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A review of Bone Preparation Techniques for Anatomical Studies

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ABSTRACT

The scope of modern anatomy has become very wide because it is now studied by all possible techniques which can enlarge the boundaries of the anatomical knowledge. So, bones are essential part of anatomy teaching curriculum and are unsurpassed in the ability to provide three dimensional instruction in Osteology as well as understanding the sites of soft tissue insertion and the course of neurovascular structures in a region. Many techniques have been employed over the years for preparation of bones. These methods include the use of insects, chemicals and enzymes. Bone preparation involves soft tissue removal, maceration, bleaching and labelling of the bones. The present study conducted with the aim of evaluating the least time-consuming and effective method of bone preparation from embalmed and wet specimens.

Key words: *Osteology, maceration, soft tissue removal, bleaching, degreasing.*

INTRODUCTION

Bone is one third connective tissue. It is impregnated with calcium salts which constitute the remaining two-third part. The inorganic calcium salts make it hard and rigid, which can afford resistance to compressive forces of weight-bearing and impact forces of jumping. Collagen fibres makes it tough and flexible. Which can afford resistance to tensile force. If calcium salt is removed by putting the bone in acid become flexible.

Through the ages, anatomy evolved as a foundation of medical education. Osteology i.e., study of bones is very essential and integral part of anatomy teaching curriculum.[1] Human bones are unsurpassed in the ability to provide three-dimensional instruction in the study of Osteology. In addition, bones also assist in understanding the sites of soft tissue insertion and the course of neurovascular bundles in the region.

Bone preparation essentially involves soft tissue removal, bone bleaching, bone de-greasing and labelling. The time required for these processes vary depending on the size of the human dead body.[2]

There are different techniques are used in bone preparation, which mainly include insect consumption, cold or warm water maceration. Which have been used as standard maceration techniques and requires between 2 days to 8 weeks depending on the amount of bacteria present, the size of material being macerated and the temperature of the environment during the maceration. Boiling method and subsequent mechanical cleaning of skeletal material method is also used.[3] Organic and inorganic chemicals solutions are also used to remove soft tissue from bones. Inorganic chemicals used are antifoaming, ammonium

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hydroxide, sodium hydroxide and other alkaline solutions. Maceration with organic chemicals can be performed with enzymes such as pepsin or with washing powders containing enzymes[4] as well as burying in soil.

Original human bones are not available in the market due to government's policy of not licensing any vendor to deal in human bones. In the absence of a standardized method of bone retrieval, a large repository of human bones is lost, as most medical colleges do not process the bones after the dissection of human cadavers. Original human bones sold by unauthorized persons are excessively expensive and without a valid invoice. Artificial bones made of POP (plaster-of-Paris) or resins available in the market are inferior in terms of details of the morphological features of the bones.

The authors, having tried different methods, have been able to standardize their own method and presents the method of retrieving bones from cadavers using a combination of various bone cleaning techniques for anatomical studies.

Following steps are followed for the Retrieval of bones from human cadavers

Step 1: Removal of soft tissue

Scrub, pick and (gently) scrap away the loosened muscles, ligaments and soft tissues using a scalpel, opening up all the joints of extremities (Limbs) and separating the bones. Articulation of hand and foot, vertebral column and pelvis should kept intact at this stage as any attempt to separate them at this stage may cause damage to the bones.

Step 2: Maceration

- Soak the bones in water for 24 hours for softening. About 30-40 litres of water may be used for bones from one cadaver or depending on the size and weight of the body.
- Remove the soft tissues by gentle scrapping.
- Put the bones in water in an appropriate container ensuring that the bones are completely immersed (about 80 - 100 litres of water) and Boil for 2 hours. Boiling the bones in water must be done carefully and should be checked often. Once the boiling has started, the bones should be gently simmered to prevent damage.
- Add Potassium Hydroxide (KOH) pellets 200 - 250 gm in case of male bones and 150 - 200 gm in case of female bones. Add Common Salt half kg to the solution and keep it simmering for 1½ - 2 hours.
- Remove vertebral column and separate the vertebrae. Similarly, the hand and foot can be disarticulated and clean off more of the tissue.

- Remove skull, clean off more of the tissue, and repeat this process until the skull is clean. Tying a string to the skull will facilitate picking it out of the hot water through the Foramen magnum, the brain must be cut into pieces with scalpel and the brain with duramater should be scooped out. Boiling too long can damage skull and may dissolve bone tissues.
- Wash and rinse the bones in water at room temperature. Check the bones and remove any

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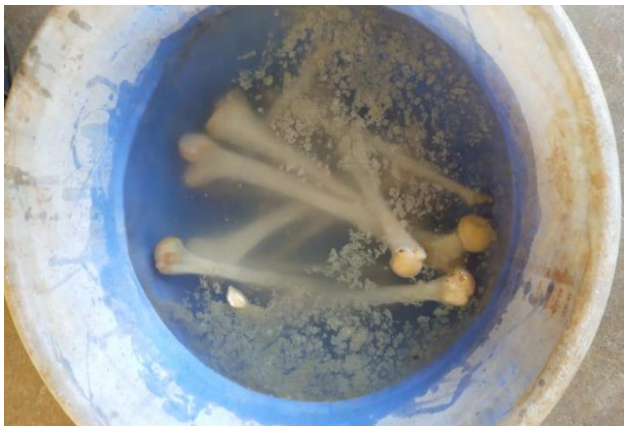
soft tissue which still adherent to the bones with the help of scalpel.

- Soak the bones in water at room temperature for at least 12 hours.

Step 3: Bleaching

Wash and rinse each individual bones. Soak all the bones from one cadaver in 30 - 35 litres of Hydrogen Peroxide (H_2O_2) 30% w/v solution ensuring all the bones covered with H_2O_2. Cover the container with a lid and keep for 12 - 14 hours. Over-bleaching will make the bones brittle.



Step 4: De-greasing

Wash the bleached bone thoroughly with water. Make sure thoroughly rinse the bones in fresh water immediately after subjecting them to the bleach, because the chlorine will continue to degrade the bone surfaces even after they are allowed to dry. Immediate reboiling in fresh or soapy water will also be used to remove the bleach.

Soak them in Acetone for 12 hours.

Step 5: Drying

Remove the bones from Acetone and wash with water. Spread the bones on blotting paper and let the bones dry in normal room for 4-5 days.

Step 6: Finishing

After completely drying, finish the procedure by painting with a mixture of half litre lacquer and half litre lacquer thinner (alternately by painting with Johnson Touchwood®). This will prevent erosion at the ends of the bones.

Salient features

- The chemicals used in the method of bone preparation are easily available in any Anatomy department of a medical college or easily available at any vendor dealing in chemicals.
- The chemicals used in the method are inexpensive.
- Soft tissue removal by chemicals solutions found to be the most effective since it macerates bone in a remarkably fast and odourless way. In addition, it

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has a less degradative effect on bone DNA than the other cleaning processes.

- The method is suitable with including Formalin-fixed cadavers as it does not involve using Insects to clean bones, as insects do not eat into formalin fixed tissue.
- The method includes degreasing as grease must be removed because it will smell, seep through the bone, and may attract dust and grime.
- The method does not damage the bone and preserves all morphological traits of the bones.

DISCUSSION

Osteology i.e., study of bones is very essential and integral part of anatomy in teaching curriculum. Human bones are unsurpassed in the ability to provide three-dimensional instruction in the study of Osteology. In addition, bones also assist in understanding the sites of soft tissue insertion and the course of neurovascular bundles in the region. By utilizing a combination of dried bones with textbooks and atlases as well as laboratory dissection, the important aspect of bone anatomy learned most efficiently.[5]

Bone preparation essentially involves soft tissue removal, bone bleaching, bone de-greasing and labelling. The time required for these processes vary depending on the size of the human dead body. There are different techniques used in bone preparation, which include insect consumption, cold or warm water maceration, which have been used as standard maceration techniques and requires between 2 days to 8 weeks depending on the number of bacteria present, the size of material being macerated and the temperature of the environment during the maceration. Boiling and subsequent mechanical cleaning of skeletal material is also used. Organic and inorganic chemical solutions are also used to remove soft tissue from bones. Inorganic chemicals used are antiformin, ammonium hydroxide, sodium hydroxide and other alkaline solutions. Maceration with organic chemical solutions can be performed with enzymes such as pepsin or with washing powders containing enzymes as well as burying in soil.

Maceration

Bones were macerated in solutions of varying P^H to observe the effects of varying fresh water P^H on bone. Since little is known about the decomposition of remains in aquatic environments of varying P^H, and even less is known about the specific effects of these environments on bone. Bovine bones were placed in solutions of pH 1, 4, 7, 10, and 14 and observed over a period of 1 year. All solutions eventually removed or dissolved the soft tissues from the external surface of the bone. The pH 7 and pH 10 solutions had little effect on the bone, but the other solutions affected the bone to varying degrees.[6]

Extreme P^H levels may be destructive, while more moderate P^H levels have lesser but significant effects.

Additional simmering in borax helped to break down cartilage and collagen, followed by soaking in xyol to remove residual fats.

Maceration using insects: It has been shown that dermestid beetles are useful as a technique to clean bones, especially for the parts of the skeleton, which are difficult to dissect by hand.

It cleaned various parts of the skeleton of normal and osteoporotic rats using Dermestes maculates beetles or manually and compared them with respect to their dry weight, ash weight, and calcium content.

Use of chemicals: Human skull is important in osteology because they aid in understanding the sites of soft tissue insertion and their intricate course of neurovascular structures in the skull base. Recent geopolitical developments in Asia have led to extreme difficulty in obtaining human skull specimens. A method for the preparation of dried human skulls from fresh and frozen cadavers using commonly available chemicals has been described. The technique, requiring about 8 weeks total time and basic equipment, consists of maceration accelerated by several enzymes followed by defatting, washing and bleaching. The skulls produced are of excellent quality and durability with no preparation artefacts.

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An economical source of skulls has now re-established to facilitate learning of the intricate relationships of the skull base. This method found to be the best in terms of time required to complete the procedure, number of bones recovered, colour of the bones and odour of the preparation. However, the chemical method has the disadvantage of dissolving, cracking the bones if the concentration used is high, and prompt attention not given to the preparation.[7]

Detergent maceration

Osteological assessment of human remains forms an essential part of forensic work, especially during the examination of extensively decomposed, dismembered or burnt bodies.

In a study to assess the effectiveness of detergents for the purpose of soft-tissue removal from animal derived specimens, it was shown that such a means is comparable to enzymatic maceration but with fewer health and safety issues and greater advantages regarding transportation and availability of materials when an investigator is in a fieldwork scenario.[8]

The forensic biologist called upon to conduct DNA analysis if identification is not possible from visual inspection of skeletal remains. The possibility of downstream DNA testing needs to be considered when skeletal preparation techniques are employed to deflesh human remains, as they have the potential to strongly impact genetic analyses and subsequent identification.

There has been considerable debate among anatomists and anthropologists as to how the remains should be macerated. Many feel that boiling is much too destructive a process. However, we have been experimenting with this method and feel that boiling, when carefully applied, is far superior to no-heat or low- heat methods, beetles, and caustics (bleach, ammonia), and is also quicker. Soft tissues and grease best removed through simmering and boiling. Skin, large bundles of tendon, muscle, and ligaments should be carefully removed with dissecting tools, taking care not to damage the bone surfaces - the boiling will be doing most of the work.[9-11]

Conclusion

We conclude that retrieval of bones using our method is very effective combining various bone-preparation techniques for anatomical studies. The yield of the bones thus retrieved by this method is of very superior quality in terms of morphological details and can be used for anthropological and morphometric studies.

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