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Comprehensive Study of the Structural Components of the Skin: From Routine Methods to Modern Microscopy Methods

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Abstract

Background: Modern methods of microscopy expand our capabilities to detail objects and move to the study of native tissue. The varieties of laser microscopy, which are becoming more and more popular, have broad prospects in the study of morphological properties, combining high resolution and minimal exposure to aggressive media during sample preparation. However, in the scientific literature, the aspects of the structure of individual structural components of the skin or morphofunctional changes in various pathological conditions are not well covered. In this regard, the purpose of our study was a multilevel analysis of structural components using both classical and modern morphological methods.

Methods and Results: The material for this study was skin fragments obtained from laboratory male Wistar rats. The study of the structural components was carried out by the methods of light microscopy, scanning electron microscopy, and laser scanning microscopy. The results of our study indicate that the most effective way to obtain complete information is an integrated approach to the study of tissue morphology, where the researcher requires deep knowledge and the use of not only modern methods, but also the possibility of combining them with existing classical methods.(**International Journal of Biomedicine. 2021;11(2):216-219.**)

Key Words: skin • light microscopy • scanning electron microscopy • laser scanning microscopy

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Abbreviations

MPM, multiphoton microscopy; **LM**, light microscopy; **SEM**, scanning electron microscopy; **LSM**, laser scanning microscopy; **H&E**, hematoxylin and eosin; **TEM**, transmission electron microscopy.

Introduction

Over the long history of microscopic research, a large amount of information has been accumulated about the microstructure of tissues and organs, which we can obtain from scientific journals, monographs, and educational literature. At this stage, modern morphology seeks to obtain information about the structure of living tissues of organs. The main components that determine the quality of the "research - result" sequence are sample preparation and technical characteristics of the optical device. In this regard, each of these stages undergoes its perfection—the quality of optics, increase in resolution and in sample preparation, reduction in destructive effects.

At present, morphologists have in their arsenal a fairly large set of microscopic methods, any of which can be used depending on the task at hand. However, there is no complex data in the available literature that allows one to compare the data obtained using different methods. In this regard, the

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purpose of our study was a multilevel analysis of structural components using both classical and modern morphological methods.

Material and Methods

The material for this study was skin fragments obtained from laboratory male Wistar rats. The study of the structural components was carried out by the methods of light microscopy (LM), scanning electron microscopy (SEM), and laser scanning microscopy (LSM).

Results

Among the routine methods of microscopy, the main one is still LM with H&E staining,[1,2] the result of which is staining of nuclei in colors from purple to blue (the shade depends on the type of hematoxylin and the duration of staining), and of cytoplasm and extracellular matrix in colors from pink to red (the shade depends on the pH of the eosin and the duration of the staining) (Figure 1A). This technique is intended rather for an overview, histological or pathological examination and further morphometric processing. The next stage for the study of tissue is the use of histochemistry, as a method of histology, which makes it possible to isolate individual structures due to the specific chemical interaction of tissue components with a dye or mixture of dyes. The most popular methods for studying the fibrous components of the skin are staining according to Van Gieson (Figure 1B) and Mallory. Due to the non-absolute specificity of the methods, it is not always possible to determine the biochemical nature of tissues. In order to selectively stain fibers, it is necessary to carry out careful differentiation.[3-6]

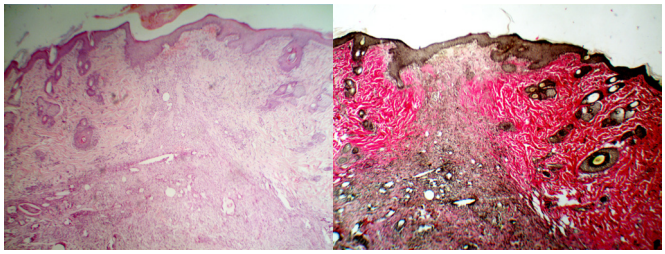

Fig. 1. *Micrograph of the skin: a section of the connective tissue scar. A - H&E staining (Magnification ×40). B - Van Gieson staining (Magnification ×40)*

Of course, light-optical methods are applicable for microscopy at low magnification. The use of electronic techniques allows "depth of penetration" and expands knowledge of the ultra-microstructure of an object. But each method has its own target priorities. For SEM, this is primarily the acquisition of information about the shape and surface of the structure, whereas TEM adds knowledge about the intracellular, more detailed (at higher magnification) structure.[7,8] All of the above methods give an idea of the morphological picture, taking into account the errors caused by numerous stages of sample preparation—the action of formaldehyde, alcohols, xylenes, etc.

The beginning of the study of the morphology of living tissue was the study of samples isolated from cultured cells of tissues by the phase-contrast method. Improvements on this method—Hoffman modulation contrast and Nomarski interference contrast—make it possible to study the cultured cells, as well as the structure of living cells, at the maximum resolution (×1000) of the light microscope.[9,10] The use of an inverted microscope with a cell incubator and time-lapse imaging makes it possible to study the structural dynamics of cultured cells from the moment of their seeding to the formation of a confluent monolayer, and changes in the shape of cells in a wide range from spherical to elongated and flattened, which confirms their high plasticity (Figure 2A). The process of cell division, the interaction of cells with each other, and adaptation to new cultural conditions have been studied in detail, all of which can be regarded as cells' acquisition of a peculiar cultural phenotype (Figure 2B).

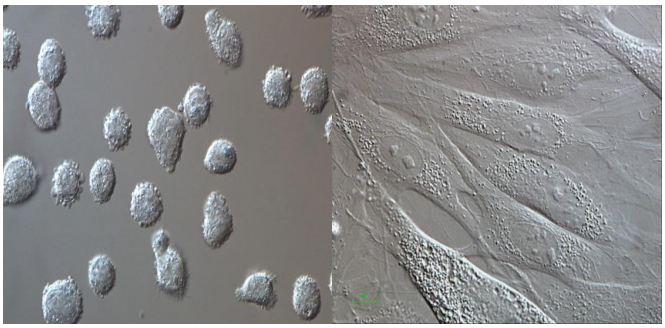

Fig. 2. *Micrograph of the culture of isolated fibroblasts. A - at the attachment stage. 3 hours after reseeding. B - at the monolayer stage. Nomarski interference contrast (Magnification ×600).*

Another method for the study of cultured fibroblasts (the method of intravital microscopy) is the study of cultures by direct, confocal, scanning multiphoton microscopy. Cell structures containing fluorophores will fluoresce in the green or yellow range, depending on the wavelength of infrared radiation.[11-13] Confocal microscopy expands the range of our capabilities for a detailed study of other cellular structures, as well as their chemical composition, and allows the addition of immunocytochemical methods, in particular the use of fluorescent proteins.[14] It is applicable to both the study of fixed preparations and intravital study.

Concerning the morphology of living tissue, various types of laser microscopy are used, and one of the most promising methods is MPM, which is based on the ability of some tissues to autofluorescence.[15-18] The main advantage of MPM over electron microscopy is the identification of cellular and fibrous components with absolutely preserved architectonics and dimensional data of the object under study (Figure 3A). With MPM of the dermis, the cells in it fluoresce in the green and yellow spectral channels. A sufficiently pronounced signal comes from the fibrous elements of the extracellular matrix surrounding the cells (Figure 3 B).

A similar glow caused by infrared photons will be generated by the fibrous structures of the connective

tissue—elastic and collagen fibers. The latter, in addition to multiphoton autoluminescence, generates radiation called the second optical harmonic. In this case, some of the infrared photons are not absorbed by electrons, but are reflected from the electron shells of collagen atoms and scattered as radiation with half of the wavelengths of infrared photons. This radiation generated by the reflection of infrared photons will be differentiated by special filters in the form of a blue-blue glow. Elastic fibers with autoluminescence properties will give a green or yellow glow.

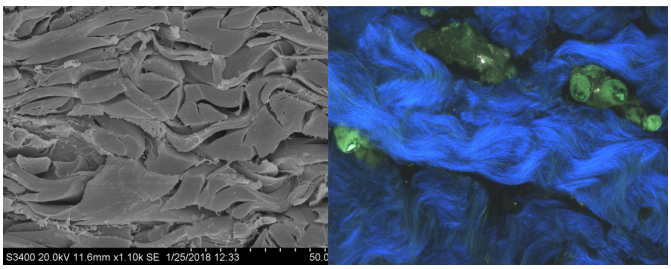

Fig. 3. *Micrograph of the dermis of the skin. A – SEM (×1100). B - Two-photon laser scanning confocal microscopy (×400)*

The nature of the second harmonic is associated with the anisotropic structural organization of collagen fibers. Thus, we can identify the biochemical nature of the fibrous structures. Considering the ability of an enhanced generation of infrared radiation at a greater depth, with MFM we have the possibility of layer-by-layer scanning of the examined skin (Figure 4).

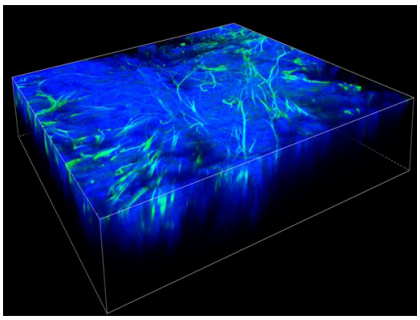

Fig. 4. *Micrograph of the dermis of the skin. Two-photon laser scanning confocal microscopy (3D reconstruction), × 400*

Discussion

Nonlinear optical microscopy is a convenient tool for biomedical imaging due to the physical principles underlying the creation of contrast between individual objects. One of its main advantages is the ability to reconstruct a volumetric image, which, in particular, makes it possible to perform morphological studies ex vivo (on biopsy samples) and in vivo. And in the future, the results of such studies will become important diagnostic criteria in clinical practice. As a result of the above, we can conclude that modern methodological capabilities are gradually bringing us closer to the visualization of living tissues without the use of sample preparation. The knowledge accumulated by classical histology will help us in interpreting the data obtained using new methods, which can take a key place in the integration of cell biology, biochemistry, physiology, molecular genetics, and proteomics in solving fundamental problems and applied problems of biomedical research. A fairly wide variety of such methods allows one to choose the optimal technique for specific needs, taking into account the advantages and disadvantages of specific methods, and important diagnostic criteria in clinical practice, as well as existing methodological limitations.

Competing Interests

The authors declare that they have no competing interests.

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