Histological stability of the Malolyakhovsky mammoth tissues to permafrost conditions

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Abstract

This work presents a histological analysis of adipose, muscle, cartilaginous tissue, walls of blood vessels and tendons of the Malolyakhovsky mammoth, found on the island of Maly Lyakhovsky of the New Siberian archipelago in 2012, for signs of preservation and stability of tissue structures to permafrost conditions. We have also performed a comparative analysis of the obtained histological data with the data from other woolly mammoths from the available literature. It was revealed that the tissues which were most preserved and resistant to permafrost conditions were the ones with a compact structure of connective tissue. The results of the work and the data obtained are of great importance for further research in the field of paleontology. In the future, they can be used in a comparative analysis with other representatives of woolly mammoths and mammals of the mammoth fauna.

Keywords: Malolyakhovsky mammoth, soft tissues, histological analysis, comparative morphological analysis, adipose tissue, muscle tissue, circulatory tissue, cartilaginous tissue, tendons

Introduction

The permafrost on the territory of Republic of Sakha (Yakutia) to this day preserves not only the skeletal remains of fossil animals, but also their carcasses from the Ice Age, representing great scientific and cultural value. Most of the finds come from the Arctic zone of Yakutia, where ice sediments of the Pleistocene period are widespread (Boeskorov et al., 2013b, 2016; Grigoriev et al., 2017a; Lazarev et al., 2004; Lazarev, 2007, 2008; Mashchenko, Tikhonov, and MacPhee, 2005; Tikhonov, 2005). These sediments were formed during extremely cold glaciations in the Pleistocene, reaching a thickness of several hundred meters, and have been preserved in a little-modified form due to the cold climate prevailing in the northeast of Eurasia to the present time. Due to the frozen state of soils, not only bones can be preserved in their depths, but also whole corpses of long-dead animals (Boeskorov et al., 2012, 2013a; Lazarev et al., 1998; Tikhonov, 2005; Vereshchagin, 1979; Vereshchagin and Tikhonov, 1990).

In recent years, research institutions in Yakutia, in particular the Lazarev Mammoth Museum (Institute of Applied Ecology of the North, North-Eastern Federal University), have been conducting comprehensive studies of a number of unique remains of mammoth carcasses and other fossil animals. Such stud-

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ies make it possible to obtain comprehensive scientific information on burial conditions, features of the structure of internal organs and external appearance of extinct animals, paleoecological conditions of the Ice Age, palynological features, ancient microflora, genome, etc (Boeskorov, Tikhonov, and Suzuki, 2007; Kornienko et al., 2019; Mashchenko, 2002; Plotnikov et al., 2015).

One of the main unique treasures of the Lazarev Mammoth Museum is the carcass of a woolly mammoth (*Mammuthus primigenius*) found in 2012 on Maly Lyakhovsky Island of the New Siberian Islands by tusk ivory pickers. As a result of excavations in May 2013, the lower half of the mammoth carcass was removed from the frozen ground. Morphological signs indicate that the animal was an old female approximately 50–60 years old, the age of the find is 32.500–33.000 year (Fisher et al., 2012; Galanin et al., 2015; Grigoriev et al., 2017; Kornienko et al., 2018). The main distinguishing feature of a more thorough examination of the Malolyakhovsky mammoth was the unique preservation of soft tissues, as well as the presence of unfrozen tissue fluid in the body, which looks like blood (Garmaeva et al., 2014; Grigoriev et al., 2017; Kornienko et al., 2019).

Such objects need a thorough study to reveal the uniqueness of the preservation of their cellular and tissue structures in permafrost conditions. The analysis of domestic and foreign literature shows that histological studies of ancient animals are few. According to the literature, the preservation of tissues of ancient animals in other regions of the planet is practically absent, however, the data of histological studies are of great relevance in biomedical science for studying the preservation and stability of ancient tissues, cloning, cryopreservation of organs, phylogenetic analysis, etc (Boeskorov et al., 2013a; Garmaeva, Buzinaeva, and Grigoriev, 2019).

Material and methods

In 2014, as a result of an autopsy of the Malolyakhovsky mammoth, soft tissue samples were taken from various parts of the body. In total, about 99 different histological preparations were counted, ranging from the lateral surface of the left forelimb to the adipose tissue of the foot. On the basis of the samples taken from the mammoth remains, histological specimens were prepared according to the standard methodology (Peshkova and Akulinin, 2012; Yanin, Bondarenko, and Sazonova, 2015), capable of ensuring the preservation of tissues and cellular structures of the material under study for histological analysis. The preparation of histological sections with a thickness of 3–5 μm was carried out on a Leica SM2010R microtome. In this study, preparations of the skin of the forearm of the right forelimb, adipose tissue of the left foot, skeletal muscle, cartilage tissue, tendon, arterial and venous vessels were selected.

The preparation of the histological specimen was carried out according to the generally accepted method. The mammoth tissues were cut with great difficulty due to the sublimation of tissues, as a result of which the samples were compacted and had a pronounced mummification. Hematoxylin-eosin staining was used as material for histological sections. Microscopic morphological examination of histological sections was performed using a Nikon Eclipse Ci-E biomedical microscope. A pronounced mummification was observed on all histological preparations of the tissues of the studied ancient animal.

Morphological analysis

Adipose tissue of the left foot. Histological observation of adipose tissue showed a white tissue consisting of medium light cells — adipocytes, large drops of oval lipids with a well-preserved membrane, tightly adjacent to each other. Loose fibrous connective tissue is noted between adipocytes. The nuclei are not rendered (Fig. 1).

Skeletal muscle. On a histological sample of the skeletal muscle of the right forearm, fibrous-muscular tissue is visualized in a longitudinal section. In myofibrils transverse striation is not visualized, but basal-brightly colored shadows of irregular oblong shape are clearly visible along the muscle shapes (Fig. 2).

On other histological samples of skeletal muscle tissue, numerous longitudinal sections of muscle fibers are visualized. In the cytoplasm, myofibrils with traces of transverse striation are arranged in an orderly manner. Nuclei are not visualized on these samples (Fig. 3).

Arterial and venous vessels from the extremities. On the surface of the inner lining of the arterial vessel, the endothelium is clearly visible, lined in the form of folds. Medium shell made of well-preserved circular muscle fiber. The outer shell is represented by a well-preserved loose fibrous connective tissue (Fig. 4).

On a histological sample of the superficial venous vessel of the forelimb a loose fibrous connective tissue is visualized. The middle shell is formed by muscle tissue. The inner shell (endothelium) is partially destroyed. A homogeneous brick-colored mass is visualized in the vessel cavity, which is probably a blood clot (Fig. 5).

Cartilaginous tissue. On a histological specimen hyaline cartilage with a perichondrium and a zone of young chondrocytes are visualized. Groups of isogenic cartilage cells with signs of preservation of the nucleus shadow are visible. Cartilage cells in an amorphous substance are observed either singly or in isogenic groups, but mainly in groups of isogenic cartilage cells (Fig. 6).

Tendon. Tendon is visualized built mainly of dense formed connective tissue, which consists of a large amount of collagen and a small amount of amorphous substance. In a densely formed connective tissue, collagen fibers lie in an orderly manner (Fig. 7).

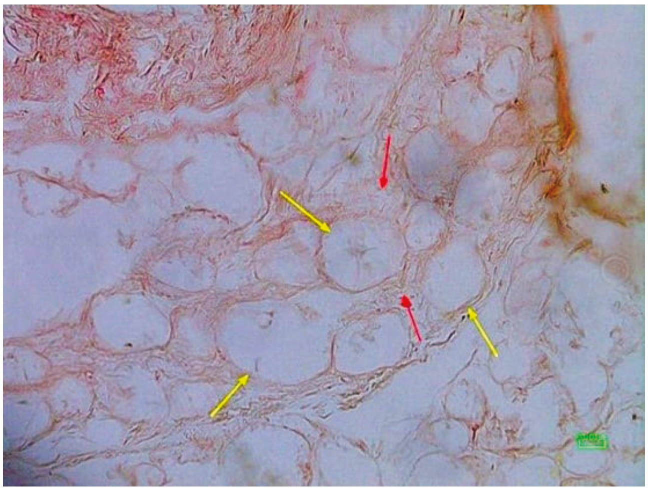

Fig. 1. Adipose tissue of the left foot stained with hematoxylin-eosin, 40x. The yellow arrow indicates coated adipocytes. The red arrow indicates loose fibrous connective tissue.

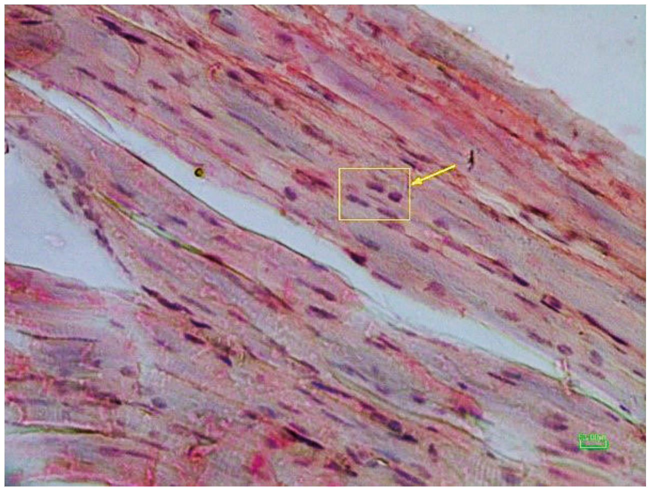

Fig. 2. Skeletal muscle of the forearm of the right forelimb stained with hematoxylin-eosin, 40x. The yellow arrow indicates the area of basal brightly colored nuclei shadows.

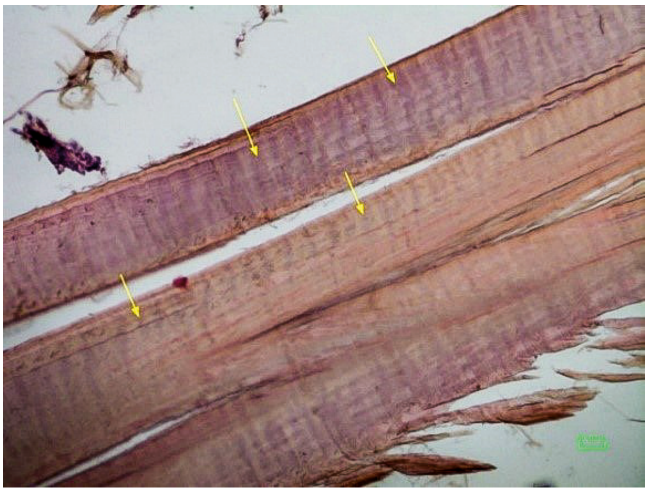

Fig. 3. Skeletal muscle tissue stained with hematoxylin-eosin, 10x. The yellow arrow indicates the locations of the transverse striation of muscle fibers.

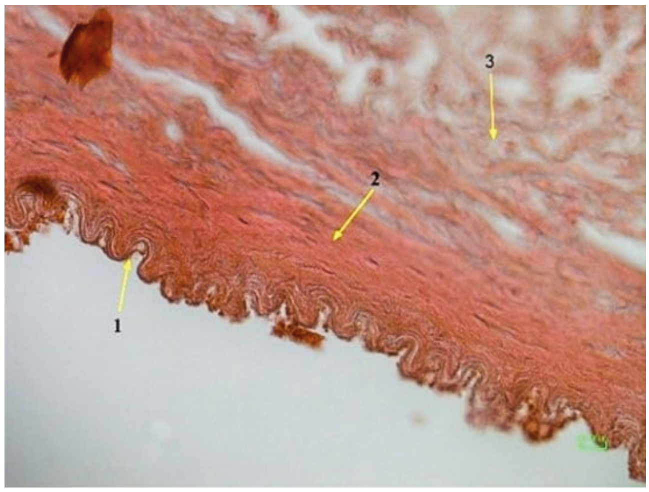

Fig. 4. Arterial vessel stained with hematoxylin-eosin, 20x. The yellow arrow indicates: 1 — endothelium; 2 — middle shell; 3 — outer shell.

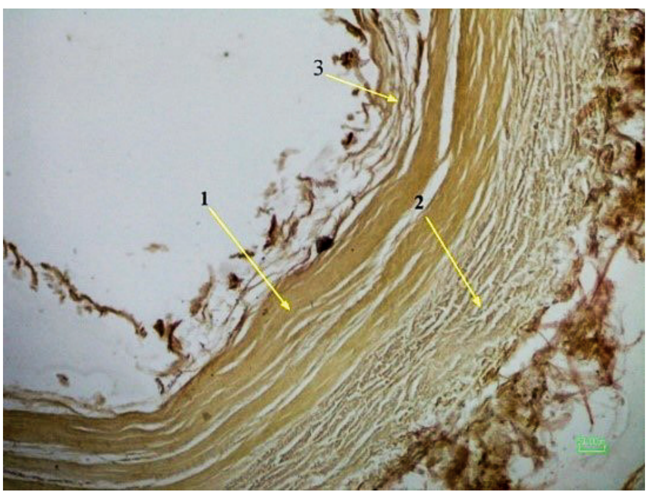

Fig. 5. Venous vessel of the forelimb stained with hematoxylin-eosin, 10x. The yellow arrow indicates: 1 — the middle shell; 2 — outer shell; 3 — endothelium.

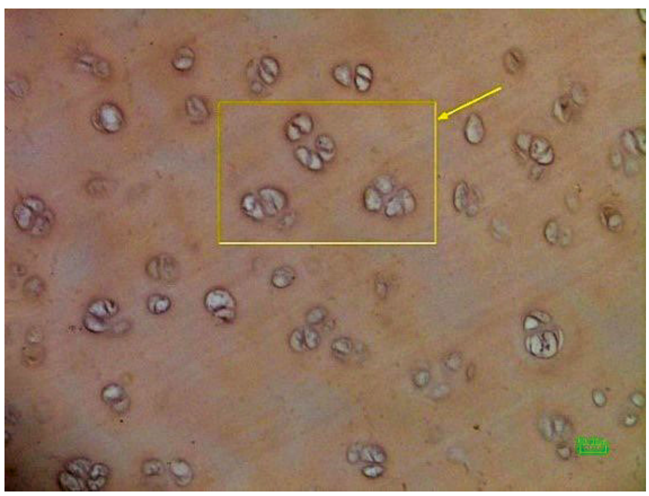

Fig. 6. Cartilage tissue stained with hematoxylin-eosin, 40x. The yellow arrow indicates the area of isogenic groups.

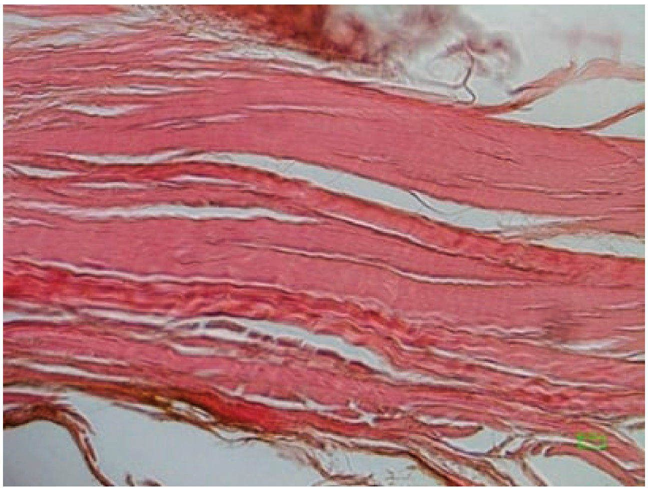

Fig. 7. Tendon stained with hematoxylin-eosin. Magnification x40.

Comparative analysis

The analysis of domestic and foreign literature showed that histological data on studies of adipose, cartilaginous, muscle tissue and blood vessels of woolly mammoths are insufficient (Boeskorov, Tikhonov, and Suzuki, 2007; Garmaeva et al., 2014; Garmaeva, Buzinaeva, and Grigoriev, 2019; Hattori et al., 2021; Kharlamova et al., 2015; Papageorgopoulou, Link, and Rühli, 2015).

In this regard, an attempt was made to compare the adipose and muscle tissue of the Malolyakhovsky mammoth with the results of the histological studies of:

1) the Kirgilyakh mammoth Dima, found in the valley of the Kirgilyakh stream of the Magadan region in 1977 with an age of 40,000 years old;

2) the mammoth Lyuba, found in 2007 in the course of the Yuribey River on the Yamal Peninsula, which is 41,800 years old;

3) the African elephant (*Loxodonta africana*).

Additionally, a comparative analysis was performed with muscle tissue and a blood vessel of the Maksunuokhsky mammoth, and also with a blood vessel of the Yukaghir and woolly mammoth found on the Maksunuokha River in 2002 (Boeskorov, Tikhonov, and Suzuki, 2007; Kato et al., 2009; Papageorgopoulou, Link, and Rühli, 2015; Repin et al., 2004; Shilo, Lozhkin, Titov, and Shumilov, 1983; Vereshchagin and Mikhel'son, 1981).

Numerous adipocytes with a well-preserved shell, tightly adjacent to each other, are visualized on the histological sample of the adipose tissue of the Malolyakhovsky mammoth. Loose fibrous connective tissue is observed between adipocytes (Fig. 8A). A micrograph of the subcutaneous adipose tissue of the mammoth Lyuba visualizes rather compact adipocytes, without a clear cell wall (Fig. 8B). The walls of adipose cells and collagen are clearly visible on the specimen of an African elephant (Fig. 8C). According of the results of histological study of Dima's mammoth, most of the shells of adipose cells of the subcutaneous layer, including connective tissue fibers that are located parallel to the skin surface, and adipose lobes have been preserved (Fig. 8D).

Based on these data, it follows that the adipocytes of the adipose tissue of the Malolyakhovsky mammoth have the best preservation than those of the mammoth Lyuba and are most similar to the samples of the African elephant.

On a histological specimen of skeletal muscle tissue of the Malolyakhovsky mammoth, fibers of skeletal muscle tissue are visualized in a longitudinal section, myofibrils have traces of transverse striation, basal-brightly colored shadows of an irregular oblong nuclei are visible along the muscle fibers (Fig. 9A, B). Mummified longitudinal and oblique muscle fibers, which do not have nuclei and traces of transverse striation, are visualized on the sample of the Lyuba's mammoth muscle tissue (Fig. 9C). In the Maksunuokhsky mammoth, the shadows of the nuclei are visualized without traces of transverse striation (Fig. 9D). On the sample of the African elephant in the longitudinal striation of muscle fibers, one can see several cell nuclei, which shows the similarity with the muscle tissues of the Malolyakhovsky mammoth (Fig. 9E). In addition, the muscle tissue of the Malolyakhovsky mammoth is compared with human muscle tissue six months after exhumation (according to Garmaeva D.K.) (Fig. 9F). Comparative analysis showed that the human muscle tissue after exhumation is strongly mummified, has no transverse striation and basal-brightly colored shadows of the nuclei, which is

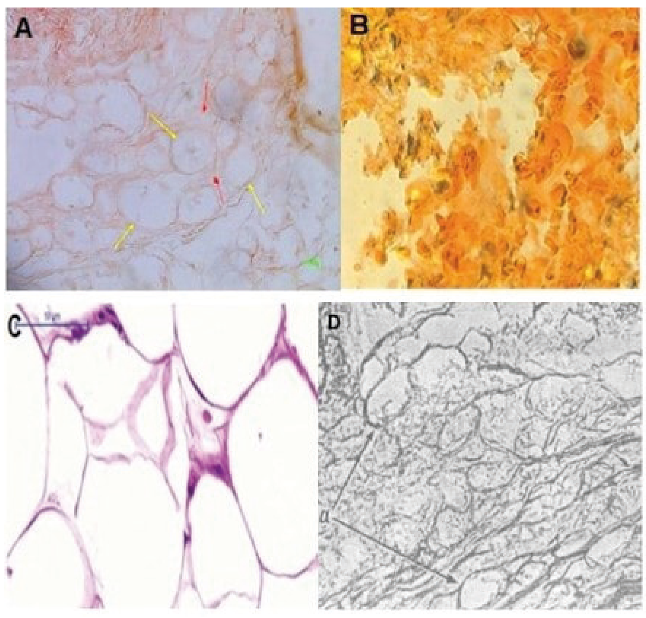

Fig. 8. A — adipose tissue of the left foot of the Malolyakhovsky mammoth; B — subcutaneous tissue of the mammoth Lyuba (Papageorgopoulou, Link, and Rühli, 2015); C — adipose tissue of the African elephant (Papageorgopoulou, Link, and Rühli, 2015); D — adipose tissue of the mammoth Dima (Shilo, Lozhkin, Titov, and Shumilov, 1983).

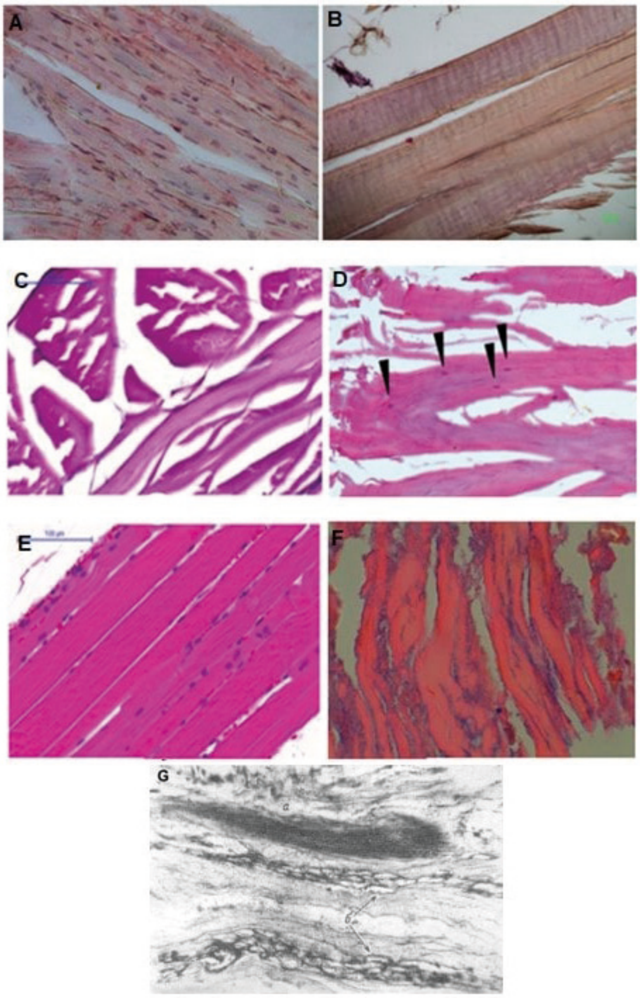

Fig. 9. A, B — muscle tissue of the Malolyakhovsky mammoth; C — muscle tissue of Lyuba mammoth (Papageorgopoulou, Link, and Rühli, 2015); D — muscle tissue of the Maksunuokhsky mammoth (arrows indicate the remains of the nucleus) (Kato et al., 2009); E — muscle tissue of the African elephant (Papageorgopoulou, Link, and Rühli, 2015); F — muscle tissue of the human six months after exhumation (according to Garmaeva D. K.); G — muscle tissue of the mammoth Dima (Shilo, Lozhkin, Titov, and Shumilov, 1983).

observed in the muscle tissue of the Malolyakhovsky mammoth. Also, on the histological specimen of muscle tissue of Kirgilyakhsky mammoth Dima, bundles of striated muscles are visible, but without transverse striation. Fibrous structures (collagen, reticulin and elastic fibers) are also visualized in its tissues (Fig. 9G).

As a result of the comparative characteristics of the muscle tissue, it was revealed that the tissue sample of the Malolyakhovsky mammoth has the best preservation and is structurally similar to the muscle tissue of the African elephant.

Discussion and conclusions

The results of the histological analysis showed the best preservation of the tissues of the Malolyakhovsky mammoth, such as adipose tissue with undisturbed adipocyte walls; hyaline cartilage with chondrocytes, where the shadows of the nuclei are visualized; skeletal muscle tissue with well-preserved shadows of the nuclei; walls of arterial and venous vessels. As a result of the comparative analysis, it was determined that the tissues of the Malolyakhovsky mammoth are better preserved, compared to other woolly mammoths from the literature data. Apparently, this is due to the fact that the mammoth fell into an ice trap, as a result of which there was an instant cooling of the tissues and subsequent thawing did not occur.

Thus, the results obtained show that, in contrast to parenchymal organs, muscle, adipose and cartilage tissues, the walls of adipocytes and blood vessels are most resistant to the effects of mummification and deformation of tissues over time in permafrost, due to the denser and more arranged structure of the connective tissue.

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