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Review Article

Procurement and processing of human bones in medical schools for teaching purpose: A narrative review

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ABSTRACT

Bones are an indispensable learning resource to learn osteology. This study reviews the various bone preparation techniques and the ease of procurement and processing of human bones for teaching medical students and osteology research. Steps of bone preparation essentially involve soft tissue removal, bone bleaching, bone articulation and labelling.[1] Embalmed cadavers may be ideal for bone preparation because it eliminates the risk of infection. Detergent maceration may be the ideal method of bone maceration because it is a cheaper technique and gives good results. Both acetone and hydrogen peroxide may be the ideal degreasing and bleaching agents, owing to their superior outcomes.

Key Messages: Embalmed cadavers may be ideal for bone preparation because it eliminates the risk of infection. Among all, detergent maceration can be considered as the ideal method because of its cost-effectiveness and good results. Both acetone and hydrogen peroxide can be considered as the ideal degreasing and bleaching agents, owing to their superior outcomes.

1. Introduction

Bones are an essential part of the anatomy teaching curriculum.[2] Knowledge of osteology, which forms the foundation for understanding skeletal muscles' origin, insertion, and action, greatly influences human anatomy. Human bones have the ability to provide 3-Dimensional information in osteology. Bones help understand the sites of soft tissue insertion and the course of neurovascular structures present in a particular region.[3] Medical colleges procure bones to enable the faculty to teach osteology to the students using bones. Sometimes the bones are also issued to the students from the anatomy department for their self-study, which they return at the end of their academic year. Such an issuing of bones to the students for self-study will be done by some medical colleges with an adequate and excessive number of bones. In contrast, certain medical colleges that do not have a sufficient number of bones will not issue bones to the students for self-study. Such an inability to issue bones to the students for self-study significantly limits their opportunity to learn osteology at a comfortable pace. If the medical colleges could provide bones to the students for self-study, this exponentially enhance osteology's foundational knowledge. On the contrary, if the medical colleges cannot offer bones to the students for self-study, that markedly limits their foundational knowledge in osteology. This can adversely impact the medical student's overall knowledge in anatomy and the understanding of other subjects where anatomy forms the foundation. Therefore, the capacity of medical colleges to provide bones to the medical students for self-studying osteology plays a critical role in the knowledge outcome and performance of the medical students, in extension, future doctors. Medical colleges can do this by

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two means, purchase of readymade human articulated and disarticulated bones or by in-house preparation of the bones from the cadavers available at the medical college. The procurement of a prepared human skeleton is a costly affair, with the price often reaching INR 650000 (in the year 2021) for a single articulated skeleton. So such a huge cost of the purchase of human skeletons by the medical colleges makes them a costly proposition. Alternatively, the in-house preparation of bones from the cadavers available at the medical college will be a cost-effective option (approximately INR 2500 in the year 2021) to provide an adequate number of bones for teaching purposes.

In this paper, we review the preparation method and the feasibility of different techniques in the procurement and processing of the human bones utilized to teach medical students and osteology research. We specifically look to find out which method of bone preparation involves a more straightforward technique that is cost-effective, less labour intensive and that requires minimal infrastructure, and that yields a better outcome of bones.

2. Discussion

The study of osteology is fundamental in understanding anatomy and its application in Orthopaedic and surgery practices.[4] Software's such as Anatomy 3D atlas, Osseous system in 3D, 3D4 Medical, Acland's Anatomy Atlas and websites such as https://3d4medical.com, https://www.biodigital.com, https://anatomylearning.com, https://www.visiblebody.com, https://www.zygotebody.com are some of the electronic resources for learning osteology. However, learning through physical bone is paramount for thorough understanding. Although plastic bones are available comparatively cheaper for the students, they cannot completely replace the original human bones as a learning resource. Plastic bones provide a basic idea of the structure of bones. But, they lack intricate details that are necessary for a deeper understanding of osteology. They are prepared by using moulds and are sometimes prone to errors in the bony details. Moreover, they do not help conduct scientific research or for designing bone-related surgical instruments. Hence, the procurement of bones from cadavers plays a vital role.

The various stages of preparation of bones include procurement of cadavers, maceration, degreasing, bleaching, drying, and finishing.

2.1. Procurement of cadavers

The sources of cadavers for bone preparation include the embalmed cadavers dissected by students, embalmed un-dissected cadavers, and fresh un-embalmed cadavers. Cadavers for embalming and dissection are generally procured by the Department of Anatomy in each medical college as a part of a voluntary whole body donation program. Cadavers are also sourced from unclaimed dead bodies from the hospital attached to the medical college. The voluntary whole body donation program can be augmented to increase the cadaveric input and availability for dissection and consequently the availability of bones.

2.2. Maceration of cadavers

Maceration is defined as controlled putrefaction wherein the proteins of the body's cells are broken down and consumed by bacteria in anaerobic conditions. A constant optimal temperature is required to be maintained throughout the process. Since it generates a strong malodour, the process is usually carried out in a closed container in a ventilated area. The various types of maceration include hot /cold water maceration, maceration by burial method, chemical maceration, insect maceration, detergent maceration, maceration by manual cleaning, microwave maceration.[5]

1. Hot water maceration involves cooking bones in boiling water.[6] In this step, all the internal organs of the cadaver have to be removed, and the bones are required to be defleshed as much as possible.[6] Partially defleshed bone specimens are immersed in water and boiled at 100 0C for about 5 hours, and cooled overnight.[6] The boiling causes rapid breakdown and disintegration of proteins in the soft tissues. Although boiling speeds up the process of maceration, it may cause shrinkage, discolouration of bones and damage the ends of the bones.[6]
2. Coldwater maceration involves soaking partially defleshed bones specimens in water, which allows the buildup of the microbes, leading to the breakdown of proteins and the decomposition of soft tissues.[6] This process may require an additional step of intermittent defleshing of bone specimen for about 10-12 rounds to hasten the disintegration and decomposition of soft tissues.[6] The cold maceration causes more minor damage to the bones, but it retains the natural colour of the bones and prevents shrinkage.
3. Maceration by burial method involves the burial of cadavers in the burial ground containing sand for a prescribed amount of time to facilitate bacterial action and decomposition of the soft tissue. Before the burial, the bones have to be manually defleshed using a scalpel, forceps, and scissors in order to reduce the soft tissue burden.[2] Alternatively, before the burial, the bones can also be boiled for about 7-8 hours or can be immersed in warm water at 650C for 24 hours with or without detergent to facilitate this process.[2] The structure of the burial ground plays a vital role in the acceleration or deceleration of the decomposition of the soft tissues buried in the ground. The ideal dimensions of the burial ground

include an area not less than 10x8x4 feet.[4] Burial ground filling is done with loam sand which allows the atmospheric air to pass through the different strata of soil, which will facilitate the decomposition process.[4] Cadavers will be buried at variable depths from 1-4 feet for about 8 months to 3 years, depending upon the type of procurement of cadavers, pre-burial process, and humidity of a particular geographical region.[4] Embalming tends to preserve the cadavers for longer duration; hence embalmed cadavers may have to be buried at shallow depths for a longer term. After the burial, cadavers will be excavated and subjected to subsequent steps of chemical cleaning.

4. Chemical maceration: Both organic and inorganic chemicals are used for bone maceration.

   (a) Organic chemicals including pepsin, trypsin, papain, and Adolph's/ Palmolive are used.[7] Organic (enzymatic) maceration is the most convenient method of maceration. Adolph's/Palmolive is a fully constituted formulation that is an alternative to pepsin, trypsin, and papain,which are only available in powder form.[7] A 1% aqueous solution of pepsin, trypsin, and papain is made in which the partially defleshed specimens will be immersed at 60^0C for 24-48 hours.[8] This method completely digests the fat, muscle, connective tissue, tendons, ligaments and cartilage, resulting in clean, intact bones. Intermittent stirring of the enzyme solution is required to achieve the optimum activity of enzymes.[7] Drawbacks of this method include a significant health and safety risk to the operator and strong malodour emitted during the maceration process. Moreover, prolonged exposure of bones to these enzymes makes them brittle, fragile and leads to loss of bone feature details.

   (b) Inorganic chemical maceration: This method involves denaturation of protein and breakdown of collagen due to the chemical action in the soft tissues. Commonly used inorganic chemicals are antiformin, sodium hydroxide, calcium hydroxide, hydrogen peroxide. Other chemicals used for bone maceration include ammonium hydroxide, potassium hydroxide, Ether.

      i. Antiformin: It is a stock solution made from 10% sodium hypochlorite (NaOCl), 45% potassium hydroxide (KOH), and distilled water.[9] Sodium hypochlorite is pale greenish-yellow in colour, and its diluted solution is commonly used as a bleach or liquid disinfectant.[10] The active compound present in bleach is chlorine, and it is a sodium salt of hypochlorous acid.[10] The anhydrous compound of sodium hypochlorite is highly unstable, which decomposes explosively.[10] Hence it is always preserved in a crystallized form as pentahydrate.[10]

      For maceration, partially defleshed specimens are treated with Antiformin solution for 24 -48hours.[9] Followed by immersion in 3% hydrogen peroxide and Ether for 24-48 hours in each solution.[9]

      ii. Sodium hydroxide (NaOH): It is available as white powder or pellets.[11] It is highly soluble in water and absorbs carbon dioxide from the air.[11] It causes protein decomposition at ambient temperatures.[11] Varying concentrations and normality of sodium hydroxide have been tried for bone maceration. The partially defleshed specimens are immersed in 0.5N NaOH for 24-48 hours, or 1N NaOH for 48 hours, or 5% NaOH for 4-7 days at 70^0C, followed by immersion in 3% hydrogen peroxide at 70^0C for 24 hours.[9] Finally, bones are treated with Ether for 24-48 hours in an airtight jar.[9] The advantage of this method is that it yields intact bones and retains the natural colour of the bones. The drawback of this method includes softening and cracking of bones. When it comes in contact with bare skin, it can cause severe burns. Hence, care should be taken while handling it.

      iii. Calcium hydroxide ($CaOH_2$): It is a colourless crystal or white powder produced when calcium oxide (quicklime) is slaked with water.[12] Hence, it is commonly called as slaked lime/builder's lime.[12] Saturated calcium hydroxide in water is called Limewater, a corrosive agent that denatures the proteins.[12] It is soluble in acids and glycerol and forms a solution that acts as a moderate base. It forms salts while reacting with acids. The partially defleshed specimens are immersed in calcium hydroxide solution for 4-7 days under sunlight.[13] This method yields white coloured bones without any damaging effects such as cracks/brittle, and this process are associated with slight malodour.

      iv. Hydrogen peroxide (H_2O_2): This chemical is used for both bone maceration and bleaching. In its pure form, it is a pale blue liquid that is slightly more viscous than water.[14] It is commonly used as an oxidizer, antiseptic, and bleaching agent

at a lower concentration.[14] Concentrated hydrogen peroxide is used in industries.[14] Concentrated H2O2, when heated, decomposes explosively, and hence it is used as a propellant in rocketry.[14] It also decomposes steadily when exposed to light.[14] Hence it is stored in a dark bottle to prevent light and its decomposition.[14] Defleshed specimens are immersed in H_2O_2 solution of varying concentrations, such as 50% H_2O_2 for 48 hours at room temperature or 3% H_2O_2 for 24 hours at 70°C.[9] This method makes the bones brittle and leads to the formation of white powder residues on the surface of bones when higher concentrations of hydrogen peroxide are used.[9]

5. Maceration by insects: It is a very effective method of bone cleaning which employs the use of carpet beetle belonging to Dermestes species. These beetles feed on the soft tissues adherent to the bones. A colony of thousands of beetles are reared in a container measuring 30 cm x 50 cm x 30 cm.[8] The temperature has to be maintained at 22°C – 28°C to ensure adequate insect activity.[8] When the beetles are not used for maceration, they must be fed with pieces of meat or industrial fat by-products.[15] When exposed to a medium-sized cadaver, the beetles can completely clean the specimen within 24-48 hours.[15] This method is valuable in cleaning small and delicate bones of the skull. However, care has to be taken to monitor the insects' progress because when they are deprived of meat, they can even digest the bones.[15] Moreover, maintaining a colony is difficult, time-consuming and expensive.[16] Furthermore, the beetles tend to live within the bones for days to weeks.[16] Because of this, the bones require extreme temperature treatment, alcohol bath and several weeks of quarantine to eliminate all insects before the bones are taken out for use.[16]
6. Detergent maceration (sodium carbonate): It is a variant of enzymatic maceration. The rationale behind the use of detergents for bone maceration is that they have active biologic enzymes.[8] The detergents are available readily throughout the world, and they are ideal for fieldwork.[8] Detergent causes saponification of muscle, connective tissue, and tendons and also helps in degreasing the bones.[8] Since detergents contain deodorants, it reduces the malodour emitted during the maceration process.[8] Specimens are immersed in a detergent solution for about 24-48 hours at 40-60°C.[8] This method has fewer health and safety issues for the handler.
7. Maceration by manual cleaning: Maceration in this method involves the removal of the bulk of the soft tissues manually rather than by putrefaction. This is a simple method that requires a scalpel, forceps, scissors, brush and scouring pad to strip off the periosteum.[8] This method is helpful for cleaning long bones using limited resources. But it is a labour-intensive process with a significant risk of injury to the handler, and furthermore, it also creates artifactual damage to the bones by the instruments.[8]
8. Maceration by microwave: The defleshed specimens are placed in the microwave-safe dish and covered with loose lids or plastic wraps/aluminium foil to prevent the dehydration of soft tissues.[9] They are heated at 1300W, 2450MHz microwave at 1-minute intervals until all the soft tissues adherent to the bones are easily stripped off[7] /heated at different temperatures (80°C, 100°C, 120°C/ 140°C) with top and bottom heat for 1-5 hours.[17] Soft tissue adherent to the bone is removed every 60 minutes, followed by rinsing in warm water for 2 minutes immediately after the oven treatment and dried for three days at room temperature (22 °C).[17]

2.3. Degreasing

Degreasing involves the removal of grease from bones.[6] This process is aimed at the removal of all the oily contents from the macerated bone in order to make them oil-free.[6] Bones devoid of degreasing looks dirty, attract dust and do not bleach well. The commonly used degreasing agents are Chloroform, Benzene, Acetone, Trichloroethylene and Ammonia. Many chemicals have been used for degreasing, of which Acetone and chloroform are widely used for degreasing. Acetone is a highly volatile organic compound that is miscible with water and serves as an important organic solvent.[18] Acetone is the active ingredient in nail polish remover and paint thinner.[18] Acetone has to be kept away from the surface and the open flames since it is combustible.[18] Disposal of Acetone is vital after its use, and it must be disposed of in a leak-proof metal container which has to be lined with a plastic bag to keep it away from other things which could possibly ignite it.[18] Chloroform is a colourless clear volatile liquid and has a sweet, pleasant odour.[19] It is soluble in water, alcohol, ether, acetone, benzene and petroleum.[19] Since it has high vapour pressure, it evaporates readily.[19] Chloroform is a suspected human carcinogen and a reproductive toxin.[19] It also causes depression of the central nervous system.[19] Hence, it must be disposed of as hazardous waste within the fume hood in the appropriate organic container.[19] The macerated bones are immersed in the degreasing agent in a plastic container for a period of at least four hours.[6] The degreasing period depends on the quantum of greasiness on bones.[6] The process is usually carried out in an open surrounding to avoid inhaling poisonous chemicals by the

handlers.

2.4. Bleaching

Bleaching involves the whitening of bones. The commonly used bleaching agent is 4% or 6% H_2O_2.[6] Hydrogen peroxide is mixed with water in a ratio of 1:20. The degreased bones are immersed in the bleaching agent for a period of 24 hours in a dark area to prevent the decomposition of Hydrogen peroxide.[20] Then the bones are taken out and washed with running tap water and detergent to prevent the bones from becoming brittle and prevents the formation of white powder residues on the surface of the bones.[20] Hydrogen peroxide can be disposed of by discarding it in general drainage.[14]

2.5. Drying

The bleached bones are spread out in blotting paper and are allowed to dry in the air at room temperature for 4-7 days or under the sunlight.[3] Care must be taken while drying the bones in sunlight so as to prevent the access of bones to stray animals.

2.6. Finishing

The dried bones will be painted using lacquer and lacquer thinner or varnish or turpentine oil.[3] This prevents the erosion of the ends of the bones and also increases the shelf-life of the bones.[3] Finished bones are articulated to construct the entire skeleton for display in the museum. Bones are articulated using wires, adhesive tapes and Dunlop.[6] The bones are articulated in a way that facilitates movement at the joints. A flexible iron rod will be passed through the sacrum to enter the vertebral canal and the skull. The ribs are then attached to the vertebra posteriorly and to the modified costal cartilage and sternum anteriorly. Upper and lower limbs bones are then connected using wires. Finally, the mandible is attached to the skull.

The bone processing methods should not negate the DNA extraction during forensic assessment in medico-legal cases.[7] Maceration methods such as hot water maceration, microwave maceration, detergent maceration and enzymatic maceration result in the minimal degradation of mitochondrial and nuclear DNA and facilitate further DNA amplification using the PCR technique.[7]

Ideal bone processing methods for a limited resource setting:

1. Cadaver procurement: Cadavers received in the Anatomy department that are unfit for teaching purposes can be utilized to procure bones. Some of the long bones can be sourced from formalin embalmed cadavers after their utilization for teaching purposes.
2. Maceration: Ideal qualities of a maceration agent and maceration are as follows: It should be a method of maceration which consumes less time, with readily available consumables and that involves the least amount of workforce and that is climate independent, that does not emit a foul odour, and that yields intact bones with minimal degradation of DNA. Maceration by detergent fits the bill of the above-said criteria.
3. Degreasing: Acetone and Chloroform may be the preferred degreasing agents because of the relative ease they can be disposed of after their use.
4. Bleaching: Hydrogen peroxide may be the preferred bleaching agent because of the relative ease they can be disposed of after use.
5. Drying: Drying bone in sunlight may be the preferred method.

2.7. Duration of bone preparation

The approximate time duration for bone preparation is 2 days to 8 weeks depending on the number of bacteria, the size of the material being macerated, the temperature of the environment during the maceration.[3]

2.8. Maintenance of prepared bones

Once the bones are prepared, they are good for 15-20 years, depending upon the usage. However, varnishing the bones once in 10 years helps to maintain the durability of the bones.

2.9. Institutions and personnel involved in bone preparation

Generally, individual human bones and articulated human skeletons are required to teach medicine, dental, physiotherapy, yoga and naturopathy, pharmacy, nursing, laboratory technology and allied health sciences.

2.10. Precautions to be taken during bone preparation

There is a significant risk of injury to the handler while defleshing the cadavers. So, care should be taken while using sharp instruments while defleshing.

Chemicals such as benzene are carcinogenic, and prolonged exposure to sodium hydroxide causes lung damage, and chemicals such as trichloroethylene are poisonous if inhaled.[8] Hence appropriate care should be taken to minimize the exposure of handlers to those chemicals.

2.11. Common Problems during bone preparation

When bones are exposed to a higher temperature during defleshing using boiling, the long bones and flat bones are prone to bending. Excessive boiling can also lead to cracking, splitting, scorching of bones.[21] When the bones are macerated using a microwave, shrinkage of bones was observed.[22]

2.12. Steps for preventing problems during bone preparation

Care should be taken to maintain the temperature at 100 degrees for the appropriate amount of time. Time duration for boiling during defleshing should not exceed 8 hours. Moreover, for certain short, long bones and flat bones, the time duration for boiling is less than 5 hours. The actual amount of time needs to be standardized in each laboratory for each type of bone based on size and the amount of flesh that needs to be removed.

Apart from teaching in medical colleges, human bones are also used for display in schools and museums to pique the scientific zeal in the students.

Human bones are used to design prostheses and the instruments used to fix fractured bones, such as K-wire and intramedullary nails.

2.13. Scope of utility

Apart from anatomists working in medical colleges, anthropologists and taxidermists benefit from bone preparation techniques. In India, Sanjay Gandhi National Park is at the forefront of taxidermy. The literature search revealed that the studies on bone preparation techniques that have been reported so far have been relatively less. The current literature search revealed that the studies on bone preparation methods had been reported sporadically from different parts of the world. The majority of the studies have been reported from countries such as India, Nigeria, US, UK and Germany and in India from states such as UP, Maharastra, Chattisgarh, Punjab, Rajasthan, clearly not covering the entire spectrum of the regions. There is a felt need that the anatomy department in each medical college conduct the bone preparation techniques and publish the results to understand the effects and influence of climatic conditions such as temperature and humidity on the methods of preparation of bones. Such studies will also shed some light on the influence of the race and ethnicity of the human bones chosen for the preparation of human skeleton. Processing of bones is a value for money proposition for teaching medical institutions rather than the purchase of processed skeletons. This is because they have the entire infrastructure required for doing so.

There have been many methods of bone preparation that have been described. Some of the methods are costly, and some of them are time-intensive, while some of them are infrastructure intensive techniques. There is no information regarding the method of bone preparation that is simple to conduct, cost-effective, less labour-intensive and with minimal infrastructure requirements that produce better results. Lack of such information brings us to the question of which one is ideal for a particular setting. Attempting a bone preparation technique that is not so ideal for a particular setting, results in poor outcomes and wastes a very precious resource of human cadavers from which bones are prepared. Poor results in bone preparation may sometimes drive us to total abandonment of attempts to prepare bones, ultimately resulting in the purchase of the bones from vendors, which involves exorbitantly huge costs. On the other hand, if there is optimal information as to which is the ideal method of bone preparation for a particular setting, then it not only saves the money involved in purchasing the bones from vendors but also enables the preparation and maintenance of an adequate number of bones to cater to the needs of not only the teachers but also the students to whom bones are issued for self-study.

3. Conclusion

Human bones and skeleton remain indispensable learning resources for medical students, which makes the human bones' availability a critical component of medical education. A cost-effective way of processing bones involves the following steps: Embalmed bodies after the completion of dissection can be macerated using detergent, degreased using Acetone, bleached using hydrogen peroxide and finished using thinner for better results.

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5. Conflicts of Interest

The authors declare that they have no competing interest.

6. Author's Contributions

Kalaivani K: Definition of intellectual content, literature search, data acquisition and analysis, Manuscript preparation, review and editing. Rajasekhar SSSN: Concept, design, manuscript preparation, review and editing. Dinesh Kumar V: Manuscript preparation, review and editing.

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