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**Research Article**

# An innovative approach for manual preparation of slides of compact bone tissue

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## ABSTRACT

**Background:** Bone is a rigid form of connective tissue in which the extracellular matrix is impregnated with inorganic salts, mainly calcium phosphate and carbonate, that provide hardness. Because of its hardness, bone is difficult to cut in the microtome; therefore, special techniques must be used for its study.
**Methods:** Grinding of transverse sections of compact bone was done by using flint paper (size 0) which is simple, extremely cheap, rapid and easily available.
**Results:** Under low power magnification, we observed a complete haversian system with volkmann's canal and interstitial lamellae.
**Conclusions:** This method of preparation of ground bone slides is not only helpful to undergraduate and post graduate students but also to osteoarchaeologists, palaeopathologists and forensic anthropologists.

**Keywords:** Bone, Microtome, Haversian system

## INTRODUCTION

Bone is one of the hardest tissues of the human body and second only to cartilage in its ability to withstand stress.[1]

Bone is a rigid form of connective tissue in which the extracellular matrix is impregnated with inorganic salts, mainly calcium phosphate and carbonate, that provide hardness.[2]

Light microscopy of bone is a routine complementary diagnostic tool for osteoarchaeologists, palaeopathologists and forensic anthropologists.[3] It is also used in the diagnosis of bone tumors, hemopoietic disorders, metabolic bone diseases and infections.[4]

Techniques for the demonstration of bone and its components are possibly more varied and difficult than for any other tissue. They include:

A. **For decalcified bone**:- frozen, paraffin, or celloidin sections and transmission electron microscopy.
B. **For mineralized bone**:- frozen, plastic for microtomed or sawn ground sections, scanning and transmission electron microscopy.[4]

Because of its hardness, bone is difficult to cut in the microtome; therefore, special techniques must be used for its study. One of these consists of grinding thin slices of bone with abrasives until they become transparent. The preparation thus obtained is referred to as a ground section. This technique does not preserve the cells but it does permit detailed study of the matrix, its lacunas, and its canaliculi. Owing to differences in refractive index between lacunas and canaliculi (which are both filled with air) and the medium used in mounting, light rays striking the lacunas and canaliculi are deflected and do not penetrate the objective lens of the microscope. Lacunas and canaliculi consequently appear black in ground sections.[1]

Techniques of ground bone preparation have been explained in many histology textbooks. But these techniques require a lot of time, expensive instruments and knowledge of plastics. To overcome this, we have modified the technique of grinding sections of compact bone by using flint paper which is simple, extremely cheap, rapid and easily available. We have been using this technique for preparing compact bone slides in our histology laboratory since last few years.

## METHODS

### *Materials (Figure 1)*

**Figure 1: Materials used in procedure.**

1) Normal dried calcified long bone
2) Saw
3) Forceps
4) Warm water
5) Flint paper (size 0)
6) Painting brush (No. 0)
7) Scalpel
8) Reagents including 50% alcohol, xylene and DPX
9) Petridish
10) Cover slip
11) Slide

### *Specimen Collection*

A normal dried calcified ulna was taken. With the help of saw, approx. 1 inch thick transverse section was cut from its diaphysis (Figure 2). The bone section was then cleaned with warm water by holding it with forceps. A complete imaging of the complex three-dimensional structure of bone requires multiple transverse, longitudinal and radial sections. But we have concentrated on the preparation of a transverse bone section.

**Figure 2: Transverse section taken from ulna.**

### *Grinding*

The transverse bone section was placed on the flint paper (size 0), which was kept on a stable flat surface. With the palmar aspect of the tip of a finger, the bone section was rubbed on the paper with horizontal to and fro movement maintaining a sustained pressure. Frequent changing of fingers was done to avoid any cuts or scratches. Repeated changing of surface of section was done to provide uniformity to both surfaces. After the section becomes transparent (25-30 micron thick), grinding was stopped and then the section was washed under running tap water.

### *Trimming*

The transverse bone section was then viewed under microscope and the portion of the section which was found to be most clear was trimmed using scalpel and forceps.

### *Dehydration and Clearing*

The trimmed section was now kept in a petridish containing 50% alcohol for 5 minutes for dehydration. Dehydrated tissue was then transferred to another petridish containing xylene for clearing and was left in xylene for 24 hours.

### *Mounting*

The tissue was then transferred to the slide using painting brush (Figure 3) and mounted with thick DPX and cover slip avoiding air bubbles.

**Figure 3: Bone tissue on slide after grinding.**

## RESULTS

When the slide is viewed under the low power magnification, following observations were made (Figure 4):

a) Ring like osteons containing concentric lamellae.
b) A Haversian canal in the centre of each osteon.
c) Lacunae in between the concentric lamellae containing osteocytes.
d) Volkmann's canals connecting haversian canals in a ladder rung-like fashion.
e) Interstitial lamellae in the intervals between haversian system.

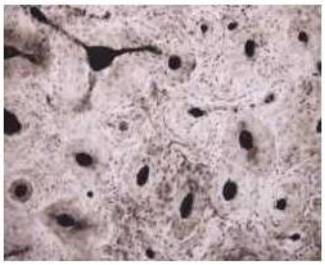

**Figure 4: Histological demonstration of Haversian system under low magnification (10x).**

## DISCUSSION

The thin grinding is the most difficult part of the sample preparation and it will require some practice. If the bone section becomes too thin there will be nothing left to look at; if it is too thick, the structures near the surface will be confounded by features that are buried inside the section.[5]

Mukherjee et al have also prepared ground bone slides from undecalcified skeleton but they have used honing stone for grinding.[6]

We may also use foot filer for coarse grinding of bone section but we have used flint paper which produces more smooth sections. Its application is simple, extremely cheap, rapid and reliable and the final product is a beautiful and intact 25–30 micron or less thin section. One advantage of this method is that the preparation of bone specimens does not require the use of dangerous chemicals for fixing and staining.

This method of preparation of ground bone slides is highly helpful to undergraduate and post graduate students and also to their teachers who want to take a closer look at one of the most astonishing, and astonishingly complex, natural materials.

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