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Comprehensive X-ray analysis of foreign bodies in the human tissues

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For the first time, a complex of X-ray methods: XRD, XRF absorption analysis and the Compton scattering method – was used to study foreign bodies in human tissues. The surface of the fragments on opposite sides was visually significantly different: on one side it was smooth, on the other – rough. At the same time, no differences in the structure of the crystal lattice and the composition of the main impurities were revealed by the XRD and XRF methods. Both methods certified the material of the objects as aluminum alloy AMC. The Compton scattering method showed a change in the effective atomic number of the surface: Z_{eff} =13.15±0.55 on the smooth side and Z_{eff} =11.27±0.46 on the rough side, which corresponds to aluminum (Z_{eff}=13) and its oxide Al_2O_3 (Z_{eff}=10). The thickness of the oxide layer of 4 μm was determined by the absorption of the Al-*K*α line. The fluorescence emission spectrum of the rough side revealed the Br-*K*α line. It has been suggested that it is the increased bromine content (20-50 ppm) that promotes additional formation of sulfylamine to increase the tensile strength of the collagen IV molecular corset. This leads to accelerated restoration of the architecture of the cell basement membrane and tissue development.

Keywords: X-ray diffraction (XRD), X-ray fluorescence absorption analysis (XRF), Compton scattering, effective atomic number.

Комплексне рентгенівське дослідження сторонніх тіл в тканинах людини. *І.Ф Михайлов, А.І. Михайлов, С.С. Борисова, В. Негодуйко, Н. Ю. Селюкова*

Для дослідження сторонніх тіл вперше застосовано комплекс рентгенівських методів: рентгенівська дифракція (XRD), рентген-флуоресцентний абсорбційний аналіз (XRF) та метод комптонівського розсіювання. Поверхня уламків з протилежних сторін візуально суттєво відрізнялася: з одного боку вона була гладкою, з іншого – шорсткою. Водночас методами XRD та XRF не виявлено відмінностей у структурі кристалічної решітки та складі основних домішок. Обидва методи сертифікували матеріал об'єктів як алюмінієвий сплав АМЦ. Метод комптонівського розсіяння показав зміну ефективного атомного номера поверхні: Z_{eff} =13,15±0,55 на гладкій стороні та Z_{eff} =11,27±0,46 на шорсткій стороні, що відповідає алюмінію (Z_{eff}=13) та його оксиду Al_2O_3 (Z_{eff}=10). Товщина оксидного шару 4 мкм була визначена по поглинанню лінії Al-Kα. Спектр випромінювання флуоресценції шорсткої сторони показав лінію Br-Kα. Є припущення, що саме підвищений вміст брому (20-50 ppm) сприяє додатковому утворенню сульфіламіну для підвищення міцності на розрив молекулярного корсету колагену IV. Це призводить до прискореного відновлення архітектури базальної мембрани клітини та розвитку тканин.

1. Introduction

For the first time, a comprehensive X-ray approach based on four natural phenomena: diffraction, fluorescence, scattering and absorption of X-rays by a substance, was used to analyze a foreign body that was removed from the human body several hours after injury. An unexpected result of the X-ray fluorescence study was the discovery of traces of bromine on one side of the fragment.

Bromine is found ubiquitously in animals as the ionic bromide, but has no known important function. Bromine is a necessary cofactor for peroxidase-catalyzed formation of sulfilimin crosslinks, a post-translational modification required for development of tissue and basement membrane architecture found in collagen IV. Bromide, turning into hydrobromic acid, forms a bromosulfonium ion intermediate, which energetically forms sulfilimine. Thus, it has been established that bromine is necessary for the formation of sulfilimin in collagen IV. From the above, it can be stated that bromine is an important microelement for all animal species, and its deficiency may be related to changes in the basement membranes, which is observed in various diseases [1]. What is new is the discovery that bromine acts as a cofactor in this reaction, so bromine clearly has an important biological function and can now be classified as an essential trace element for multicellular organisms that have basement membranes.

Basement membranes are multi-layered cell-adhesive extracellular matrices that form part of the tissue architecture, facilitating both embryonic differentiation and maintenance of adult functions. These matrices serve as an extension of the plasma membrane, protecting tissues from disruptive physical stresses, and provide an interactive interface between the cell and the environment that can transmit local and distant signals within and between these compartments [2]. Basement membranes are dense thin layers of extracellular substance that line most epithelial, endothelial, and muscle tissues. They coordinate branching morphogenesis and determine the architecture of epithelial tissue, facilitate tissue recovery after injury, and direct pluripotent cells in tissue-engineered organ regeneration [3]. Basement membranes have been found to influence stem cell behavior as well as tissue behavior and may play a beneficial role in promoting stem cells through differentiation and maintaining the biological activity and stability of engineered tissues. Basement membranes have been found to influence stem cell behavior as well as tissue behavior and may play a beneficial role in promoting stem cells through differentiation and maintaining the biological activity and stability of engineered tissues. Inside the basal membranes, a sulfilimine cross-linked collagen IV framework is embedded, which provides multi-functionality to the matrix of multicellular tissues in animals of all types [4]. Collagen IV is the main component of the basement membrane together with laminins, proteoglycans and nidogens, etc. Collagen IV is secreted in the form of triple helical protomers that self-oligomerize into large networks. Compared to other components of basement membranes, the collagen IV network is uniquely reinforced by intermolecular covalent cross-links [5]. Due to the extensive cross-links in collagen IV, it is often suggested that it determines the stiffness of bone tissue, especially with tensile or circumferential stress. In support of this hypothesis, loss of collagen IV function leads to mechanical destruction of basement membranes [1].

The sulfilimine linkage between Met^{93} and Hyl^{211} is a cross-link of collagen IV [6]. Recent work has shown that peroxidase catalyzes the formation of sulfilimine cross-links in collagen IV. In Drosophila, loss of peroxidase and sulfilimin cross-link function disrupts the integrity of intestinal muscle tissue, leading to perforation and death of larvae [1]. These data demonstrate that sulfilimine cross-links of collagen IV support basement membrane stability but not mechanics. Thus, it has been experimentally proven that sulfilimine crosslinks in collagen IV contribute to the overall stiffness of basement membranes.

Unlike well-known works on microelements, the object of our research is the foreign body itself, and not biological tissues. The study of encapsulated foreign bodies that were in the human body for a long time until the completion of all four phases of healing is presented in [7].

The aim of this work is to study the influence of the organism on a foreign body in the first phases of wound healing.

2. Experimental

The objects of the study were metal fragments of ammunition that were in the soft tissues of the limbs for 10-15 hours. An initial examination conducted immediately prior to surgical debridement was inconclusive.

Table 1. Integral intensity of Compton I_C and Rayleigh I_R scattering peaks from different sample surfaces, intensity ratio and effective atomic number Z_{eff}

	CO-1 AL standard	Light side		Dark side		Al_2O_3 standard
		sample 1	sample 2	sample 1	sample 2	
I_C, counts	347 520	325 486	324 905	352 773	361 500	342 480
I_R, counts	168 922	155 151	164 306	160 949	157 536	143 902
I_C / I_R	2.057	2.098	1.977	2.192	2.294	2.380
Z_{eff}	13	12.6	13.7	11.74	10.81	10

Foreign bodies in the soft tissues of the limbs were not detected either by X-ray of the limbs or by magnetic detection of the wound area. After the objects were removed, visual inspection showed that they were metal fragments of complex shape. On different sides, their surface had different degrees of development. On one side it was smooth, light and shiny, and on the other it was dark and noticeably rough.

Interaction with the body's aggressive environment can manifest itself in changes in structure and composition, the appearance of adsorbed layers, etc. To identify the results of the body's reaction, both sides were certified using a set of X-ray methods: XRD, XRF, and the Compton scattering method (measuring the integral intensity ratio of the peaks of incoherent (Compton) and coherent (Rayleigh) scattering).

The type of crystal lattice and impurity phases in the samples were identified using XRD on a DRON-3 diffractometer in Cu-Kα radiation. The chemical composition was analyzed by X-ray fluorescence analysis on a SPRUT-SEF 0.1 energy-dispersive spectrometer with an Amptek X-100 SDD detector. An X-ray optical setup with a secondary niobium target was used for the measurements.

To identify elements with a low atomic number (oxygen), the effective atomic number of a foreign body was determined by the ratio of the integral intensities of the Compton/Rayleigh peaks using the method reported in [8].

3. Results and Discussion

3.1 Analysis of the material structure

Phase analysis carried out after extraction of the objects gave identical results for both sides of the sample: the diffraction pattern corresponds to the face-centered cubic lattice of aluminum (Fig. 1).

3.2 Elemental analysis of the material

As for the elemental composition of the objects, X-ray fluorescence analysis showed that

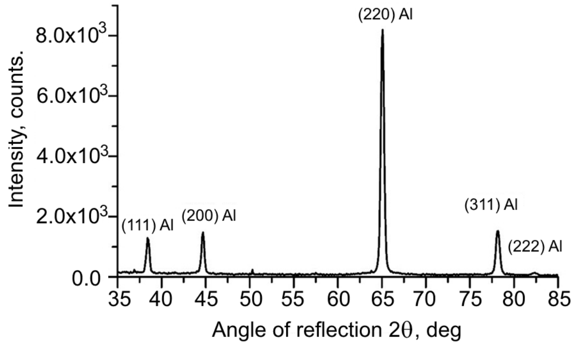

Fig. 1 Results of XRD phase analysis

the base of the material is aluminum, other impurities: Mn (0.8 wt %); Fe (0.3 wt %); Zn (0.1 wt %); Cr (0.05 wt %), Ti (0.2 wt %) and Pb (0.01 wt %). This composition is completely consistent with the aluminum alloy AMC.

Thus, traditional X-ray methods failed to reveal differences in the structure and chemical composition of the surfaces of the objects, although visually the differences were quite clearly observed.

3.3 Compton scattering method

To characterize the surfaces for the presence of elements with low atomic numbers, the Compton scattering method was used [8]. We have found a significant difference in the ratios of the integrated intensities of the peaks of incoherent I_C (Compton) and coherent I_R (Rayleigh) scattering on different sides of samples (Table 1). Let us discuss the results of Table 1. According to the effective atomic number Z_{eff}=13.15±0.55, the light side corresponds to the standard of pure aluminum with Z = 13. The dark side is characterized by a value of Z_{eff}=11.27±0.46, which is probably associated with the oxidation of the aluminum alloy as a result of the explosion.

3.4 Detection of oxygen by absorption method

Non-destructive assessment of oxygen content in a foreign body is a major challenge.

Table 2. Integral intensity of Al-***K***α and Mn-***K***α lines from the light and dark sides of the samples

	Light side		Dark side	
	Sample 1	Sample 2	Sample 1	Sample 2
$I_{Al\text{-}K\alpha}$, counts	6643	6786	1610	2334
$I_{Mn\text{-}K\alpha}$, , counts	34759	39200	29799	37400
$I_{Al\text{-}K\alpha}$ / $I_{Mn\text{-}K\alpha}$	0.191	0.173	0.054	0.062

Indeed, it cannot be quantitatively measured by the intensity of the X-ray fluorescence line due to the known limitation of this method in terms of the range of chemical elements analyzed. Therefore, to confirm our assumption, we studied the absorption properties of the surface layer.

The mass coefficient of attenuation of aluminum radiation by oxygen atoms $\mu_O^{Al-K\alpha} = 1530$ is several times greater than for aluminum atoms $\mu_{Al}^{Al-K\alpha} = 422$. Therefore, the oxide layer should noticeably absorb the fluorescent radiation of the sample bulk, leading to a significant decrease in the intensity of the Al-*K*α line (λ=8.3Å) on the dark side compared to the light side. Let us estimate the ratio of the Al-*K*α line intensities for the light and dark sides of the sample. When oxidized to Al_2O_3, the mass coefficient of attenuation of Al-*K*α radiation by surface atoms will be: $\mu_{Al_2O_3}^{Al-K\alpha} = C_{Al}\mu_{Al}^{Al-K\alpha} + (1 - C_{Al})\mu_O^{Al-K\alpha}$ where $C_{Al} = 0.65$ is the mass fraction of aluminum in Al_2O_3. Using the formula $I^{Al-K\alpha} \sim C_{Al}/\mu_{Al}$ we get $I_{Al_2O_3}/I_{Al} = C_{Al_2O_3}^{Al}\dfrac{\mu_{Al}^{Al}}{\mu_{Al_2O_3}} = 0.33$.

The experimental data presented in Table 2 correspond to this assessment.

The depth of the information layer for the Al-Kα line in Al_2O_3 is about 4 μm. Normalization to the intensity of the manganese impurity line, which is present in the alloy, allows us to take into account the influence of roughness on the weakening of the aluminum line. Thus, on the dark side of the foreign body there is aluminum oxide Al_2O_3, the thickness of which exceeds 4 microns.

3.5 Microelement analysis

An unexpected result was the detection of a trace amount of bromine on the dark side, which has a developed rough surface. Its Br-*K*α line is clearly visible along with two lines of lead present in the alloy (Fig. 2). It is noteworthy that this line is not visible from the light side.

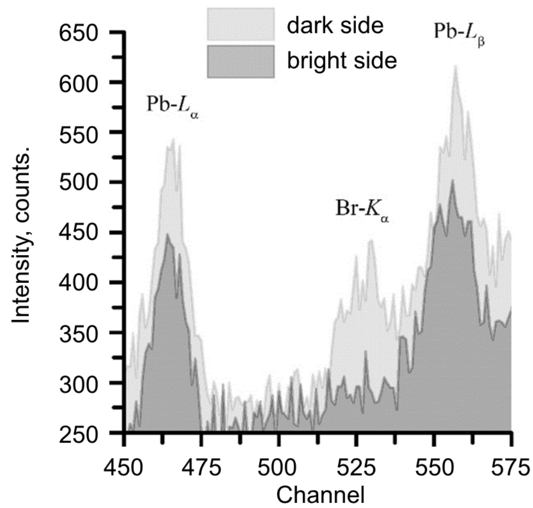

Fig. 2 Fragments of the X-ray fluorescence spectra of the dark and light sides of a foreign body

Experimental estimation of the concentration of bromine gives the value from 20 to 50 ppm. The origin of this micro-impurity is of greatest interest. Bromine is not included in the composition of the main material – AMC alloy. A possible source could be the products of the combustion of an explosive substance, however, no traces of other microelements characteristic of an explosive substance (sulfur, phosphorus, etc.) were detected in the fluorescence spectrum. In addition, bromine has a low evaporation temperature of 255ºC and must completely evaporate at the high explosion temperature. Obviously, the source of bromine on the surface of foreign bodies is the human body.

3.6 Possible functions of bromine trace impurities

The concentration of bromine in the blood does not exceed 12 ppm; in soft tissues it is significantly less. According to the concepts of the kinetics of heterogeneous reactions [9], the adsorption of bromine from the blood (electrolyte) will primarily occur on the more developed (rough) surface of the foreign body. It is known [10] that bromine is a strong oxidizer of metals and forms bromides ($AlBr_3$, Al_2Br_6, etc.). In materials science, it is used in microquantities in electrolyte solutions to accelerate the etching reaction and electropolishing of metal surfaces. The metal fragments were in the soft tissues for only a few hours. Apparently, the accumulation of bromine on the developed surface is the primary reaction of the body to a foreign body, which promotes the formation of collagen [11] and subsequent encapsulation of the foreign body.

We did not find any traces of calcium, which is part of the capsule, on the surface of foreign bodies. This means that the process of encapsulation of a foreign body in soft tissues does not occur during the first hours after injury. Thus, the primary reaction of the body to a foreign body is the appearance of bromine on its most developed surface. Subsequently, bromine activates the formation of collagen and encapsulation of the foreign body.

4.Conclusions

For the first time, a complex of X-ray methods: XRD, XRF and the Compton scattering method – was used to study foreign bodies extracted from soft tissues. The fragments were removed from the body before the wound healing process began.

We observed increased bromine levels at the site of damage to human physiological tissue. It is the increased content of bromine that contributes to the additional formation of sulfilamine to increase the tensile strength of the collagen IV molecular corset. And this leads to the accelerated restoration of the architecture of the basement membrane of cells and the development of tissues. Perhaps the increased level of bromine in damaged human tissues stimulates the production of tissue hormones that will act on the restoration of damaged cells. This is speculation, but it is known that bromide stimulates the production of thyroid hormones. This idea requires further research.

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