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Preparation of thin histological sections from archaeological bone and tooth samples

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PŘÍPRAVA TENKÝCH HISTOLOGICKÝCH ŘEZŮ Z ARCHEOLOGICKÝCH VZORKŮ KOSTÍ A ZUBŮ

ABSTRAKT Histologická analýza osteologických nálezů pocházejících z archeologických výzkumů je časově a finančně náročný proces, který ve svém výsledku přináší data a informace, jež jsou obtížně získatelné z makroskopického pozorování (např. taxonomické určení fragmentů bez morfologicky diagnostických znaků), nebo vedou k získání zcela nových informací o životní historii jedince (přes hodnocení markerů vývojového stresu ze zubní skloviny a určení chronologie jednotlivých událostí). Mikroskopická analýza vzorků kostí a zubů klade důraz na zhotovení kvalitního histologického preparátu, který je následně možné analyzovat za pomocí optického mikroskopu. Dosud bylo publikováno široké spektrum metodických postupů, jak zhotovovat histologické výbrusy z kalcifikovaných tvrdých savčích tkání, lišících se a) v závislosti na typu tkáně, ze které je preparát zhotoven, b) stavu zachovalosti vzorku, c) přítomnosti laboratorního vybavení. Naše metodika je podmíněna již publikovanými protokoly a postupy, které jsou navíc pozměněny tak, aby vyhovovaly našemu laboratornímu vybavení a požadavkům zpracovávaného vzorku. Výsledkem je tak opakovatelný protokol, sestávající se z několika hlavních kroků, které jsou proveditelné v laboratořích se stejným nebo podobným vybavením.

KLÍČOVÁ SLOVA tvrdé tkáně savců; histologie; médium pro zalévání; repliky z epoxidové pryskyřice; příprava tenkých řezů

ABSTRACT Histological analysis of osteological remains from archaeological excavations provides data and information that can be difficult or impossible to obtain from macroscopic description and examination. Furthermore, the microscopic perspective provides important evidence for taxonomically indeterminate samples lacking morphologically diagnostic marks, e.g. determination of human/non-human bone origin, and can provide further information about analysed individual, e.g. studying of developmental stress in dental enamel. Microscopy of bone and tooth samples requires preparation of good quality thin histological sections for transmitted and polarized light microscopy examination and analysis. This paper presents detailed methodology description in several main steps which were modified to fit our laboratory. Finally, we suggest an easily repeatable protocol and know-how fitting in similar or identical laboratory conditions, including equipment, consumables, and other items.

KEY WORDS mammal hard tissue; histology; embedding medium; epoxy resin replicas; thin section preparation

INTRODUCTION

Despite the time consuming and financially demanding process of obtaining thin histological sections, microscopic examination provides specific data that cannot be obtained from macroscopic observation. Histological analysis is useful, for example, in determining whether indeterminate bone fragments are of human or non-human origin (Cattaneo et al. 2009; Sawada et al. 2010; Urbanová – Novotný 2005), taxon determination of fragmentary animal bones (Martiniaková et al. 2006), more accurate estimation of age at death (Fitzgerald – Rose 2007; Reid et al. 1998; Singh – Gunberg 1970),

Period	Site	Number of specimens	Animal/Human	Bone/Teeth	Burned/Unburned
Gravettian (34 - 29 ka cal BP)	Dolní Věstonice I	4	animal	bone	burned
	Dolní Věstonice II	3	animal	bone	burned
	Dolní Věstonice II	2	animal	teeth	unburned
	Milovice IV	3	animal	bone	burned
	Milovice IV	1	animal	bone	unburned
	Pavlov I	1	animal	teeth	unburned
	Pavlov I	1	animal	bone	burned
Epigravettian (22 ka cal BP)	Stránská skála IV	4	animal	bone	unburned
Early Middle Ages (1000 - 1300 AD)	Brno - Vídeňská	20	human	teeth	unburned
New Ages (1500 - 1700 AD)	Přibice	2	human	teeth	unburned
Early Middle Ages - Modern Period (1000 - 1700 AD)	Unspecified archaeological sites	6	human	teeth	unburned

Table 1: Composition of prepared samples.

study of taphonomic changes on microscopic level (Boriová et al. 2020; Hanson – Cain 2007), evaluation of survived stress events, and reconstructing the life history of an individual (Humphrey et al. 2008; Gamble et al. 2017; Lorentz et al. 2019).

Methodological procedures for thin section preparation from calcified mammal hard tissues differ depending on a) the tissue type from which the section is prepared, b) preservation of the specimen which correlates to the time frame and taphonomic context as well as c) the laboratory at which the sections are prepared. These conditions determine our orientation in a large spectrum of published recommendations for preparation of bone (Goldman et al. 1999) and thin tooth sections (Marks et al. 1996; Reid et al. 1998). It is possible to obtain good results following a rapid and financially non-demanding technique (Frost 1958) which was developed for fresh bone sections and has been successfully applied on well-preserved dry bone samples (Beauchesne – Saunders 2006; Maat et al. 2001). However, if specimen fragility is expected (especially in Late Pleistocene samples), the usage of embedding medium and good saturation of a sample cannot be underestimated (Chinsamy – Raath 1992). This methodological paper presents a procedure leading to quality thin sections from dry bone and tooth samples appropriate for subsequent histological analysis. Additionally, we compare the quality of the results obtained by using different laboratory equipment and consumables. Our methodology follows the previously published protocols and approaches (Bancroft et al. 1996; Caropreso et al. 2000; Chinsamy – Raath 1992; García-Donas et al. 2017; Hupková et al. 2015; Mahoney 2010; Marks et al. 1996; Nedorost et al. 2009) and is gently modified to our laboratory needs and sample demands, leading to the most effective procedure.

METHODOLOGY

Studied samples

In total, 47 human and faunal samples were analysed, including 19 archaeozoological and 28 anthropological samples (Table 1). Faunal remains were selected from osteological assemblages from the Mid-Upper Palaeolithic micro-region settlement area Dolní Věstonice I, II – Pavlov I, and Milovice IV (dated between 34 and 29 ka cal BP; (Svoboda 2016) and Late-Upper Palaeolithic site Stránská skála IV (dated to 22 ka cal BP; Boriová et al. 2020; Svoboda et al., 2020). All samples are currently deposited at the Centre for Paleolithic and Paleoanthropology in Dolní Věstonice at the Institute of Archeology in Brno, Czech Academy of Sciences (IA Brno CAS). Anthropological samples originated from archaeological site Brno – Vídeňská street, (excavated by Archaia o.p.s. dated to 1000 – 1300 AD; Sedláčková 2013) and Přibice (excavated by Department of Anthropology, Masaryk University, dated to 1500 – 1700 AD, Unger 1973). We also sampled dental collection with unspecified archaeological provenience and dating (between Middle Ages – Modern period). All anthropological samples are currently deposited at the Department of Anthropology, Faculty of Science, Masaryk University, Brno (DA FS MU).

THEORIES AND REASONING

We applied the protocols listed above at the DA FS MU, then further modified the methods at IA Brno CAS's Histological Laboratory.

Laboratory equipment at DA FS MU is more focused on an-

thropological dental samples and includes a Proxxon vertical band saw with diamond blade Micromot MBS 240/E and a Proxxon grinding machine TSG250/E. The silicone form for anthropological dental samples embedding was shaped from the plastic form approximating human teeth dimensions originally designed by authors (MK, SV) with valuable contribution of Dr. Mikoláš Jurda and printed on a 3D printer at the Laboratory of Morphology and Forensic Anthropology (LaMorFA DA FS MU).
The laboratory at IA CAS, however, is focused on Pleistocene samples where high mineralization and fragility is expected. Equipment includes a Struers circular saw with diamond blade Accutom 100, Logitech lapping machine PM5, Struers vacuum machine CitoVac, and an Ultrasonic cleaner VGT-1620Q.

Sample composition

Specimens were intentionally selected to cover a wide spectrum of tissue types and preservation levels to test, adjust, and unify laboratory protocols. Specimens without archaeological context were included to test suitability of embedding mediums. Specimens with known archaeological provenience were processed as well for specific histological analysis. Results from histological examination of archaeozoological specimens are published in Boriová et al. (2020); Sazelová et al. (2020a, 2020b), and are not part of this study. Histological examination results of chosen anthropological specimens are available in Vacková et al. (2021) and in unpublished thesis by Pelikán (2018).

Sample documentation

Due to the destructive nature of the preparation process, all samples were carefully documented before sectioning. In case of extremely valuable specimens the micro-CT sample scanning should be proceeded, however this type of examination is financially demanding, especially when a large number of specimens are prepared. Therefore, the documentation of our samples included photographs and osteometric evaluation and in order to preserve morphological and metric data, casts of specimens were prepared from dental plaster and epoxy resin (Araldite 2020). Replicas of bone specimens were not prepared due to the fragmentary nature of assemblage; however, we suggest that replicas from bone specimens only be produced when closer anatomical determination of fragment is possible and when spongious tissue is not exposed due to the risk of damaging the specimen during replication.
Two types of silicones standardly used by dentists were tested for replica preparation: condensation silicone (Zetalabor) and addition-curing two component silicone (Interduplicast). Both silicones have dimension stable properties, high precision in drawing details, and neither leaves residue, grease, or colour changes on the sample surface.

Sample cleaning

Cleaning specimens with water and a brush is inappropriate due to the high risk of damage and unwanted specimen hydration. We dehydrated most specimens in ethanol row (approximately 6 hours in 70 %, 16 hours in 90 %, 8 hours in 96 %, 18 hours in 99.6 %, depending on the laboratory's time options and specimen type), in order to clean and degrease the specimen surface, ensure better adherence, saturation, and prevent an exothermic reaction with epoxy resin. We avoided dehydration of several anthropological tooth specimens to observe differences between dehydrated and non-dehydrated specimens. Non-dehydrated anthropological specimens were cleaned with a cotton swab soaked in acetone (as recommended by Hupková and Králík, 2015).

Sample embedding and sample solidification

Bones and teeth from archaeological excavations present fragile tissue, so embedding was necessary to strengthen and stabilize specimens for further manipulation and processing. Two types of embedding mediums were tested: two component methyl methacrylate resins, Dentacryl and Duracryl Plus, that are commonly used in dental laboratories; and two component epoxy resins, Araldite 2020 and Epo-Tek, that are commonly used as adhesive for bonding a wide variety of materials.
Methyl methacrylate resins were tested on four anthropological tooth specimens, two specimens for Dentacryl (one dehydrated, one hydrated), two specimens for Duracryl plus (same procedure as in Dentacryl). Epoxy resin Araldite 2020 was tested on two anthropological tooth specimens (one dehydrated, one hydrated) and Epo-Tek was tested on four archaeozoological specimens (all dehydrated before embedding).
Specimens were placed in silicone forms and fixed with a drop of cyanoacrylate (instant glue) or a pea size amount of dental wax and embedded by epoxy resin. The correct cutting plane is necessary to obtain valuable microscopic records. Section plane was chosen for bone specimens based on anatomical determination, preservation, and further histological analysis. In our samples, the plane led perpendicularly to the longest axis of the fragment. Dental specimens cutting planes lead parallel to the vertical axis of the tooth and cross a particular tooth cusp (Hillson 2014). Solidification of the blocks with archaeozoological specimens took place in the vacuum machine (teeth: 0.8 Bar for 2 - 5 hours; bones: 0.5 – 0.7 Bar for 5 hours) to minimize air bubbles and provide better saturation of specimen by epoxy resin (necessary due to fragile nature of Late Pleistocene samples). Anthropological specimens hardened without vacuum machine due to their good preservation state. Solidification took 24-36 hours per specimen (both archaeozoological and anthropological).

First sample sectioning

Sectioning was done with two different saws: a) Proxxon vertical band saw and b) Struers circular saw, and both machines used distilled water cooling. The Struers saw also uses an anticorrosive solution.

First sample grinding and polishing

To remove surface irregularities and obtain an absolutely flat plane, specimens were ground and polished by waterproof sandpaper with a grit size of P500 – P4000, Proxxon grinding machine for dental anthropological specimens and Logitech lapping machine for archaeozoological. Finally, anthropological dental specimens were polished by diamond pastes with grain size of 6 – 0.7 μm and by felt and silk disk. Diamond pastes were not used in archaeozoological specimens due to possible specimen colour change, which could harden observation of taphonomical changes at the microscopic level. Between each step, specimens were cleaned with distilled water or airflow and by the end of polishing with Ultrasonic cleaner (40 kHz).

First sample bonding

The polished side of the specimens embedded in resin were mounted on a cleaned and degreased slide with epoxy resin Araldite 2020 and using Logitech bonding holder.

Second sectioning, second grinding and polishing

This step finalized the thickness of bone specimens around 40 – 100 μm, depending on preservation and type of analysis to be used in the next step of histological evaluation, and 80 – 110 μm in dental specimens. Similar steps to the first sectioning, grinding and polishing were used.

Second sample bonding

All steps were repeated as they had occurred during the first bonding. Finally, all archaeozoological specimens were covered with a cover slide to fill small surface irregularities and to protect the specimen. Anthropological dental specimens were not covered due to additional analysis of sample applied after histological examination.

RESULTS

The preparation of 47 specimens took approximately 8 weeks, with an overage duration of 90 hours per dehydrated specimen and 40 hours per non-dehydrated specimen. The preparation process was first tested on 13 specimens modifying chemicals or laboratory equipment. Table 2 represents each step of the process and the differences between laboratory equipment used (e.g., dehydration in ethanol row proceeded/not proceeded, using circular/band saw etc.). Process testing resulted in the final methodology (Figure 1), which we preferentially used for producing next 36 thin sections. Testing different approaches in thin section preparation resulted in following outcomes:
Sample documentation: Producing epoxy resin replicas from Zetalabor and Interduplicast silicone forms has proved to be a suitable method of measuring anthropological dental samples and morphologic data preservation. Considering bone specimens, epoxy resin replicas can be produced when closer anatomical determination of fragment is possible and when spongious tissue is not exposed due to the risk of damaging the specimen during replication.
Sample cleaning, embedding and solidification: Well preserved anthropological teeth samples indicated no difference between dehydrated and hydrated specimens. Epoxy resins were more appropriate for embedding compared to methyl methacrylate resins. Use of the vacuum machine for sample solidification is not necessary in well preserved anthropological tooth samples.
Sample sectioning: We obtained acceptable results using the Proxxon vertical band saw but the specimens' surface had several irregularities. The sectioning process was more skill-demanding than using automatic circular saw. Automatic Strues circular saw produced plane sections with minimum amount of surface irregularities.
Sample grinding: Using the Proxxon grinding machine sped--up the grinding process, however it elevated risk of specimen destruction because of the heat ("burning" the specimen). Still, it is suitable to use the machine carefully in case of severe surface irregularities on the block. Grinding in hand is more time-consuming process and it demands skilled technician but there is minimal risk of the specimens' destruction. Finishing the grinding process in lapping machine showed as the best option in terms of efficiency and accuracy, because it can be used to check the amount of ground material through a lapping jig with a digital gauge.
Sample polishing: Using polishing diamond pastes can result in specimens' colour change which can harden the observation of taphonomical changes at the microscopic level. The surface smoothness of the specimens finished using diamond pastes and those finished using sandpaper and felt and silk disk was not basically different.
Sample bonding: Epoxy resin Araldite 2020 proved to be suitable bonding medium. specimen bonded to the microscopical slide were almost without air bubbles. Sporadic presence of air bubbles was caused rather by misplacing the bonding holder than by epoxy resin used.
Thin sections produced by final methodology (Figure 1) provided good quality, and all necessary histological structures and presence of potential taphonomical changes were observable under a light microscope (Figure 2) at: a) low magnification level, i.e. 20x or 50x for basic bone microstructure, such as primary/secondary osteons, plexiform bone, reticular bone, and identifying accentuated lines in dental enamel; b) mid-level of magnification, i.e. 100x for specific bone microstructures such as osteons, Haversian canals; closer examination of taphonomical modifications; and measurements taken in dental

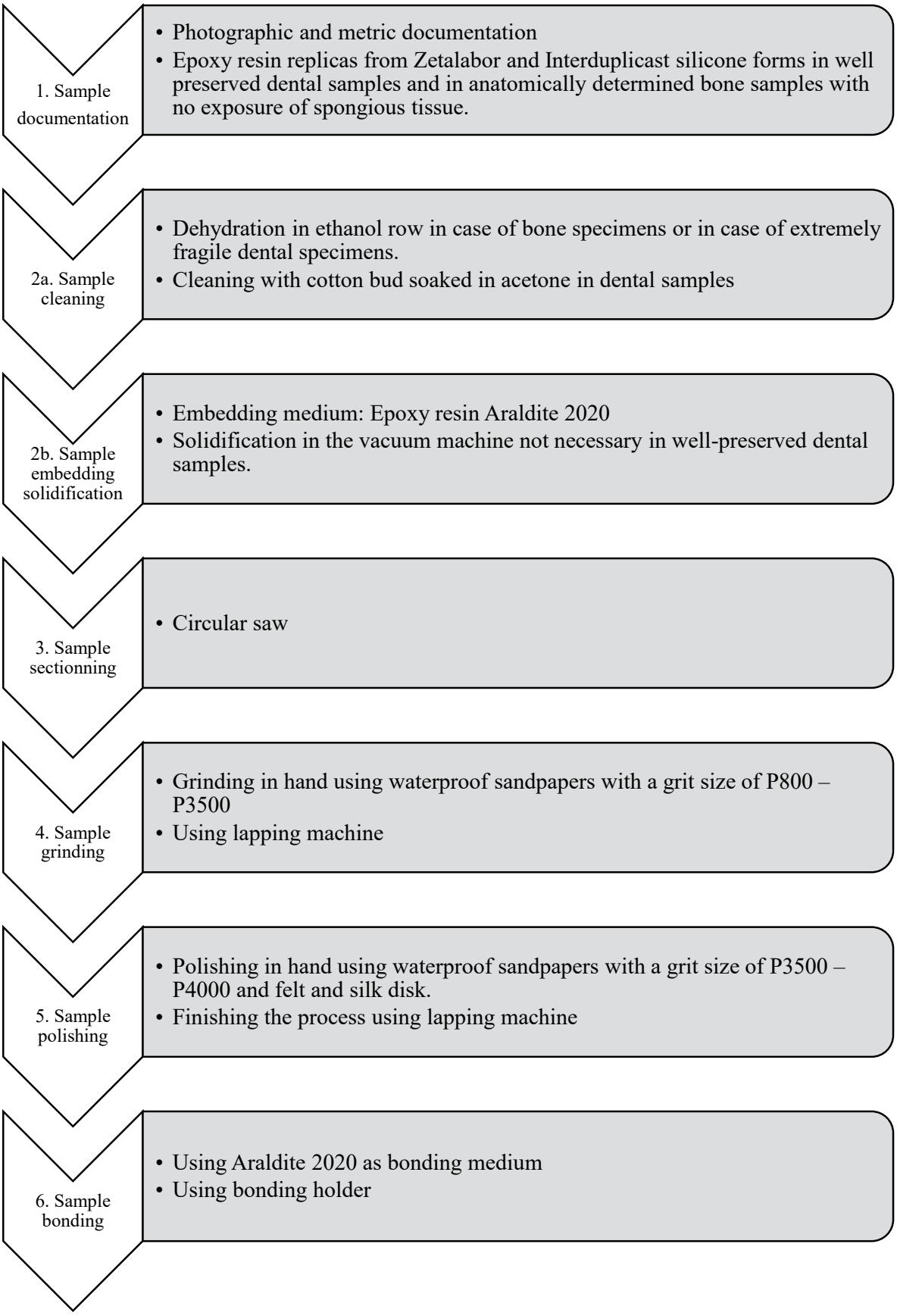

Figure 1: Methodological steps and protocols used in our laboratory in the histological bones and teeth thin sectioning.

		Type and number of samples (n)							
		Human tooth (n= 1)	Human tooth (n= 1)	Human tooth (n= 1)	Human tooth (n= 1)	Human tooth (n= 1)	Human tooth (n= 1)	Animal bone burned/unburned (n= 4)	Animal teeth (n= 3)
	Macroscopic sample description	Adult tooth, without visible damage at the macroscopic level. Specimens were no originally cremated/burned.						Fragments of burned and unburned spongious and compact bone, mostly indeterminate to skeletal part or taxon, the burned bones displayed changes in colour, some of the unburned specimens had black stained surface	Adult teeth, fossilized, colour changes visible of the crown surface due to taphonomical processes, several cracks visible on the enamel surface. Specimens were not originally cremated/burned.
STEP 1	**1. Sample documentation**	Photographic and metric, epoxy resin replicas from Zetalabor and Interduplicast silicone forms							
	1. Outcome:	Producing epoxy resin replicas proved to be suitable way to preserve specimens morphological and metric data of the specimen.						Epoxy resin replicas can be produced when closer anatomical determination of fragment is possible when spongious tissue is not exposed due to the risk of damaging the specimen during replication.	Producing epoxy resin replicas proved to be suitable way to preserve morphological and metric data.
STEP 2	**2a. Sample cleaning**	Dehydrated in the ethanol row	Non-dehydrated in the ethanol row	Dehydrated in the ethanol row	Non-dehydrated in the ethanol row	Dehydrated in the ethanol row	Non-dehydrated in the ethanol row	Dehydrated in the ethanol row	Dehydrated in the ethanol row
	2b. Sample embedding and solidification	Embedding medium used: Methyl methacrylate resin Dentacryl Solidification without the vacuum machine		Methyl methacrylate resin Duracryl Solidification without the vacuum machine		Epoxy resin Araldite 2020 Solidification without the vacuum machine		Epo-Tek Solidification in the vacuum machine	Epoxy resin Araldite 2020 Solidification in the vacuum machine
	2. Outcome	Strong exothermic reaction with methyl methacrylate resins, several bubbles in the block, yellow colour of the block. Dehydration in the ethanol row had no influence on the final specimen qualities.				No exothermic reaction with epoxy resin, clear block with minimal amount of air bubbles. The usage of vacuum machine had no influence on the amount of air bubbles in the final block with the dental specimen. Dehydration in the ethanol row had no influence on the final dental specimen qualities.			
STEP 3	**3. Sample sectioning**	Machine used: Proxxon vertical band saw				Machine used: automatic Struers circular saw			
	3. Outcome	Several surface irregularities				Plane surface with minimal amount of irregularities			
STEP 4	**4. Sample grinding**	Proxxon grinding machine with waterproof sandpaper with a grit size of P800 - P1200				Grinding in hand with waterproof sandpaper with a grit size of P800 – P3500		Grinding in hand with waterproof sandpaper with a grit size of P800 - P1200, finished in Logitech lapping machine	Grinding in hand with waterproof sandpaper with a grit size of P800 – P3500
	4. Outcome	Speeding up the process but risk of sample burning. Helpful in case of extremely irregular plane after cutting.				Grinding in hand is time consuming but there is minimal risk of sample destruction.		Finishing the sample grinding in the lapping machine is automatic process, so not so time demanding.	Grinding in hand is time consuming but there is no risk of sample burning.
STEP 5	**5. Sample polishing**	Polishing in hand with waterproof sandpaper with a grit size of P1200 – P4000, finishing with diamond pastes with grain size of 6 – 0.7 µm and felt and silk disk				Polishing in hand with waterproof sandpaper with a grit size of P3500 – P4000 and felt and silk disk.		Polishing in hand with waterproof sandpaper with a grit size of P1200 – P4000 and felt and silk disk. Finishing the process in the lapping machine.	Polishing in hand with waterproof sandpaper with a grit size of P3500 – P4000 and felt and silk disk.
	5. Outcome	Smooth and plane surface of the block with the specimen, without visible scratches on the microscopic level. The specimens' colour changed due to colour of paste used.				Smooth and plane surface of the block with the specimen, without visible scratches on the microscopic level.			
STEP 6	**6. Sample bonding**	Bonding medium used: Epoxy resin Araldite 2020 Bonding holder used							
	6. Outcome	Solid connection with the microscope slide, sporadic aur bubbles visible on the final thin section under the transmitted light microscope.							
	Final description of thin(s) section prepared	Thin section slightly coloured due to using diamond polishing paste, however incremental structures examined have good visibility. Several air bubbles make microscopic evaluation difficult, especially on the high-level of magnification (200x and 400x).				Good quality thin section, microscopic structures clearly visible even on the high-level of magnification (400x)			

Table 2: Qualitative description of testing several approaches of hard tissue thin section preparation process.

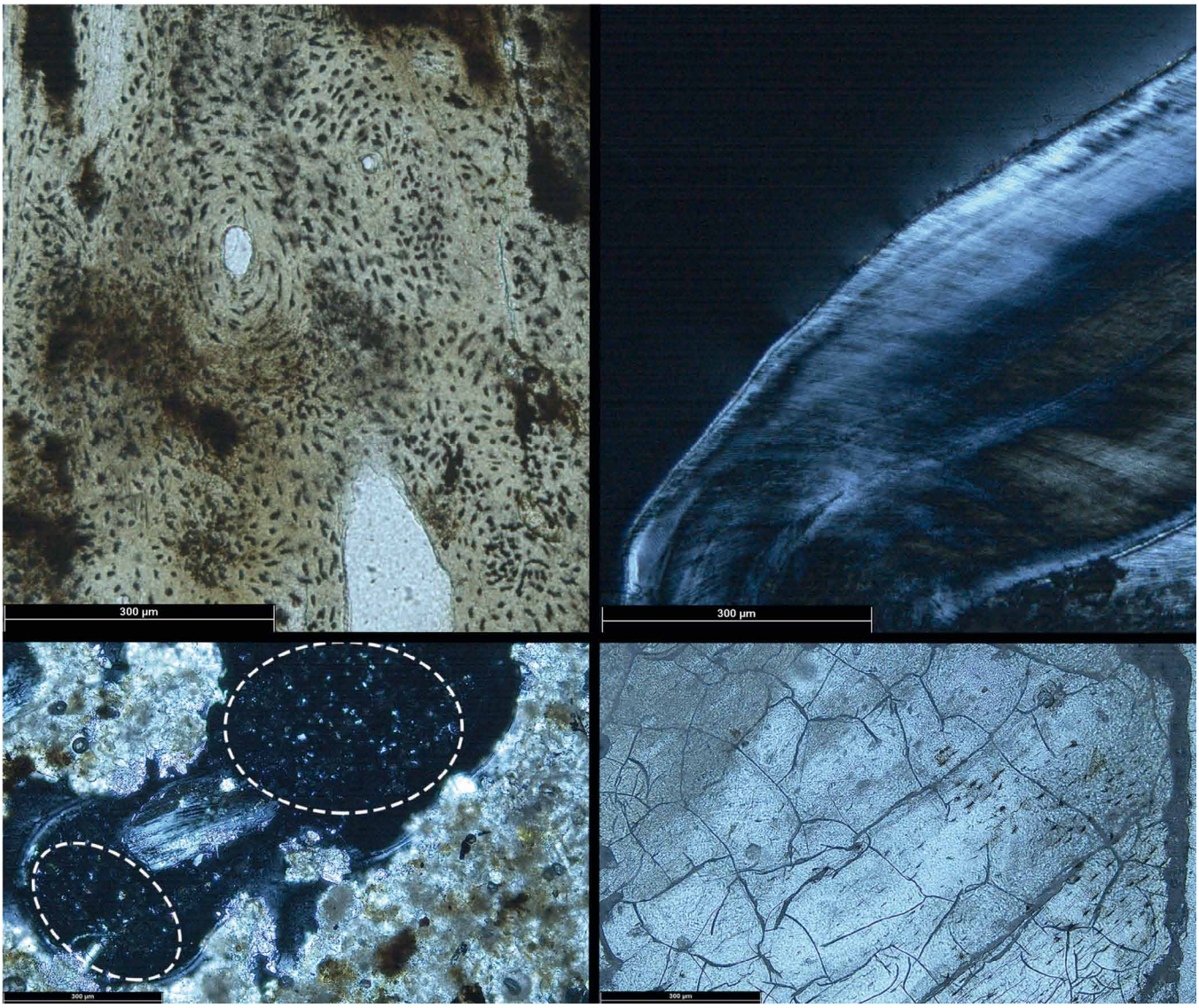

Figure 2: Thin histological sections following our protocol, magnification 50x. Upper part presenting ideal stage of the final products. Upper left: bone fragment, transmitted light, good visibility of osteons and lacunas. Upper right part: cuspal part of dental enamel, polarized light, good visibility of accentuated lines in dental enamel. Lower part represents lacking and/or mistaken protocol steps (lower left: bone fragment, polarized light, in dotted ellipse residues of abrasive) or unadvised sample treatment (lower right: bone fragment, transmitted light, cracks at the sample edge caused by over-grinding; which can be misinterpreted as burning).

enamel; and c) high-level of magnification, i.e. 200x or 400x for examination of smaller bone microstructures such as lacunae and measurement of daily increments in dental enamel. Application of the specific microscopic analysis of Late Pleistocene specimens was published in Sazelová et al. (2020a,b), with part of the anthropological samples microscopic analysis available Vacková et al. (2021) and in the thesis by Pelikán (2018).

DISCUSSION

Thin section preparation represents a destructive process; therefore, proper sample documentation must be done prior to starting. Producing replicas from epoxy resin is a suitable way to preserve morphologic and morphometric data. When extremely valuable specimens are processed, another expensive and time-consuming non-destructive analytical method (such as micro-CT) would be preferred.

Silicon embedding forms created for human teeth are advantageous because they allow for repeated usage, optimal consumption of epoxy resin, and comfortable resin block extraction from the form. Additionally, the cuboid shape of the final block makes fixation to the cutting machine holder easier, which ensures the correct cutting plane of the tooth.

The plasticine-like nature of Zetalabor silicone before solidification facilitated handling tooth during form preparation, but its rigid nature after solidification made whole tooth extraction from the form more difficult and consequently increased

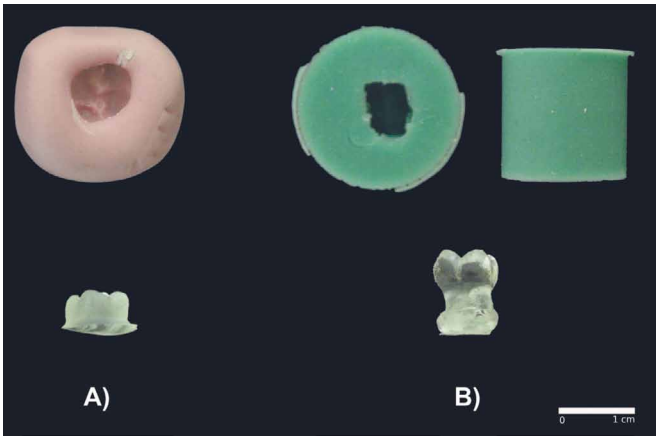

Figure 3: Silicone forms and replicas made from the epoxy resin of our dental samples. A) Zetalabor form for tooth crown replicas, B) Interduplicast form for the whole specimen replicas. We used a small piece of dental wax to fix the sample in its appropriate position.

Figure 4: A) Strong exothermic reaction between dental sample and its embedding medium (Duracryl Plus). Tooth embedded in a similar block lacks visibility and the sample is thus destroyed. B) Yellow coloration occurs in the embedding medium (Dentacryl) after its solidification. Colour change in such a degree is inappropriate due to the low visibility of cut line in dental samples and misinterprets observed taphonomic phenomena.

risk of tooth damage. Therefore, we suggest Zetalabor as more suitable for tooth crown replicas. Interduplicast silicone nature is viscous before solidification which made handling it more difficult, requiring the sample to be fixed in its position, but its elastic nature after solidification made replica extraction easy, so we recommend using Interduplicast for whole tooth replicas (Figure 3).

Replicas produced from dental plaster were brittle, which resulted in problematic extraction from the silicone form and surface damage while taking calliper measurements. Because of hardness as well as shape and size stability, we suggest using epoxy resin for replicas production.

Several authors recommended the hard tissue to be dehydrated before further processing (Caropreso et al. 2000; Marks et al. 1996). Other authors do not consider this step necessary due to nature of the sample (dry bone, dry tooth) (García-Donas et al. 2017). In our testing, the effect of teeth dehydration with alcohol row is consistent with the later opinion. Dehydration of anthropological dental specimens did not affect the exothermic reaction with methyl methacrylate resins or the quality of the final thin section, therefore we did not consider this step necessary in well preserved anthropological tooth samples. Durability differences were not monitored closely; however, non-dehydrated dental samples produced three years ago stay macro- and microscopically intact.

Using methyl methacrylate resins (Dentacryl, Duracryl Plus) as embedding mediums turned out to be inappropriate due to strong exothermic reaction with the specimen despite specimen dehydration before embedding and yellow colour of the block with the embedded specimen which makes it more difficult to visualize the cutting line (Figure 4). Epoxy resins (Araldite 2020, Epo-Tek) provided the best option for future manipulation with the specimen, e.g. straightening and protection and a low number of air bubbles when the vacuum usage was unnecessary.

The advantage of the Proxxon vertical band saw was the possibility of adjusting the cutting plane when the specimen in epoxy resin block was misplaced (due to incorrect fixation in resin during solidification). The Struers circular saw provided better results due to its automatic process, more accurate section, and safer handling.

The Proxxon grinding machine sped-up sample grinding, but careful sample handling is necessary to avoid uneven sample thinning or even completely removing specimen areas. Working with the Logitech lapping machine was a more time-consuming process, however there is minimal risk of the failures noted above. However, there is still risk of undergrinding the specimen when starting the grinding process for longer periods of time. Using the lapping machine is recommended for highly fragile and valuable samples.

Even with lower-cost laboratory equipment (Proxxon band saw and grinding machine in our case) it is possible to achieve good quality thin sections appropriate for further histological analysis. However, the process is more demanding than using automatic machines (such as Struers circular saw and Logitech lapping machine), and careful sample handling is necessary to avoid failures caused by unexperienced technicians. The purchase price of fully automated laboratory equipment is much higher; however, this equipment provides a more comfortable preparation process, higher certainty of a good quality result, and minimal risk of failure due to human factor.

CONCLUSIONS

Applying different methodical approaches of histology thin section preparation resulted in a basic, seven step protocol appropriate for our laboratory needs and for dry bone and tooth sample treatment. Methodology applied to our laboratory provided good quality thin histological sections suitable for further microscopic examination, which can provide further data and information about the analysed sample. Using of lower-cost laboratory equipment (Proxxon band saw and grinding machine) can lead to good quality thin histological sections of well-preserved anthropological dental samples and potentially also in well-preserved archaeozoological samples, however the process requires skilled and experienced technician due to higher risk of sample destruction caused by the human factor. Therefore, we consider using automated

laboratory equipment as a more appropriate in order to proceed highly valuable samples or samples where high fragility is expected (e.g. Late Pleistocene samples in our case).
Producing thin histological sections is a destructive process, however subsequent histological examination and analysis of archaeological and anthropological specimens can a) provide completely new information about life history based on an analysed individual or population, e.g. examination of stress markers in enamel and their chronological timing, b) supplement macroscopic information, e.g. determine more precisely age at death, and c) closer taphonomic phenomena determination, e.g. manganese concretion or microbial attack. Additionally, in extremely fragmentary or poorly preserved osteological remains, histological data can provide the only source of information about an analysed individual.

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