POSSIBLE APPLICATIONS OF SCANNING ELECTRON MICROSCOPY IN STRUCTURE AND COMPOSITION ANALYSIS OF INTRA-ARTICULAR DISTRACTED FORMATIONS OF PROXIMAL INTERPHALANGEAL JOINTS IN HUMANS

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Abstract. This research evaluates particularities of surface structural organization of surgical samples of intracapsular distracted formations in treating post-traumatic fibrous ankylosis of proximal interphalangeal joints by means of scanning electron microscopy. The research is carried out on small-fragment samples of intracapsular formations collected in the course of treatment from 8 patients of the Microsurgical Department. Morphological evaluation of the surface of two types of samples was performed: that of proximal interphalangeal joint capsules, and of the regenerate itself. Structural analysis of the surgical samples allowed to identify considerable differences both in form and size of single structure-forming elements, and in their organizational types. It was established that the regenerate has a more compact structure. In the course of microanalysis of elemental composition, it was ascertained that mass percentage of calcium and phosphorus in the examined regenerate samples is 4.4 and 2.2 times respectively higher compared to the capsule samples. These results could be used in further research into approaches to the evaluation of connective tissue regeneration after surgeries, as well as in making recommendations for operative or physiotherapeutic patient treatment in cases of joint capsule distraction.

Keywords: scanning electron microscopy, X-ray microanalysis, calcium, phosphorus, proximal interphalangeal joint, distraction, fibrous ankylosis, regeneration.

Introduction

Proximal interphalangeal joint damage is one of the most common hand injuries. (Novikov *et al*., 2017). Ankylosis is joint immobility resulting from pathological change within it. It is followed by degeneration of cartilaginous cover of articular surface with fibrous connective tissue or bone tissue overgrowth. There is a number of works dedicated to the description of methods of approaching treating post-traumatic contractions in proximal interphalangeal joints of hand finger bones (Fasakhov *et al.*, 2022). The main difficulty in treating post-traumatic finger joint deformations, often followed by the development of fibrous ankylosis of proximal interphalangeal joints, lie in the fact that it is necessary both to eliminate finger deformation and to restore joint mobility for the hand to be functional. In order to achieve this, a two-step approach is currently applied, where first a transosseous apparatus is applied and a gradual broadening of joint space is achieved, and later, the apparatus having been removed, arthroplasty with removal of intra-articular formations blocking the movement is performed (Oak & Lawton, 2013).

In practical orthopedics, to control the activity of distraction osteogenesis, as a rule, radiography is used, less often ultrasonography, and even more rarely biochemical blood tests.

The level of mineralization is assessed using quantitative computed tomography (QCT), single photon absorptiometry (SPA), dual energy x-ray absorptiometry (DEXA) (Markel & Chao, 1993). The presence «ischemic» distraction regenerate in patients with bone defects and false joints, characterized by the predominance of the connective tissue layer over the area of the bone sections, was confirmed by X-ray and sonographic research methods, as well as coherence tomography data (Borzunov & Shastov, 2019). Some features of the mineralization of the organic matrix of distraction regenerates were studied using biochemical

methods (Kamerin *et al.*, 1977; Matveenko *et al.*, 1981). These methods allow to make an indirect, very approximate assessment of the outcome of long-term treatment. Therefore, for many decades, studies have been ongoing to control the structure and composition of the emerging bone callus and to search for the most optimal control methods for the development of the mechanical strength of bone tissue in the treatment of patients. It is possible to study the volume-spatial organization of regenerating bone tissue, describe the ultrastructural features of osteogenic cells of various types of bone tissue, evaluate the biosynthetic activity in the areas of bone tissue growth, as well as the content of calcified components using modern electron microscopic methods.

Application of scanning electron microscopy methods allows to perform high-quality visualization and collection of exhaustive information regarding surface structure of different types of samples, including those obtained on different stages of surgery during treatment.

Purpose of the research: scanning electron microscopy evaluation of particularities of surface structural organization of surgical samples of intracapsular distracted formations in treating post-traumatic fibrous ankylosis of proximal interphalangeal joints.

Materials and Methods

The research was carried out on small-fragment samples of intracapsular formations collected from 8 Microsurgical Department patients with post-traumatic fibrous ankylosis of proximal interphalangeal joints. All patients signed a voluntary informed consent for material sampling, the presented work was a fragment of the state assignment «Justification and development of innovative technology for arthroplasty using autologous MMSC», the study was approved by the local ethics committee of the Federal State Budgetary Educational Institution of Higher Education «Privolzhsky Research Medical University» of the Ministry of Health of the Russian Federation (extract from protocol № 2/20.02.2016).

The research was carried out on two types of samples, a proximal interphalangeal joint capsule, and a regenerate. After preliminary intra-operative dissection of the capsule wall from collateral ligaments along the neutral axis on the radial or ulnar side, micro-fragment research samples (up to 1.5 mm) were extracted.

Electron microscopy research of thus prepared samples was carried out in the research equipment sharing center «New Materials and Resource-Saving Technologies» of Lobachevsky State University of Nizhny Novgorod on the scanning electron microscope JSM-IT300LV (JEOL) in low vacuum mode, which ensured neutralization of adverse influence of sample surfaces static non-stationary charge effect on the image quality during observation. Measuring current was set at low values ($\leq$ 0.1 nA). In order to reduce the impact of electron beam on the researched samples, electrone probe diameter was set up to 5 nm, accelerating voltage 20 kV. X-ray microanalysis of single local areas was carried out with the help of EDS-detector X-Max^N 20 (Oxford Instruments) using Aztec software, elemental composition calculation was carried out by fundamental parameter method.

In order to be observed in the electron microscope, the researched samples were extracted from fixing formaline solution, air-drained on filter-paper support and transferred onto carbon adhesive tape support (Fig. 1.) Additional sample preparation and staining were not applied. On examination completion samples were returned into 10% formaline solution for archiving.

Images, obtained in the backscatter detector (BED-C), allowed to evaluate compositional contrast (Z-contrast) on the researched sample areas. Within the low vacuum secondary electron detector (LVSED) signal, topographic contrast, i.e. surface irregularity of the samples, was mostly detected. A photo archive of 262 electron microscopic images of various areas of the researched samples was created. In the course of X-ray microanalysis of surgical specimens, 137 spectra were evaluated.

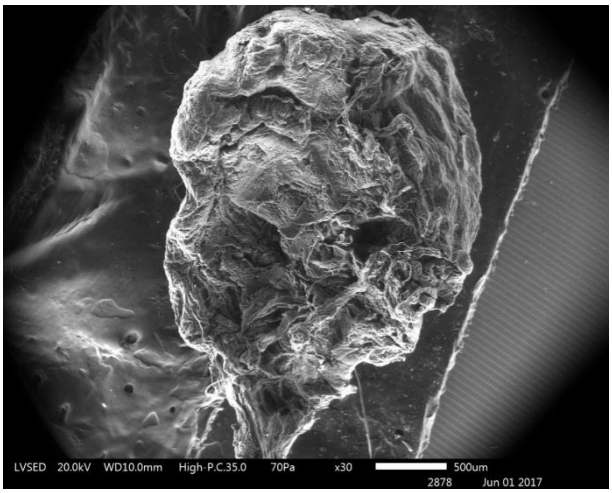

Fig. 1. An example of researched sample positioning on the carbon adhesive tape support. Zoom x30. Low vacuum mode

Statistical result analysis was carried out using specialized Statistica 6.0 (StatSoft Inc., USA) software. After elemental microanalysis data collection, arithmetic mean of calcium and phosphorus content in conclusive sample areas, as well as error of mean were calculated. Student's t-test was applied to evaluate relevant variances between readings. Differences between groups were considered to be statistically significant with $p < 0.05$.

Results

Electron microscopy image analysis allowed to identify structural particularities of the two sample types. The researched regenerate samples demonstrated compact structure, close-packed fiber arrangement with single filamentary structures, aligned with the main structure. There were some areas of surface relief changes with single cracks (Fig. 2 and 3).

In some preparations both single erythrocytes as well as their clusters were observed, mostly in the areas of relief change with fibrination (Fig. 2b) and thrombus formation on the regenerate surface (Fig. 3a and 3b, Fig. 4b). This result indicates the formation of numerous vessels in the zone of distraction regenerates.

In the works of some authors studying the microcirculatory bed in the layer of distraction regenerates (Mikhailova & Shtin, 1978; Mikhailova & Shtin, 1979), local microcirculation disorders with the release of blood cells and the formation of erythrocyte columns located along collagen fibers were also noted, which is interpreted by the authors as the presence of open blood flow in the layer.

In some preparations formation of fibrin filaments was observed in the peripheral zone of the regenerates and could be especially well visualized with high zoom definition in low vacuum mode (Fig. 4).

Study of proximal interphalangeal joint capsule surface allowed to identify irregular fiber arrangement in these types of samples, with thin filamentary structures and wide «cracks», as well as single areas without filamentary structures. High zoom values allowed to discover fibrination aligned with the fibers of proximal interphalangeal joint capsule (Fig. 6).

Thus, structure analysis of two types of operational samples allowed to identify considerable differences both in form and size of single structure-forming elements, and in their organizational type. Special attention was drawn to the central area of one of the samples with a big number of irregularly-shaped elements (Fig. 7) which on close inspection were identified as bone chip fragments, introduced into the sample during its extraction in surgery. Thereby, an X-ray microanalysis of the operational samples proved to be especially interesting.

In the course of the compositional elemental microanalysis distinctive X-ray signal mostly from carbon (C), nitrogen (N), oxygen (O), phosphorus (P), sulphur (S), clorine (Cl) and calcium (Ca) was registered. Less frequently a signal from aluminium (Al) was registered, which was connected with diffuse radiation from aluminium substrate holder. The researched samples by approximately 90% consisted of carbon, nitrogen and oxygen, but the main attention was concentrated on evaluation of composition differences in calcium and phosphorous, as the two most characteristic for this type of tissue elements, defining bone tissue formation process.

In the next step of our research, an elemental microanalysis of calcium and phosphorous con tent was carried out on the samples not containing big particles (Fig. 8) and thus, representing proper sample tissue.

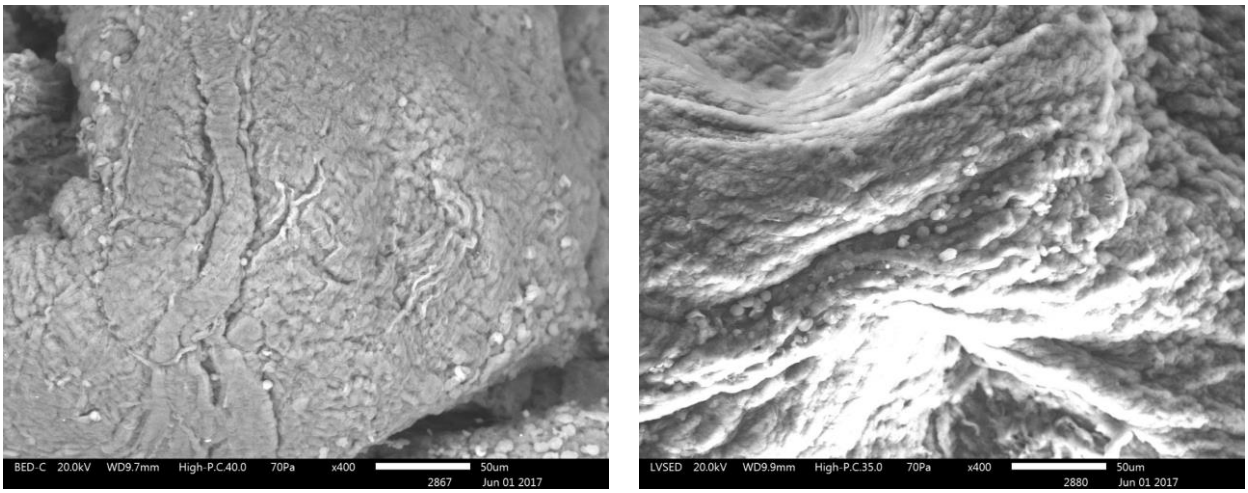

Fig. 2. Study of the regenerate sample surface by means of scanning electron microscopy. Zoom x400. a) Backscatter detector images, b) low vacuum mode

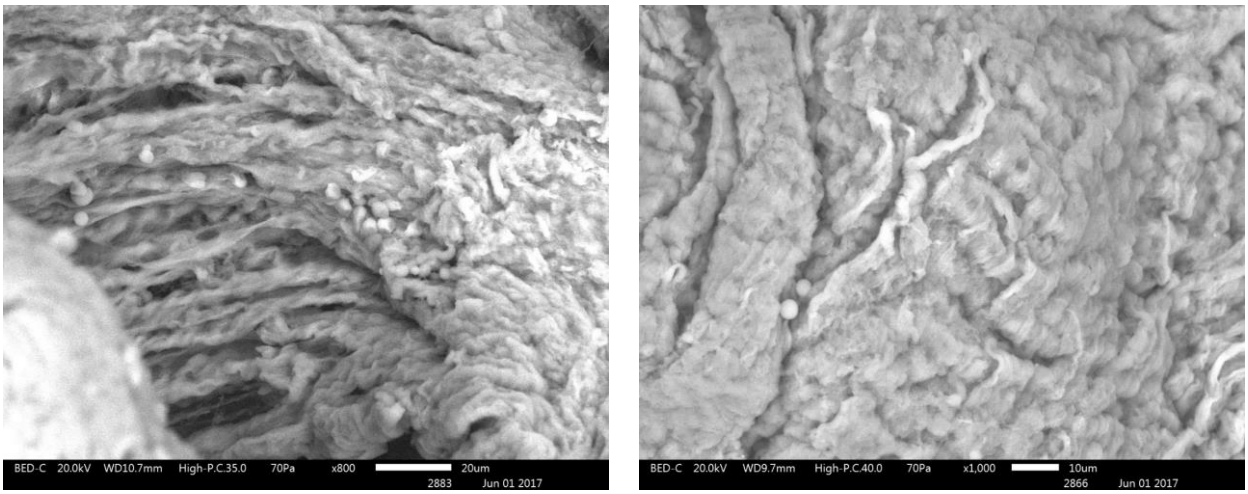

Fig. 3. Study of the regenerate sample surface by means of scanning electron microscopy. Backscatter detector images. a) Zoom x800, b) Zoom x1000

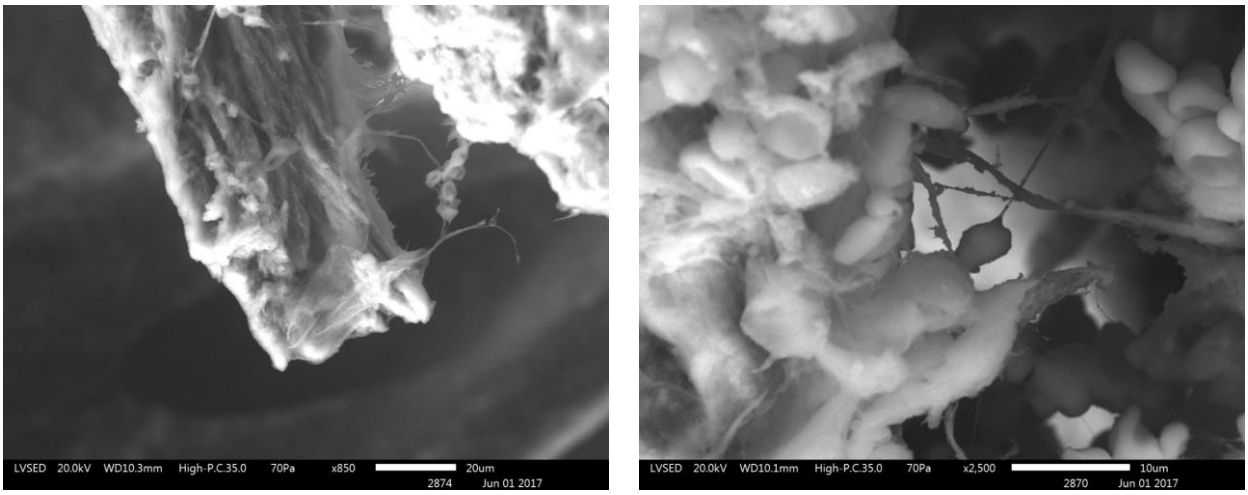

Fig. 4. Study of the regenerate sample surface by means of scanning electron microscopy. Low vacuum mode. a) Zoom x850, b) Zoom x2500

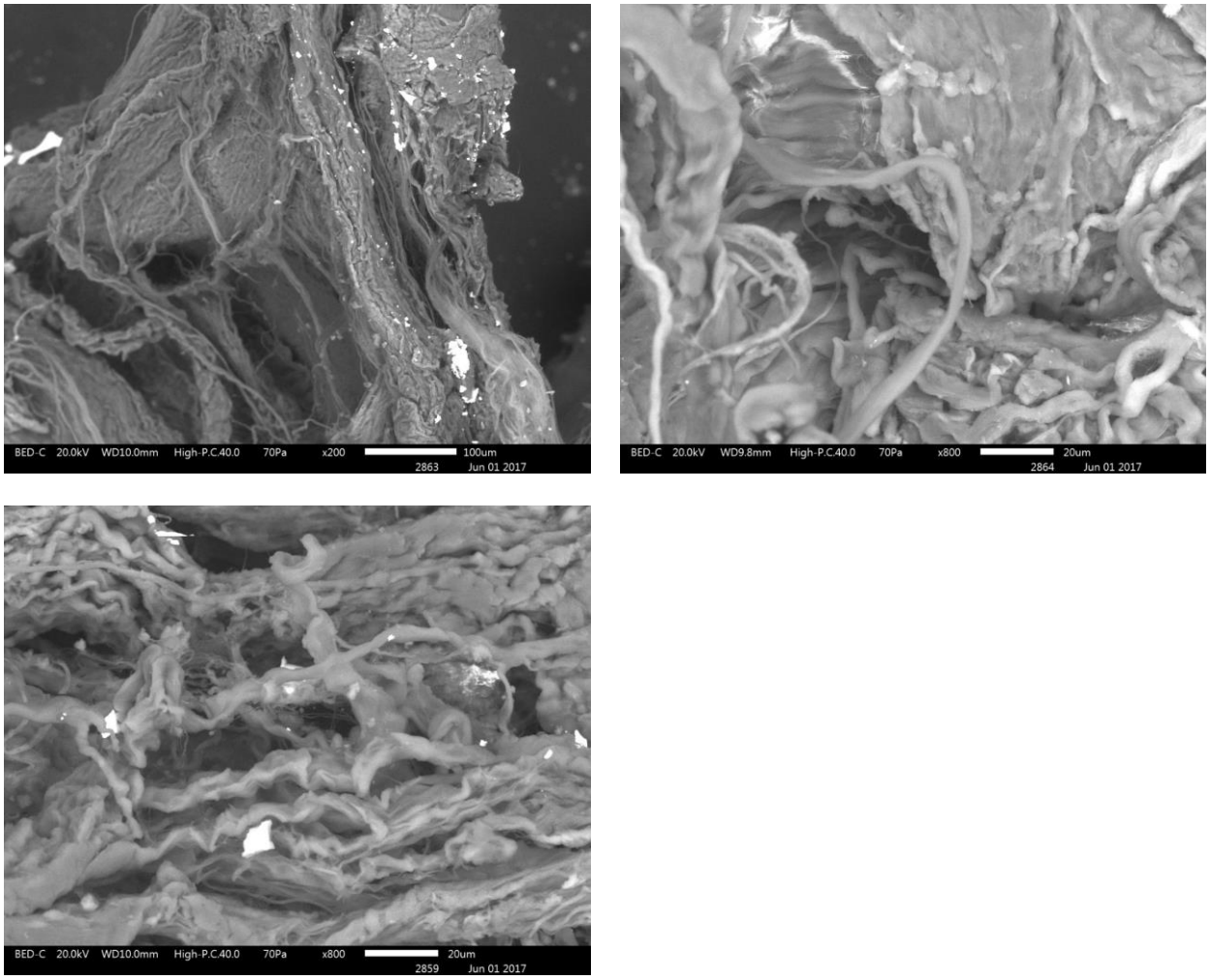

Fig. 5. Study of proximal interphalangeal joint capsule surface by means of scanning electron microscopy. Backscatter detector images.
a) Zoom x200, b) and c) Zoom x800

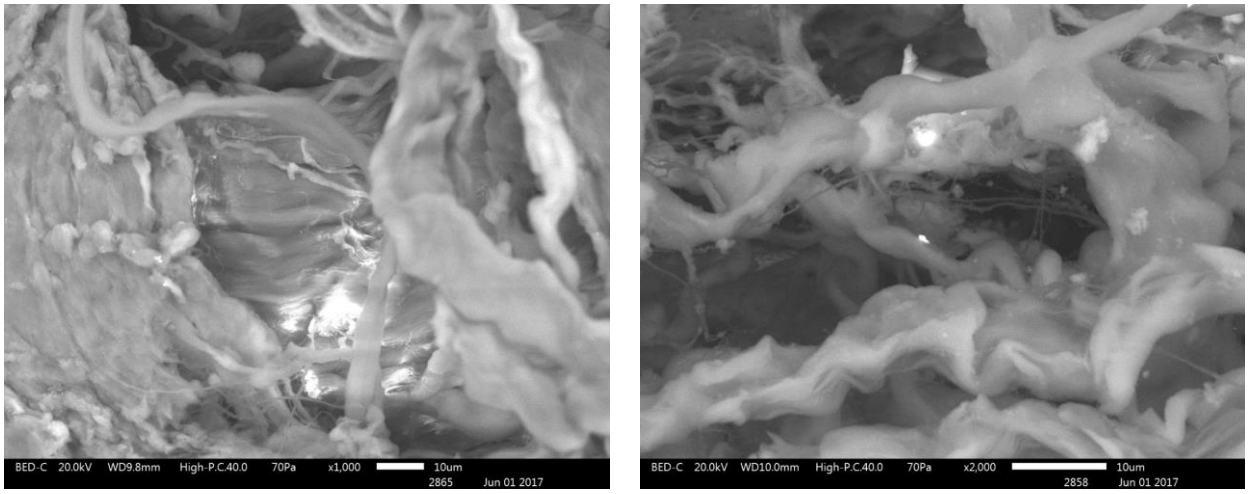

Fig. 6. Study of proximal interphalangeal joint capsule surface by means of scanning electron microscopy. Backscatter detector images.
a) Zoom x1000, b) Zoom x2000

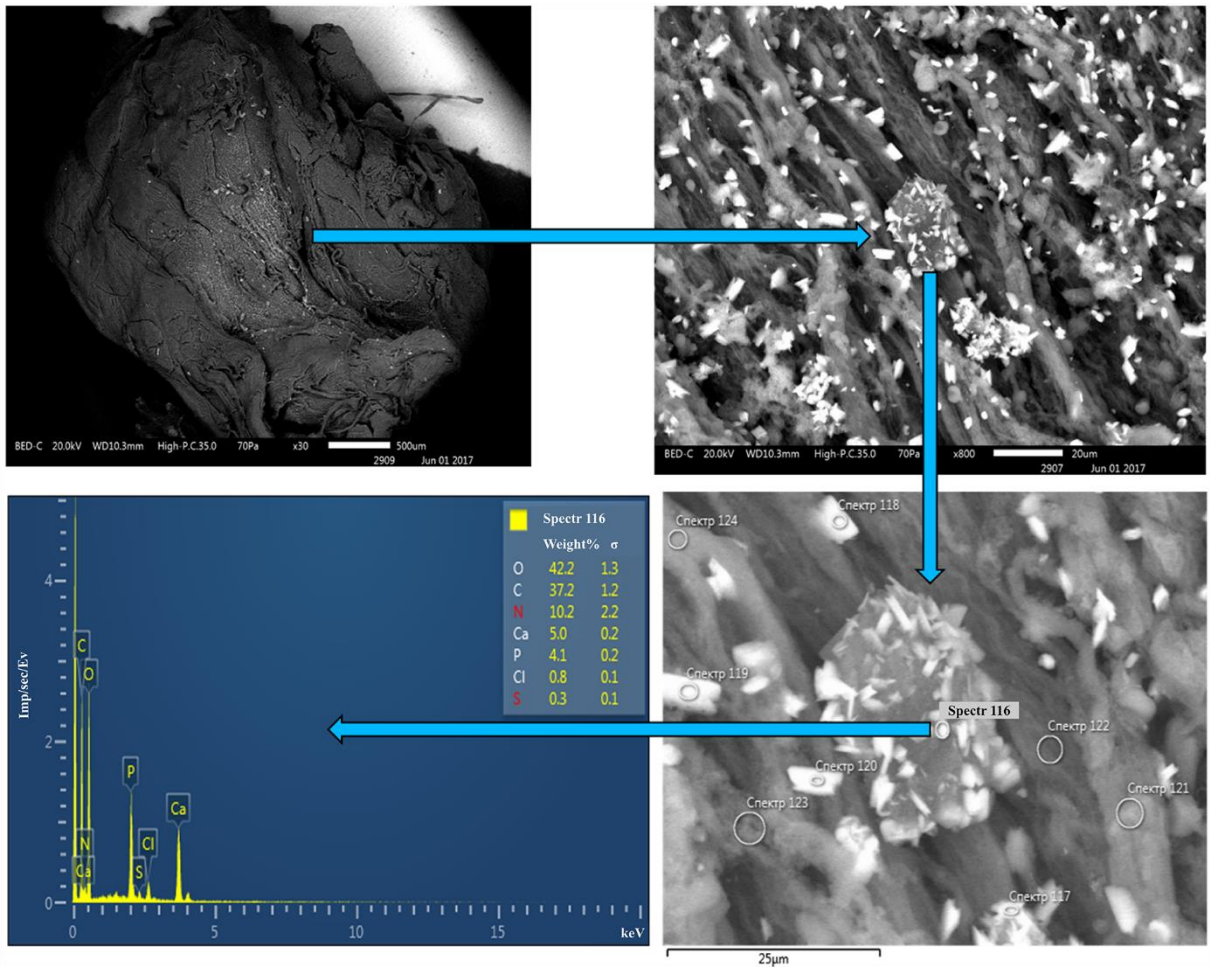

Fig. 7. Study of proximal interphalangeal joint capsule surface by means of scanning electron microscopy, the choice of area for elemental microanalysis. Spectrum example. Patient D. Backscatter detector image

Spectral analysis for informative correlation of the obtained data and statistical analysis demonstrated that calcium mass percentage is 4.4 times and phosphorous mass percentage 2.2 times higher in regenerate samples compared to those of proximal interphalangeal joint capsule samples (Fig. 9). Content difference of microelements in the capsule samples are statistically significant ($p < 0.001$).

Discussion

Despite a significant number of studies on various issues of distraction osteosynthesis, many aspects of this problem are still debatable. Information about the sources of development of osteogenic cells, the features of their proliferation, differentiation and specific functioning in distraction regenerates is contradictory. The effect of distraction on the volume-spatial organization and mineral composition of bone regenerates has not been sufficiently studied (I′ryanov, 1998). From the beginning of the use of compression-distraction devices, researchers have been interested in the issues of control in clinical practice of the processes of compaction and hardening of the regenerate, the influence of loads on the regenerate, as well as the patterns of distraction osteogenesis and the possibilities of conscious control of the biomechanical system «apparatus-segment» in the treatment of patients (Vvedenskiy, 2013).

Access to intra-articular pathological formations is currently possible in two ways. At once, by traumatic wide access through the joint

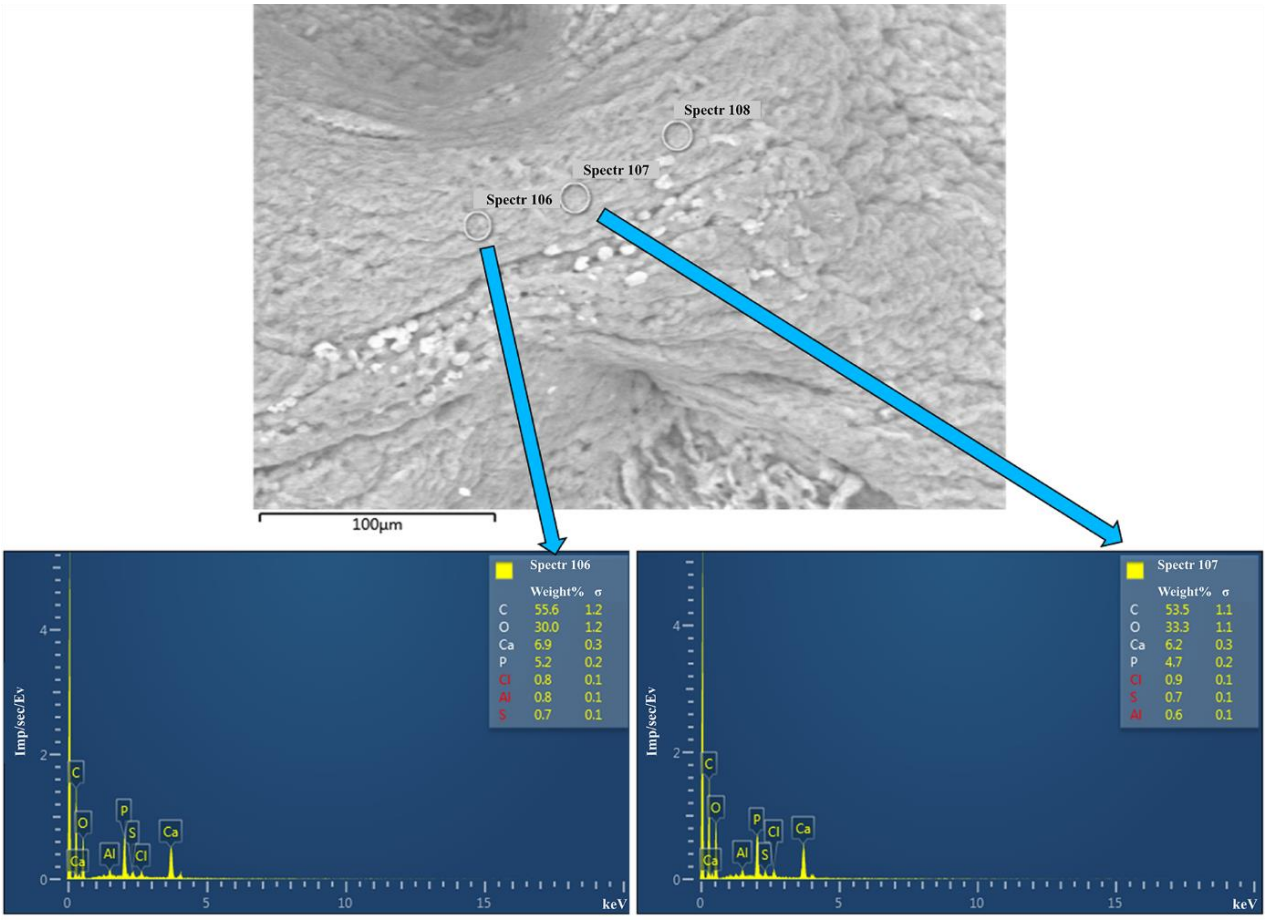

Fig. 8. An example of choosing information region,
and spectral data collection in elemental microanalysis, a regenerate sample

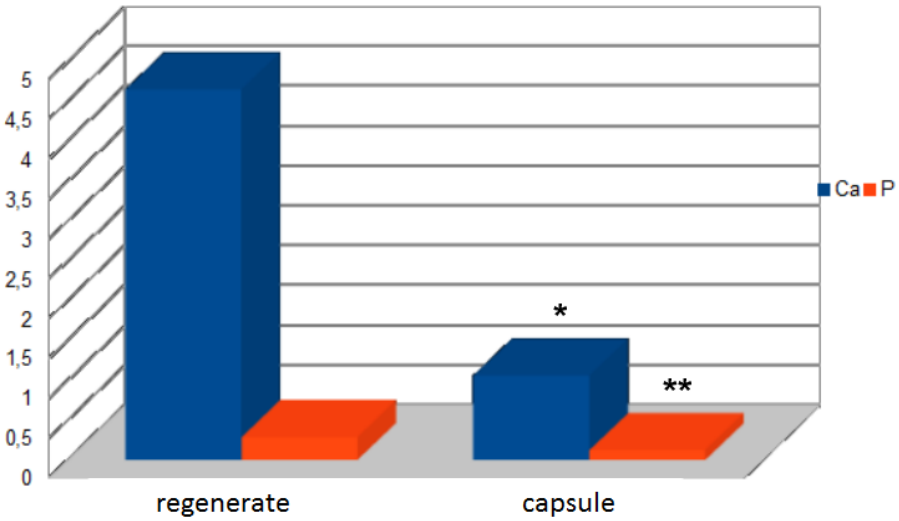

Fig. 9. Calcium and phosphorous content comparison for information regions of regenerate and capsule samples, weight % (mass percentage in the researched regions)
Note: * and ** – calcium and phosphorous content differences in capsule and regenerate samples are statistically significant ($p < 0.001$).

capsule with dissection and excision of intra-articular pathological structures that limit the mobility of the articular ends or apply a two-stage technology of arthroplasty: at the first stage, apply a transosseous apparatus, with which to gradually expand the joint space up to 10-12 mm, at the second stage, remove the distraction apparatus and through the incision in the distracted capsule along the neutral line and only in the projection of the enlarged joint space remove distracted intra-articular formations. However, resection arthroplasty with dosed distraction and the use of an external fixation device is not accompanied by restoration of the structure and function of the joint capsule (Lantsov & Malanin, 2007).

A number of studies have shown that the distraction regenerate is represented by two vascularized bone sections separated by a connective tissue layer with a poorer network of newly formed vessels (Li *et al.,* 2006; Kojimoto *et al.* 1988; Isaac *et al.,* 2008).

If the area of the connective tissue layer prevails over the bone sections then a cartilaginous fibrous tissue is formed, devoid of blood vessels, with the development of pseudarthrosis as a result at the level of the distraction regenerate (Emara *et al.*, 2011).

The occurrence of desmal bone formation and its activation in the process of distraction is associated with abundant capillarization of the osteogenesis zone; at the end of stretching, the capillary network becomes intraosseous (Shtin & Gulnazarova, 1974).

However, it has also been shown that stretching can support hypoxia, which is a specific stimulator of bone formation. At the same time, collagen fibers play the role of a «conductor» of osteogenesis and are reinforcement for the neoformation of bone structures. The authors indicate the centripetal nature of the growth of longitudinally oriented bundles of collagen fibers, along which proliferating cells of the osteobrastic series are located, acquiring signs of fibroblasts (Shtin, 1974).

In experimental studies on the dynamics of the increase in the rigidity of the regenerate, changes in its viscoelastic properties in the treatment of fractures using the method of external fixation, the non-linearity of the increase in the rigidity of the regenerate was confirmed and the two-phase nature of this process (Mark *et al.*, 2003; Chehade *et al.*, 1997; Hente *et al.*, 2003).

In the first phase, the regenerate is more viscous and plastically deformable. In the second phase, the regenerate acquires greater elasticity and resilience, which is associated with the natural morpho-functional restructuring of the emerging callus. The duration of the phases and their ratio depend on many factors, to a greater extent on the biomechanics of the «device-segment» system. Adaptive mechanisms that develop a response to distraction are accompanied by pronounced manifestations of neoangiogenesis (Shevtsov & Ir´yanov, 1998; Makarov *et al.,* 2009). The study of the features of vascularization of distraction regenerates showed that during the period of distraction and subsequent fixation in the apparatus, the formation of numerous capillary, arterial and venous vessels in the endosteum, periosteum and compacta plates occurs with their simultaneous restructuring in the newly formed area of the tibial diaphysis.

Understanding the morphological changes occurring in the distracted soft tissues would make it possible to largely determine the tactics of treating post-traumatic ankylosis and justify the use of the distraction method as a preparatory stage of bioactive arthroplasty. In the work of colleagues (Bugrov *et al.*, 2016) it was shown an increase in the volume of the microcirculatory bed, the ordering of the architectonics of the connective tissue of the capsule, an increase in the number of fibroblastic cells after distraction and, thus, the effectiveness of the stage of bioactive arthroplasty of fibrous ankylosed hand joints.

The data of electron microscopic studies of these objects are not numerous, they are mainly represented by the results of transmission, extremely rarely - scanning electron microscopy and are often contradictory, as they demonstrate different stages of the process of reparative regeneration. It was important for us to evaluate the surface of the regenerate and the capsule as an accessible material that was removed to achieve an optimal clinical result at the stage of

treatment. We evaluated the material in the terminal phase when the surgeon had already made a decision to remove the formed fragments and surgically restore the mobility of the patients' hands. The obtained electron microscopic data on the structure and composition of the regenerate and the capsule partially agree with the data available in the literature on the growth of distraction regenerates due to the «fibrous-tissue» layer with actively functioning cells in the «growth zone» of the regenerate.

Thus, it is possible to draw the following conclusions based on the research results:

1) Morphological evaluation of regenerate and capsule surfaces demonstrated that regenerate has a more compact structure with close-packed fiber arrangement and single filamentary structures. Some preparations had single erythrocytes and their clusters, mostly in the areas of relief change with fibrination and thrombus formation on the regenerate surface.

2) Study of proximal interphalangeal joint capsule surface allowed to identify irregular fiber arrangement in these types of samples, with thin filamentary structures and wide «cracks», as well as single areas without filamentary structures. It was discovered that proximal interphalangeal joint capsules have a microstructural organization particularity, fibrination aligned with the fibers.

3) In the course of elemental microanalysis, it was demonstrated that calcium mass percentage in regenerate samples is 4.4 times higher compared to the capsule samples.

4) Phosphorus mass percentage analysis demonstrated that its content is 2.2 times higher in regenerate samples than in capsule samples.

Conclusion

This research demonstrates possibilities of complex application of scanning electron microscopy for visualization, architectonics evaluation and elemental analysis of biological samples on the material of intracapsular distracted formations of proximal interphalangeal joints in humans, in different steps of treatment. These data could be used in further research into approaches to evaluation of post-surgery connective tissue regeneration process, and in working out recommendations for operative or physiotherapeutic patient treatment in cases of joint capsule distraction.

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