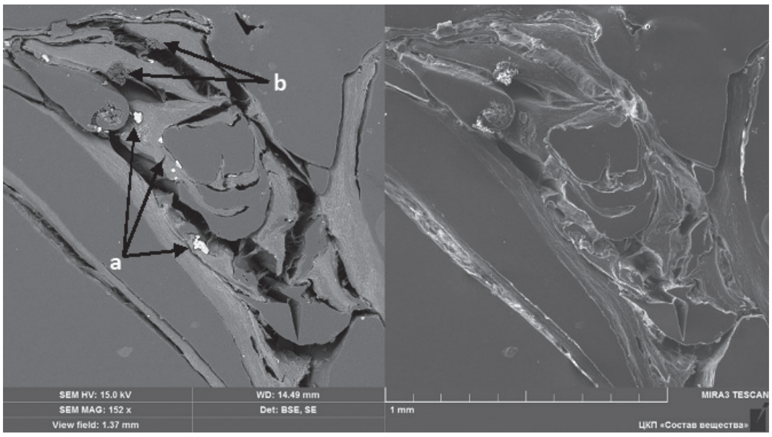

Figure 2. SEM microscopy of a slice of a rat jaw in the area of the cartilaginous and connective tissue junction of the alveolar processes of the jaws at a higher magnification in the direct projection

Note. There are determined indurated inclusions (a), foci of necrotization (b).

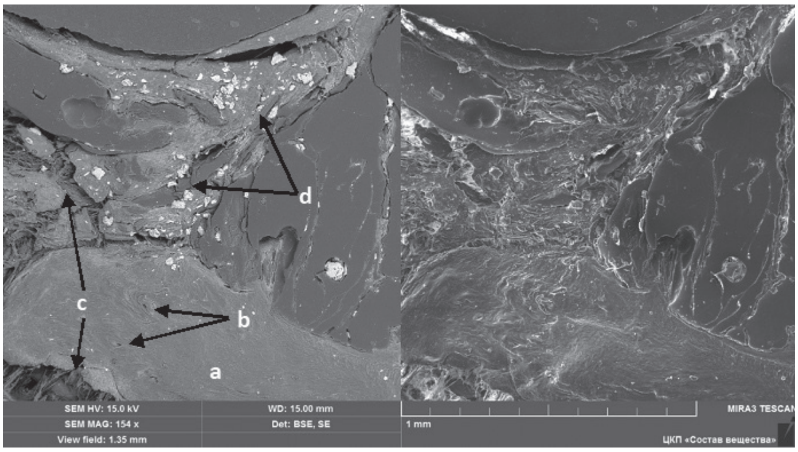

Figure 3. SEM microscopy of a section of a rat jaw in the area of the socket of the lower incisor at a higher magnification in the side projection

Note. There are determined part of the remaining cortical layer of the alveolus (a), pores (b), areas of cancellous bone (c), small and large indurated inclusions (d).

lead, iridium, titanium, zinc, mercury, molybdenum. The list of these metals corresponds to the chemical composition of the insulin needle in contact with bone tissue during injury. Insulin needles are made of steel, which includes alloying elements: Si, S, P, Bi, Pb, Ti, Zn and others. Alloy steel has improved technical characteristics: corrosion resistance, refractoriness, high hardness, etc.

Since the chemical composition of the jaw bone depends on many factors, for example, the quality of nutrition, the degree of mechanical stress, the physiological state of the body [1, 5], in addition to the indicated elements, the content of other elements in the range of 10^{-3}-10^{-4} mass percent was identified in the test sample.

It should be taken into account that any surgical intervention, in particular dental implantation, triggers a cascade of immunological mechanisms induced by immunocompetent cells due to their contact with the implant surface. The severity and duration of the immune response affects the quality of osseointegration. Isolation of inflammatory factors by immunocompetent cells determines the composition of the microenvironment and, as a consequence, the vector of development of the immune response.

Particular attention is drawn to the mechanisms of maintaining the immunological balance in the area of implantation. In particular, the degree of migration of neutrophils, thrombocytes and macrophages producing growth factors, cytokines and chemokines that promote the migration of other immune cells involved in the processes of phagocytosis and stimulation of the reparative process. There are two macrophage phenotypes: M1 – pro-inflammatory, producing IL-1β, IL-6 and TNF and inducing differentiation of osteoclasts; and M2 – anti-inflammatory, producing IL-4, IL-10, IL-13 and TGF-β, affecting the reactions of healing and bone tissue remodeling. It is likely that the emission and biocorrosion of nanoparticles into the area around the implant can cause a shift in the immunological balance towards the chronicity of the inflammatory process, which may be due to provoking the death of immunocompetent cells, incomplete processing of particles, the release of pro-inflammatory cytokines and the formation of a vicious circle.

The presence of microparticles in the bone tissue in contact with a metal foreign body indicates the possibility of provoking the development of an inflammatory process by nanoparticles and their conglomerates. The formation of complexes (conglomerates) of nanoparticles and their enlargement to micro- or submicron sizes, with the inability of immunocompetent cells to timely utilization and subsequent migration, can lead to their accumulation and provoke the occurrence of chronic aseptic inflammation. Achievement of CDNanoMP (Critical Dose of NanoMetal Particles) leads to early death of the immune system's own cells [3]. In this case, microbial contamination takes second place as an etiological factor in the occurrence of a breakdown in previously achieved osseointegration, i.e., tissue homeostasis provided by immunotolerance.

Conclusion

The emission of nanoparticles and their subsequent association to micro- and submicron sizes, their persistence, as well as biocorrosion in the areas of bone tissue remodeling can be a trigger for the development of an aseptic inflammatory process. This effect is due to both a direct damaging factor and an indirect effect through specific signal molecules produced in response to tissue damage.

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