A QUICK AND EASY APPROACH TO PREPARE GROUND BONE SLIDES FROM UNDECALCIFIED SKELETON

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ABSTRACT

Ground bone slides are not only necessary for teaching histology of Haversian system to undergraduate and postgraduate students, but also essential for rapid pathological diagnosis in related diseases as well as to be used in polarizing microscopy and studying bone architectures from fossils. This technique is also applicable for hard tissues like teeth. Present histology manual-cum-practical literatures though prefer bone-slide preparation from decalcified skeleton, but Haversian architectures with osteons are vivid in ground bone slides. Here a technique is briefed; which have practiced well to prepare the ground bone slides with very simple, cheap and easily available equipments in a very short time, which can be adopted by anatomists, odontologists and geologists for quick making of compact bone slides without hampering the bone continuity avoiding hazardous and time consuming methods or costly equipments like freezing microtome.

KEY WORD: Ground bone, bone histology, Haversian system

INTRODUCTION

Study of bones is dealt in anatomy, pathology, orthopedics and dentistry. Lamellar architecture of bones, osteocytes residing in the lacunae, calculi containing processes of osteocytes, periosteum, endosteum, ground substance etc. can be easily demonstrated in ground preparation[1]. Ground bone preparation reveals the mineralized components of the bone[2].

Distributions of collagen fibers are only demonstrable in ground bone preparation where proper precautions have been taken to prevent the collagenous swelling[3]. Normally calcified frozen sections need different reagents and specialized microtome. For satisfaction, block must be frozen hard, Carbon dioxide is preferred than thermoelectric module and sledge microtome is preferred than ordinary freezing microtome[4].

Sections of Haversian systems or osteons showing alternate dark and light circles resulting from medium magnification have been described. Collagen fibers appear bright when cut longitudinally and dark when cross sections is taken[5]. Applications of aniline blue in compact bone sections presents a contrast and colour background[6].

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Method of ground bone preparation is briefly explained in most of the histology practical manual and as well as in textbooks. Photomicrograph of thin sections of about 50 micrometer mounted in Canada-balsam though available[7], but it has been mentioned in books that hand sawing and grinding of compact bones are not enough to produce slides with required standard because of its chance of uneven sections .Mineralized bones must be cut with tungsten carbide tipped knives and need special hard support to avoid cracked or crumbling of tissue sections. Acrylic resins and plastic are now widely used as embedding media, which follows sectioning in freezing microtome[7-9]. But adopted procedure as mentioned below, has successfully produced sections of satisfactory quality to describe the Haversian system as practiced repeatedly over last few years with very cheap and easily available equipments.

MATERIALS (Fig no. 1)

1. Normal dried calcified dried human skeleton
2. Hacks' saw blade (Iron cutting)
3. Forceps
4. Honing stone (fine carborundum stone)
5. Painting brush (No.0)
6. Tap water
7. Reagents including 50% alcohol, xylene, DPX, aniline blue
8. Petridish- three such

METHODS

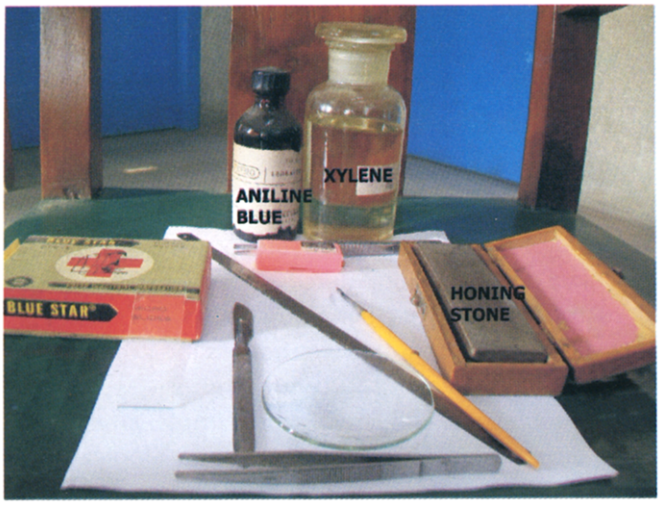

Fig-1: Essential equipments

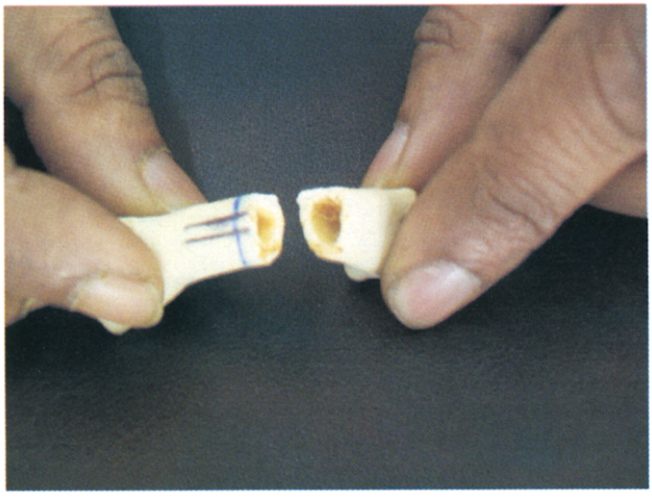

Fig-2: Sample collection: transverse section of metacarpal.

Fig-3: Sample collection: wedge section (point 'A') from femoral cortex.

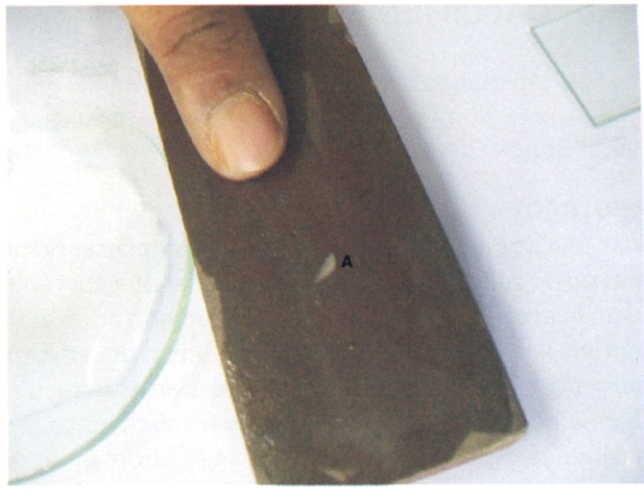

Fig-4: Grinding of the sample on honing stone. The sample bone piece is marked by 'A'.

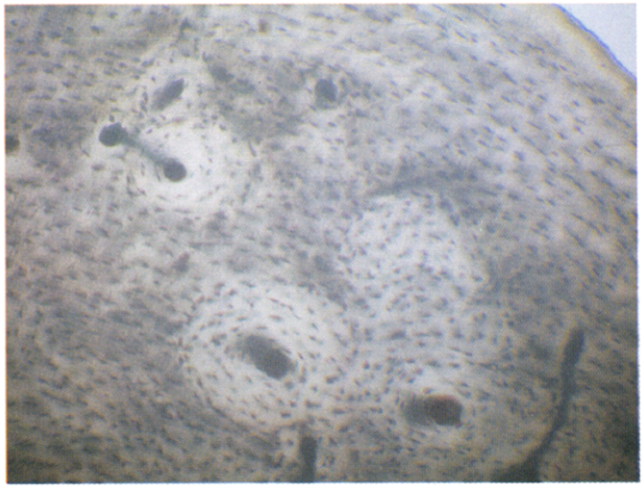

Fig-5: Histological demonstration of Haversian system under low magnification (10x nose piece lens).

Specimen collection:

Specimen of compact bone can be collected from miniature long bones like metacarpals or metatarsals (Fig. no.2). Two to three centimeter thick transverse sections can be made by the hack's saw blade in full thickness from periosteum to endosteum. If desired, longitudinal sections also can be taken by the same method. Thin wedge sections can be taken from cortical portion of any long bone without disturbing its continuity and contour (Fig no. 3). If required the portion can be repaired with synthetic glue and bone dust.

Trimming

Thin sections thus taken from bone are later trimmed in appropriate size with the help of scalpel and forceps

Grinding

The honing stone placed first on a stable flat surface and the bone chip is placed on it. With the palmar aspect of the tip of a finger (usually index or middle) the chip is rubbed on the stone with horizontal to and fro movement maintaining a sustained pressure. Water is applied frequently during the procedure with the painting brush. Water acts as the lubricant-cum-coolant, which help in rapid progress of the work. Repeated change of surface of the section with the forceps or paining brush helps in uniformity of the both surface of the specimen. Fingers can be changed to avoid the skin damage. Change of grinding pressure depends on the thickness of the section. Achievement of transparency denotes the adequate thinning (nearly 50 micron) of the specimen (Fig. no. 4).

Dehydration and clearing

Adequately thin specimen (nearly 50 micron) is now transferred to a Petridish containg 50% alcohol for dehydration for 5 minutes. Dehydrated tissue is now transferred to another Petridish containing xylene for 5 minutes. Xylene will make the tissue further transparent[4].

Check status

This can be done by transferring the tissue with the painting brush on a slide and observing it under the low power magnification. If the specimen is good enough, next step would be followed; otherwise further rubbing, dehydration and clearing steps can be followed as necessary.Impregnation and mounting Specimen is mounted with thick DPX and cover slip avoiding air-bubbles. After 24 hr drying it becomes ready to study under microscope (Fig no. 5).

DISCUSSION & CONCLUSION:

This method neither utilizes any freezing microtome nor uses any costly reagents like acrylic resins etc., but can satisfactorily produces ground bone slides with simple equipments. Though it requires a bit practice for it, but can be adopted easily. But as precaution only thing to be kept in mind is that too much keeping in xylene will make the section too transparent to study under microscope. In such case transferring the tissue in aniline containing petri dish for ten minutes will definitely enhance the contrast of the tissue, which makes convenient to be visualized. To have deep blue colour tissue can be kept immersed in aniline blue for thirty minutes and then a rapid wash in water following a smart dip in 50% alcohol gives a brilliant bluish colour. Such a procedure will be wise to be practiced by the postgraduate students and interested faculties for easy demonstration of Haversian system in compact bone.

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