MANUAL PREPARATION OF THIN SECTIONS FROM HISTORICAL HUMAN SKELETAL MATERIAL

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REZUMAT

Au fost testate patru metode și patru variante ale fiecărei metode, pentru a determina care este cea mai potrivită și mai simplă pentru cercetarea structurii microscopice a scheletului uman vechi. Rezultatele studiului indică faptul că toate cele patru metode și variantele lor pot fi utilizate în acest scop. Totuși, cea mai adecvată modalitate pentru studiul calitativ și cantitativ al microstructurii pare a fi cea care combină aplicarea cleiului de cianoacrilat pe suprafața periostală a osului înainte de secționare cu aplicarea acestuia pe suprafața de secțiune. Următoarea etapă include șlefuirea manuală cu abraziv până la o grosime finală de 100 microni, introducerea secțiunilor subțiri în apă distilată cu câteva picături de detergent, uscarea în aer, introducerea în balsam de Canada fierbinte, și acoperirea cu capac de sticlă scufundat în prealabil în m-xilen.

Cuvinte cheie: os uman vechi, preparare manuală, secțiuni subțiri, microstructură, țesut osos

ABSTRACT

We have tested four methods in four variants in order to find the most suitable and simple manual method for research of the microscopic structure of historical human skeletal material. Our findings indicate all tested methods and all variants can be used for this aim. However, the most suitable method for research of qualitative and quantitative characteristics of the microstructure seems to be the one which sums up an application of cyanoacrylate glue onto periostal surface of bone before cutting and on the surface made by the first cut. Next steps include manual grinding by carborundum to the final thickness of 100 microns, introduction of thin sections into distilled water with a few drops of detergent, air drying, sealing off into hot Canada balsam and covering with m-xylene dipped covering glasses.

Key Words: historical human bones, manual preparation, thin sections, microstructure, bone tissue

INTRODUCTION

In general, two procedures are used to prepare samples for microscopic examination of the bones. The bone fragment is first decalcified and subsequently cut by microtome or directly ground. However, decalcification of historical skeletal material is not possible, thus the preparation of ground sections is widely used in this case. The bone is cut perpendicularly to long axis and two or three millimeters thick bone fragments are consequently removed with a common lapidary saw, a handsaw, or a diamond-cutting saw.[1-3]

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Received for publication: Jul 12, 2005. Revised: Mar. 17, 2006.

The resulting fragments are then put in a low-speed diamond saw and/or in a microtome with a diamond-coated circular blade for further cutting; the thickness can also be reduced manually with various types of mineral grinding powders (e.g., SiC, finely powdered carborundum, polishing alumina).[1,4] In case of fragile specimens it is necessary to reinforce complement parts of the bone.[5] For this purpose Němečková et al. use Epon, Maat et al. employ cyanoacrylate glue, Titlbachová uses methyl methacrylate solution and Hermann et al. put samples of the bone in epoxid resin Biodur.[6-10]

After grinding, the sections may be directly glued onto standard glass microscopic slides, covered with cover glass and examined at a magnification of 50-100x.[1,11] They may also be washed, placed in 95% alcohol for several minutes, air-dried and coated with transparent liquid plastic - Parlodion solution in butyl acetate, methyl methacrylate and/or the sections can be put into distilled water with a few drops of detergent, air-dried and finally sealed off in a mounting medium.[3,7,8,12-14] Capasso et al. and Jírovec use hot

Canada balsam, Maat et al. employ Entellan.[4,7,8,13] The sections are further examined using an optical microscope at a magnification of 50-200x for the microscopy of bone tissue.

The aim of our work was to compare and to modify some manual methods for preparation of thin sections from historical human skeletal material depending on material conditions. We tried to determine which is the most suitable and simple method for research of historical human bone tissue microscopic structure.

MATERIAL AND METHODS

Our research focused on medieval human skeletal material found in Dubovany cemetery (Trnava district, Slovakia) originating from the 8th and 9th Centuries (the Early Middle Ages). Femurs of a man of 40-something years old(the grave No. 20/1995) were used for testing the methods. The bones were cut by a handsaw in a diaphyse section. Four different methods were used for grinding two millimeter-thick bone fragments. The transversal sections were ground by wet SiC[13] - method No. 1, by carborundum[3] - method No. 2, by carborundum after submergence of the cross sections into methyl methacrylate solution for 24 hours - method No. 3, and by carborundum again after aplication of a few drops of cyanoacrylate glue onto periostal surface of bone and on the surface made by the first cut - method no. 4.[7-9] Final thickness of the samples was approximately 100 microns. Altogether 16 thin sections were obtained by these method and they were subsequently modified by following variants:

1. After grinding, thin sections were glued onto standart glass microscopic slides and covered with cover glass;[1,11]

2. After covering with glass, the sections were slowly warmed up (carbonization);[12]

3. After grinding, sections were put into distilled water with a few drops of detergent, air–dried,sealed off into hot Canada balsam and covered with m–xylene dipped cover glasses;[4,7,8,13]

4. After grinding, the sections were washed, air-dried, placed into 95% ethanol, air-dried again and finally coated by methyl methacrylate solution.[11]

For the examination, an optical microscope Jenaval (Carl Zeiss Jena) with digital CCD camera (Mintrow) at a magnification of 200x were used. Photo-documentation of slides was done by computer programs Ati Player 5.2. (Ati Technol. Inc) and Adobe Photoshop 5.0. The software Scion Image (Scion Corporation, USA) allowed us to measure diameter of the Haversian canals and secondary osteons.

RESULTS

Testing of the manual methods for investigation of historical human bone tissue microscopic structure has showed that all are suitable for this purpose. However, the method No. 2 is the simpliest, cheapest and it takes the shortest time. On the other hand, this methodic makes skeletal material fragile during grinding (similar to method No. 1). Methods No. 3 and No. 4 eliminate this disadvantage, and even if the procedures are longer (method No. 3 longer than No. 4), the quality of the thin sections is better. Each method includes four variants which were also tested. Our findings are as follows:

- The second variant is the most suitable for differentiation of osteon age groups. After warming up younger osteons were getting light, older ones were getting dark.

- The third variant is the most appropriate for research of quantitative characteristics of the microstructure (e.g., measurements of Haversian canals, secondary osteons diameter). Haversian canal and concentric lamellae of Haversian systems (secondary osteons) are the most distinguishable.

- All tested variants may be used for research of qualitative characteristics of the microstructure (e.g., determination of bone tissue type in a certain part of the bone).

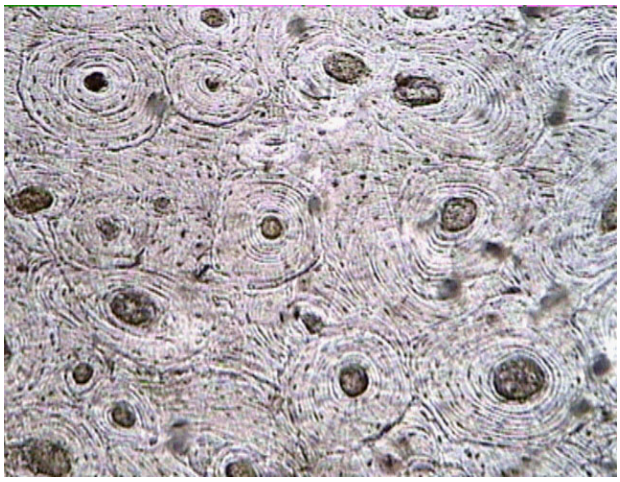

Figure 1. Completely differentiated Haversian systems (secondary osteons) connected with Volkmanns canals (magnification x 200).

According to our study the most suitable manual method for investigation of analysed bone tissue microstructure (including both qualitative and quantitative characteristics) seems to be method no. 4 combined with the third variant. We applied the method for investigation of 10 medieval adult female femur diaphyseis microstructure from Dubovany cemetery (graves No. 30A/98, 30B/98, 24/98, 15/95, 16/95).

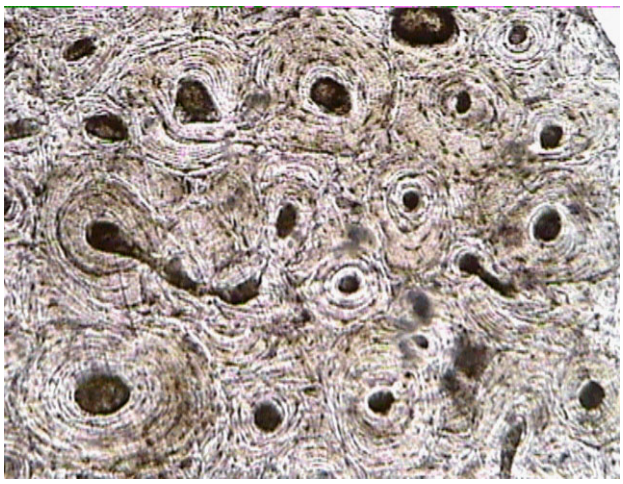

Figure 2. Numerous interstitial lamellae in between the particular Haversian systems (magnification x 200).

Figures 1 and 2 demonstrate that this method produces beautiful sections. The examined bone tissue is in general composed of completely differentiated secondary osteons, some of them are connected with Voklkmanns cannals. (Fig. 1) There are a lot of interstitial lamellae in between the particular osteons. (Fig. 2) On the other hand, there are no outer circumferential lamellae identified. Mean diameter of 200 Haversian canals and 200 secondary osteons is 32.26 ± 7.82 μm and 189.27 ± 28.49 μm, respectively.

DISCUSSION

Because bone tissue seems very hard to prepare, there is a widely spread misconception that the preparation of thin sections from historical human skeletal material is expensive, demands a lot of time, knowledge of plastics and stamina to face failed attempts to produce useful sections. As early as 1958 Frost recommended an elegant procedure for the manual preparation of thin sections from recent human bones.[14] Maat et al.applied this original technique for many years with some modifications on inhumed and cremated osteoarchaeological and forensic material.[7,8] Our results revealed that this method, with modifications, is the most suitable for research of historical human bone tissue microstructure. As in the original method the modification needs basic and cheaper product. Thin sections obtained by this procedure are very suitable not only for microscopical investigation of historical human bones but also for observation of bones which are damaged by fire and/or by biotic and abiotic environmental factors.

The studied bone microstructure is typical for adult human. There are no primary osteons identified. Furthemore, the bone is composed of completely differentiated secondary osteons with many interstitial lamellae.[2] Mean diameters of Haversian canals and secondary osteons are lower comparing with Paaver's values from an Estonian population originating in the 16th-18th centuries A.D.[12] It is remarkable that the secondary osteons and Haversian canals diameter is higher in younger population. Results obtained from recent human population are similar to our findings. Value of the mean diameter of Dubovany femur Haversian canals is clearly lower than the one of the recent human population: 52.9 μm, and/or 68.8 μm.[15,16] Fairly low values of parameters among the Dubovany human bones might result from phylogenetic evolution, mechanical stress and strain, nourishment and/or a combination of all these factors.

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