Шановні колеги! У рубриці „Методологія наукових досліджень" редакція продовжує публікацію матеріалів, що пов'язані з найважливішими аспектами наукової діяльності: організаційно-методичним забезпеченням наукових видань, загальними принципами статистичного, біометричного і математичного супроводження досліджень, а також оригінальними методичними підходами вітчизняних і зарубіжних морфологів.

Ye.V.Kuzenko

Sumy State University

Key words: tissue paraffin sections, morphological analysis, scanning electron microscopy.

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METHOD: CHEMICAL AND MORPHOLOGICAL STUDYING OF PARAFFIN SECTIONS

SUMMARY. Background. Interactions between heavy metals and cells are diverse, but can be divided into 3 major categories: 1) metals are essential for metabolism. Toxic metals can stop metabolic reactions; 2) metals can accumulate in cells: intracellular uptake and binding; 3) metals that undergo biochemical transformation (inclusive of leaching). The main objectives in this study were to develop a appropriate methodology to allow histological sections scanning electron microscopy analysis of tissue samples and to apply this and a number of other analytical techniques, to investigate the nature of calcific and heavy metals deposits in tissues and cells. **Objective.** Aim of this study was to find if scanning electron microscopy can be used in chemical composition of tissue. **Methods.** For recognition of various types of tissue paraffin sections and the rate of accumulation of heavy metals in it was used scanning electron microscope equipped with energy dispersive X-ray spectroscope. **Results.** Energy dispersive X-ray spectroscopy analysis revealed that inorganic phases of tissue paraffin sections were available for chemical analysis. Scanning electron microscopy were used for morphological analysis of paraffin sections. **Conclusion.** Rationale and description of the new method of chemical and morphological studying of paraffin sections presented in the article. Scanning electron microscope equipped with energy dispersive X-ray spectroscope can be used in chemical composition of tissue.

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✉ **kuzenko_yevhen@rambler.ru**

Кузенко Є.В. Метод: хімічне та морфологічне дослідження парафінових зрізів.
Реферат. Мета даного дослідження полягала в обгрунтуванні застосування скануючої електронної мікроскопії для визначення хімічного складу тканини. Для розпізнавання різних типів парафінових тканинних зрізів і ступеня накопичення важких металів в них використовувався скануючий електронний мікроскоп, оснащений рентгенівським спектроскопом. Рентгеноструктурний аналіз показав, що неорганічні компоненти в парафінових тканинних зрізах були доступні для хімічного аналізу. Скануюча електронна мікроскопія була використана для морфологічного аналізу парафінових зрізів. У статті представлені обгрунтування і опис нового методу хімічного та морфологічного вивчення парафінових зрізів за допомогою скануючої електронної мікроскопії та рентгеноструктурного аналізу.
Ключові слова: тканинні парафінові зрізи, морфологічний аналіз, скануюча електронна мікроскопія.

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Background

Heavy metals from manmade pollution sources are continually released into aquatic ecosystems. The contamination of heavy metals is a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain [1].

Human cells employ metals, such as calcium,

copper, zinc, and iron, to control significant metabolic, signaling functions - serum or extracellular fluid at concentrations that form a “meta-stable” solution and making them essential for life. The pathogenesis of metastatic and dystrophic calcification at the cell level is partially understood. Both processes typically involve mineral accumulation within matrix vesicles [2].

Other metals can be potentially toxic such as the heavy metals: lead, cadmium, mercury, and thallium. Lead in particular, is a neurotoxin that has been linked to visual deterioration [3], central and peripheral nervous system disorders [4], renal dysfunction [5], and hypertensive cardiovascular disease [6].

Interactions between heavy metals and cells are diverse, but can be divided into 3 major categories: 1) metals are essential for metabolism. Toxic metals can stop metabolic reactions; 2) metals can accumulate in cells: intracellular uptake and binding; 3) metals that undergo biochemical transformation (inclusive of leaching).

The main objectives in this study were to develop a appropriate methodology to allow histological sections scanning electron microscopy (ESEM) analysis of tissue samples and to apply this and a number of other analytical techniques, to investigate the nature of calcific and heavy metals deposits in tissues and cells.

Objective

Aim of this study was to find if scanning electron microscopy can be used in chemical composition of tissue.

Materials and methods

The study samples consisted of epulis. People who had clinical diagnosis of epulis (20). Only patients that had epulis formations were included into the study cohorts. Briefly, 4μm thick tissue sections were placed on the graphite plates (fig. 1).

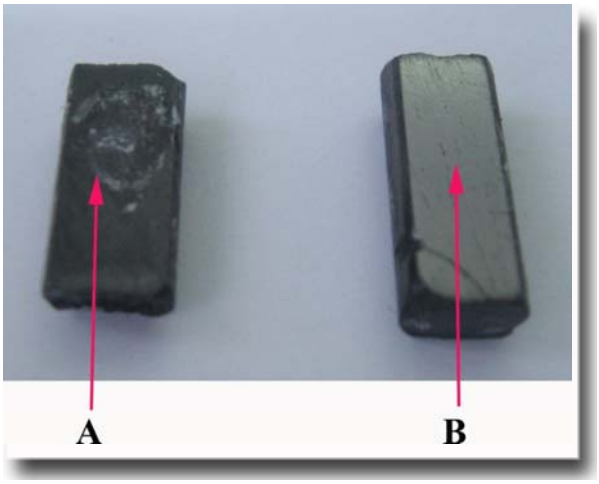

Fig. 1. A – graphite plates with tissue sections, B – graphite plates without tissue sections.

Paraffin sections are immersed into three sets of xylene for 10 minutes each followed by three sets of absolute ethanol for 10 minutes and finally rinsed with distilled water. Slides are placed into haematoxylin for 5 minutes and 96% alcohol for 10 minutes, rinsed thoroughly under distilled water for approximately 4–5 minutes.

At the specified times, the specimens were examined for topographicalchanges using Scanning Electron Microscope (SEM) equipped with Energy Dispersive X-ray Spectroscope (EDS). The surface levels of copper elements were measured as weight percentage. Each sample was exposed to radiation at the center and in 2 additional equidistant areas at a voltage of 15KV for 60 seconds and the average figure was calculated for each specimen.

These X-rays are detected by EDS and the results plotted as a spectrum. Each element has its own ‘fingerprint’ of peaks which allows both a qualitative and quantitative determination of the elements present in the selected region of the sample. Intensities are measured by counting photons and the precision obtainable is limited by statistical error. For major elements it is usually not difficult to obtain a precision (defined as 2σ) of better than ± 0.01% (relative), but the overall analytical accuracy is commonly nearer ± 0.09%.

Results and Discussion

Sections may be placed directly into the high vacuum of an electron beam instrument and immediately provide significant information. Depending on the inherent stability of the specimen, varying degrees of sample preparation will be necessary to ensure that the maximum amount of information may be derived. As with other bulk SEM specimens, biological specimens must be free of foreign particles, stable in vacuum, stable in the electron beam, electrically conductive, and must be unaltered in chemistry and morphology.

The operator uses the objective lens to focus the tissue sections. Since an increase in magnification simplythe tissue sections, it makes sense to do fine focusing at high decrease your magnification the focus will be very good.So, a sense of the highest magnification at which you wish to obtain images, corresponds to a smaller rastered area magnification stissue sections (fig. 2).

The microscopic examination of the SEM section showed a tissue with pyogenic epulis. The pyogenic epulis (fig. 2B) was presented in the form of fibrocellular structures with abundant blood supply (fig. 2C). There were observed numerous endothelium lining the vascular spaces, with apparent proliferation of endothelial cells and fibroblasts. A moderate degree of chronic inflammatory cell infiltratea, hemorrhage (fig. 2A) composed chiefly of lymphocytes and plasma cells.

For qualitative analysis the procedure were used conductive plates However, to eliminate systems peaks of aluminum and copper, it is useful to use carbon planchets or stubs for specimen mounting. For quantitative analysis the procedure is the

same as described in materials and methods, except that these samples must be "perfectly" flat, which precludes etching. The region of interest may then be found between the microhardness marks by using X-rays detector. The cut should be thin to reduce absorption effects. Carbon is good choices. Care is required to prepare the unknown sample and the microanalysis standards in an identical manner.

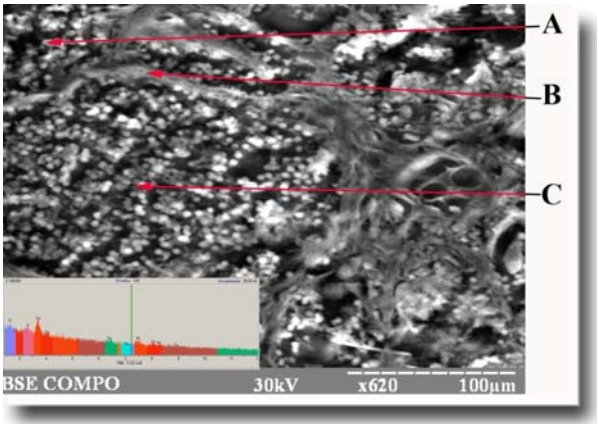

Fig. 2. Pyogenic epulis. A – hemorrhage and cell infiltrateat, B - vessel wall, C – vascular spaces.

Graphite is an allotrope of carbon. Graphite is an electrical conductor, a semimetal. Graphite is the most stable form of carbon under standard conditions. Hromatografic clean carbon plates were used in our method.

The beam electron interact with the histological section. These interactions are responsible for a multitude of signal types:backscattered electrons, secondary electrons, X-rays, Auger electrons, cathadoluminescence shown in fig. 3. The composition of metals is shown in fig 2.

Conclusion

Rationale and description of the new method of chemical and morphological studying of paraffin sections presented in the article. Scanning electron microscope equipped with energy dispersive X-ray spectroscope can be used in chemical composition of tissue.

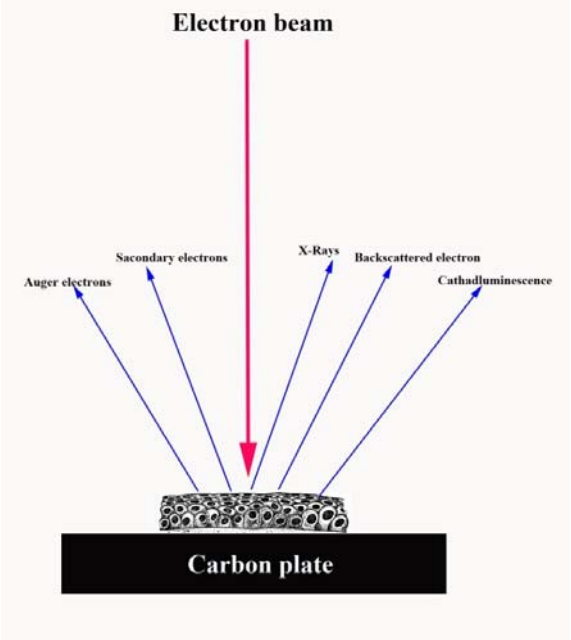

Fig. 3. Interaction scheme of the electron beam with the tissues.

Competing interests

The author declare they have no competing interests.

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Кузенко Е.В. Метод: химическое и морфологическое исследование парафиновых срезов.

Реферат. Цель данного исследования состояла в обосновании применения сканирующей электронной микроскопии для определения химического состава ткани. Для распознавания различных типов парафиновых тканевых срезов и степени накопления тяжелых металлов в них использовался сканирующий

электронный микроскоп, оснащенный рентгеновским спектроскопом. Рентгеноструктурный анализ показал, что неорганические компоненты в парафиновых тканевых срезах были доступны для химического анализа. Сканирующая электронная микроскопия была использована для морфологического анализа парафиновых срезов. В статье представлены обоснование и описание нового метода химического и морфологического изучения парафиновых срезов с помощью сканирующей электронной микроскопии и рентгеноструктурного анализа.

Ключевые слова: тканевые парафиновые срезы, морфологический анализ, сканирующая электронная микроскопия.