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Methodological Proposal for the Adequate Use of the Osteotechnics Technique

Propuesta Metodológica para el uso Adecuado de la Técnica Osteotécnica

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SUMMARY: Osteotechnics is one of the different anatomical preservation techniques and can be defined as the technique designed to prepare, clean, obtain and preserve bone structures that can be used in the teaching, museographic or research field. The osteotechnical technique procedure consists of the following phases: debulk and disjoint, maceration, cooking, cleaning, degreasing, bleaching, and labeling to obtain bone material. Seven phases will be explained in detail, as well as the materials, instruments, quantities of the substances used, and the time required to obtain human bone material. We consider that this article can serve as a guide, given that all the experimentation was carried out with human biological material. This methodological proposal could be consolidated and established based on the experience acquired during the creation of the contemporary skeletal collection of the department of innovation in human biological material (DIMBIH). Therefore, the purpose of our proposal is to provide tools that facilitate the work of those who carry out this work and fundamentally to avoid irreversible or irreparable damage to the osteological material, since it is of great value and difficult to acquire for disciplines as anatomy, veterinary, physical and forensic anthropology, medicine, dentistry and biology.

KEY WORDS: Osteotechnics; Restoration; Anatomical preservation techniques; Anatomy; Physical anthropology.

INTRODUCTION

Osteotechnics is an anatomical preservation technique and is defined as the technique intended to prepare, clean, obtain and preserve bone structures. The disciplines that mainly use this technique are anatomy, veterinary, physical, and forensic anthropology, medicine, dentistry, and biology. This technique is of great importance because it allows observing the anatomical details of the skeletal structure, facilitating its learning and analysis. The exhibition of the skeleton allows us to appreciate depressions, protuberances (and other bony processes), and the exact position of each of the bone elements in the human or animal body, and is very useful in the teaching, museography or research field.

The osteotechnical method to obtain the skeleton consists of soft tissue removal, maceration, firing, cleaning, degreasing, bleaching, and drying. The materials, instruments, chemical substances, and the time required to obtain human bone material depend on the preservation conditions of the body, for example: fresh corps, traditional embalming, or UNAM formula. However, it is pertinent to mention that throughout human history, this practice has been carried out and therefore different modifications have been presented. Some of the antecedents are the following (Santos, 2019):

1. Anatomical preservation techniques are diverse and some of them very old, such as embalming. Embalming has been linked mainly to funerary rituals, which have depended on cultural practices and the geographical region as referred to by archaeological evidence recovered in Egypt (Gannal, 1840).
2. Another area to which this technique has been linked is the medical field, and this is referred to by the different written sources that have recorded its use (Olóriz Aguilera, 1890; Thompson, 2015).

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3. Regarding other anatomical preservation techniques that can be mentioned are leather tanning, maceration, cooking and even skeletonization (Miguélez, 1805).
4. Osteotechnics also has its origins for the elaboration of personal, decorative, or work instruments from the use of human or animal bones (Arsuaga, 1999; McPherron *et al*., 2010)
5. Subsequently, this technique has been used and is linked to the funerary, anatomical, medical, and even veterinary fields for cultural, educational, research and even museography purposes (Thompson, 2015).

According to Barcat (2000), with the transition to the 12th Century, foreign corpses were cooked so that the bone remains would later be transferred to their place of origin. Thus, the crusaders had the custom of emaciating the body of their companions, boiling their bones to later transfer them. However, this resulted in a restriction by Pope Boniface's Bull "De sepulturis" issued in the year 1299 (Barcat, 2000).

Girao in 1953 mentioned that Vesalius used a chest (i.e., wooden box) in which he introduced emaciated human bones, then placed it in a stream so that the running water macerated and dragged the macerated tissue. That statement can be related to the work of Andrea Vesalio published in 1543 and entitled: De Humani Corporis Fabrica, specifically volume one that is dedicated to human osteology. Girao also makes a brief statement about those who used cooking or maceration of corpses over time, such as: Berengar and Massa who in their book published in 1536 and entitled Libri Introductorius Anatomiae Sive Dissectionis Corporis Humani recommended the cooking technique. In the work De re anatomica de Columbus he also suggested the technique of firing. Girao's text also refers to the creation of an apparatus for obtaining bones by maceration, first with Lacchi in Italy, 1902. Later, Waldeyer in 1907 reported that the Anatomical Institute of Berlin already had the installation of another apparatus for maceration. Then in 1925 Mechanik described an improvement with a "hydrothermostat" intended for the practice of maceration. Finally, it should be noted that Girao affirms that by 1926 at the Anatomical Institute of Granada, Spain, modern equipment was acquired that allowed the skeletal remains to be macerated and degreased. Undoubtedly, the construction and implementation of these devices were very sophisticated for their time, because it was a chamber in which a body was introduced and by heating the water and constantly recirculating it, the soft tissue of the body could be detached. Once the bones were denuded of soft tissue, they were placed in another device to degrease using trichlorethylene. Finally, bones were placed in the upper part of the macerating apparatus to dry them. As a result of the quality of the bones produced for study using these modern devices of the time, educational institutions in Spain, England, Italy and France adopted such equipment.

INTRINSIC AND EXTRINSIC FACTORS LINKED TO THE TECHNIQUE

The adequate application of the osteotechnical method is crucial to obtain satisfactory results, however, other factors that intervene in the results must be considered, which entail anatomical alterations or technical difficulties (Villaroel *et al*., 2017; Salazar *et al*., 2018). Intrinsic factors relative to human cadaveric material, variables such as age, sex, and pathologies, must be considered. Age is important because biological factors such as ossification, speed of growth and development, muscle mass, bone mineral mass and even menarche are linked to it. There are also age-associated pathologies such as osteoporosis. This is a disease in which there is a decrease in bone density, skeletal fragility, and an increased risk of fractures. It can manifest in male and female; the estimates of the studies report loss of trabecular and cortical bone of 20 to 30%. Females are most affected, since after menopause they not only experience low bone density with trabecular tissue loss of 20 to 30%, but also cortical bone loss of 5 to 10% (Riggs & Melton III, 1998).

Extrinsic factors include the time that the corpse was refrigerated before the application of the conservation method and its storage. Cadavers preserved with the traditional technique (which is described later) and that remained stored for some time without hydration are more likely to deteriorate during osteotechnical processes, mainly in the region of the epiphyses, which reduces the number of days to which the bone elements can be exposed to the processes of boiling, degreasing, and bleaching. This occurs due to the fragility and occasional exposure of the trabecular tissue, as well as irregularities in the surfaces of the bone elements, causing greater difficulty in cleaning. Fresh corpses present certain variables that limit their use in medical practice due to the presence of cadaveric rigidity and putrefaction, increasing the risk of exposure to microbial agents such as viruses or bacteria. Traditional embalming uses high concentrations of preservatives, especially formaldehyde. It is known that the traditional long-term preservation technique significantly hardens body tissues (Eisma *et al*., 2011). With the traditional technique, the tissues dehydrate, harden, and change their color and general appearance, making it difficult to use them in anatomy, surgery, and other techniques such as osteotechnics. On the other hand, in modern preservation techniques, less formaldehyde, isopropanol, and propylene

glycol are used, in addition to surfactants and modifiers that make the molecules more slippery. Thus, it is unlikely that they will adhere to each other and it is more likely to interact with oil and grease, some of these modern techniques are: Thiel Soft-Fiâ Technique, also called Graz fixation (1990), or the Carbowax™ Polyethylene Glycol 600 technique, or the recent dilute formaldehyde mixture developed by Anderson (2006), or the glycerin-ethanol solution developed by others authors. The currently used UNAM formula for embalming brings with it the best of the previous techniques, uses preservatives and formaldehyde in a very low concentration, a technique designed so that the tissues are excellently preserved. Corpses embalmed with formalin show discoloration compared to fresh corpses and corpses embalmed with the UNAM formula manage to preserve good color and tissue preservation, which is ideal for the application of techniques such as dissection and osteotechnics.

MATERIAL AND METHOD

Cadaveric Materials. The present work was carried out in the Department of Innovation in Human Biological Material (DIMBIH), of the Faculty of Medicine, UNAM. The data presented is from 19 subjects (14 males and 5 females), ages range from 42 to 98 years at the time of death, the information presented was obtained from the DIMBIH work log, in which the passage the osteotechnics method step by step (quantities of substances, tools, times and relevant observations of each phase and work carried out day by day). The management of the bodies adheres to the General Health Law, related to the disposal, manipulation and handling of the corpse and biological remains. In Tables I and II, the materials and methods[1] used in the bone extraction process with the Osteotechnics technique are described.

The osteotechnical process consists of seven phases (Fig. 1), which are described below:

Table I. Biosecurity material used in osteotechnics.

Biosecurity material used in osteotechnics.
Surgical gown and pajamas
Surgical cap
Safety glasses
Gloves
Face mask
Mask
Boots or special footwear

[1] Debulkeda Maceratedb Cookingc Degreasingd Bleachinge, Labelingf .

Table II. Instruments and substances used in the different phases of osteotechnics.

Instruments and substances
Scalpel handle [a]
Scalpel blades [a]
Tweezers [a]
Fixed Blade Scalpel [d]
Straight and curved mayo scissors [a]
Toothed dissecting forceps [a]
Knives various sizes [a]
Grinder [a]
Plastic and steel trays (various sizes) [b]
Cloth bags (blanket or cotton) [b/c]
Yellow bags for R.P.B.I.[a/d]
Metal chips whit ID number s BNM
Hammer
Stainless steel pot (different sizes) [c]
Plastic or glass containers of 60 CMS. long x 40 wide x 30 high [b/c]
Square acrylic sheet [d]
Nikon D3300 photo camera
Necropsy table [a]
Stove [b]
Extractor hood [c]
Stylus 0.5 mm [f]
White glue [f]
Brush [f]
Craft paper
Cardboard boxes [f]
H_2O
Powder detergent [c]
KOH [b/c]
NaCl
Liquid degreaser [b/c]
70% peroxide - H_2O_2 [e]
ARIANE® [c]
ECO Alkaline Degreaser® [c]

Phase I: Debulk and Disjoint. Debulk. Debulking is the procedure by which large segments of skin, muscles and organs that are still preserved in the individual are removed. Soft tissue removal must be done accurately and carefully, since there are bone elements that may be more fragile and greater care is needed, mainly with the ribs, piriformis orifice, orbits, hyoid bone, and auditory canal. Intervention begins in the cephalic region and ends in the feet, knives of different dimensions for example: The types of cutting instruments used according to the anatomical region are: The scalpel for the region of the face, hands, and feet. The puntilla knife for the cephalic region. Cutlet knife for the thoracic and abdominal region and the cook knife for upper and lower limbs. This is done as close as possible to the bone, trying not to damage or scratch it and thus preserve the bone structures as best as possible.

Fig. 1. Osteotechnics process according to each of its phases.

Disjoint. Disjointed consists of the separation of the axial and appendicular skeletons and more specific segments by cutting ligaments and tendons using the scalpel. First, the head is disarticulated, at the level of the C7. The rib cage will be limited to the L5. The upper members are divided into 4 sections: (1) scapula and clavicle (i.e., pectoral girdle), (2) brachium, (2) antebrachium, (4) carpus, metacarpus and phalanges. The lower members are also divided: (1) coxal, (2) femur, (3) patella, (4) tibia and fibula bones, and phalanges, (5) tarsal and metatarsus. The disarticulated body is placed in blanket bags of different sizes and according to the body segment to be placed. In the largest blanket bag that measures 100 x 100 cm, the spine whose vertebrae are articulated from C7 to L5, all the ribs and the sternum, the pelvic girdle, and the long bones are placed inside the torso first. In smaller bags measure 30 x 30 cm the hands and feet are placed. In a medium-sized bag measure 50 x 50 cm the skull is placed together with the jaw. In addition, it is supervised that there are two metal chips with ID number of the individual to avoid confusion and keep the bone material always identified. All the blanket bags are tied with a cord to prevent loss of bone samples, facilitate cleaning and lateralization of the bone segments, mainly the hands and feet.

PhaseI: Macerated. One day prior to the cooking process, the specimen is allowed to macerate in a plastic container with 60 L of H_2O, 500 ml of Master Chemical® alkaline ecodegreaser, 160 grams of Master Chemical® KOH and cover.

Phase III: Cooking. Cooking corresponds to the chemical process by which the body is boiled for approximately 19 hours with the intention of eliminating the greatest amount of soft tissue and facilitating the cleaning of bone material. The stove is turned on at a medium flame and the extractor hood is activated, the skeleton is left to boil for an hour and later the contents of the pot are constantly checked to verify that it has enough water, usually 40 to 60 L of H_2O are added. Depending on the amount of fat that each body releases, one to two water changes are made. The change consists of washing the pot well with soap and a little chlorine to eliminate the adhered fat and adding 60 L of water again, degreaser and half the quantity of KOH[2] used in principle, that is, 160 g that were used the first time, in subsequent changes only 80 g. It is important to mention that the use of KOH must be carried out with total caution because said substance used in excess and without the pertinent precautions can damage the bone material and even the container in which the boiling process is carried out. Likewise, it is suggested to have an extraction hood and when proceeding to check the material, while it is in full boiling phase, respirators with adequate filters must be used to avoid poisoning. To know if the body already has the desired consistency one must be able to clean it; it is necessary to open the bag with the stove off and check in some bone segments such as the vertebrae, if the tissue is already softened and can be easily removed. When it comes off, it should have a light brown color, which indicates that it can be removed and continue with the cleaning process.

[2] The amount of KOH is half of that used at the beginning of the process, thus we avoid damaging the bone structure.

Phase IV: Cleaning. Now, following the boiling process, the specimen must be cleaned. The blanket bag containing the skeleton is placed on the necropsy table and left to drain for a few minutes to cool down and avoid any burns. The bag is opened and with the help of fixed-blade scalpels and small knives, the bones are cleaned by careful scraping so as not to damage the bone material and avoid injuries from the use of cutting instruments. As much cartilage tissue as possible should be removed from each bone.

Phase V: Degreasing. Degreasing is the process by which the bone material is cleaned of excess fat produced during boiling. To do this, add 25 L of hot water, 500 ml of degreaser and 300 ml of peroxide to a plastic tub with a lid. Then, first place the long bones and the skull in the box to continue with the smaller, fragile bones. The bones of the hands and feet are wrapped in gauze to avoid losing (or mixing up of) pieces. When all the bones are inside the box, a square acrylic sheet is placed over the bones to keep them submerged and to reduce the time for the degreasing process. This process can last approximately 7 days depending on the size and characteristics of the individuals. After 7 days, the bones are cleaned again; with the intention of removing any tissue that may have remained attached[3]. A fixed blade scalpel and small knives are used. Once the bones are clean, they are left again on craft paper.

Phase VI. Bleaching. For the bleaching of the bones, a container of 60 CMS is required long x 40 wide x 30 high, in it we add 25 L of H_2O and 500 ml of peroxide of Master Chemical®. The bones are immersed in the solution for 7 days. The bones of the hands and feet are wrapped in gauze (separately) and these remain for 4 to 5 days. Once the process is finished, the bones are washed with running H_2O and gently rubbed with a brush to remove any remaining surface tissue residues. Then, they are left to dry again on craft paper for 15 days in a cool environment.

Phase VII: Labeling. Each bone is marked with the number assigned by the DIMBIH, for this a 0.5 mm pen indelible black ink, white glue and a brush are required. With the stylus, all the bones are marked without exception, with the brush a light layer of white glue is added, which is left to dry for an hour and the number is re-marked. In this way, the losing or mixing bone pieces between individuals is avoided, and this helps to have a better control of the osteological collection.

DISCUSSION

After carrying out the corresponding evaluations for the adequate use of the proposed osteotechnical method and its correct application in human cadaveric material for the conformation of the osteological collection, it can be concluded that the information presented suggests an efficient osteotechnical method for the conformation and conservation of collections. Likewise, this methodological proposal may serve as a guide for osteotechnics in various disciplines such as physical and forensic anthropology, veterinary science, medicine, dentistry, and biology, since depending on the size of the specimen, the quantities of the substances used can be calculated, as well as the times of each stage of the method to achieve satisfactory results. This methodological proposal has potentially significant implications, since it was observed that the intrinsic and extrinsic factors, mainly the embalming method, are important to consider, since they will determine the time that each process will require. For example, in this method, the bodies preserved through the embalming process using the Carbowaxâ technique were the ones that required the longest cooking time, 16 h on average, and the longest degreasing time, up to 15 days. In the case of the fresh corpse that was included in the sample, the cooking processes were faster[4], requiring only 3 h and 58 min, and in the degreasing process, it required only 9 days. In the same way, in the fresh corpse, the cleaning to eliminate the remaining tissue was very quick and simple, it should even be noted that the bleaching process was not necessary and even, so the results were very satisfactory, the color of the bone material is ideal and natural. On the contrary, corpses preserved with the traditional technique are more likely to deteriorate during osteotechnical processes, due to the high amounts of formalin required in this embalming technique, which reduces the number of days to which we can submit these bone elements to avoid irreparable damage (Fig. 2).

CONCLUSION

The technique can be accessible since the materials and methods do not require onerous expense or sophisticated infrastructure. The results can be optimal if the indicated times and amounts are respected. Bone collections can be

[3] The degreasing and bleaching processes in bone elements treated with phenol due to the presence of fungus should be monitored every 7 days by changing the formula with the same amounts, assessing whether the removal of fat is carried out, as well as obtaining a cleaner bone element with a more natural color, taking care that they are not damaged.

[4] A pressure cooker is not used for the cooking process, otherwise the process would be much faster in time.

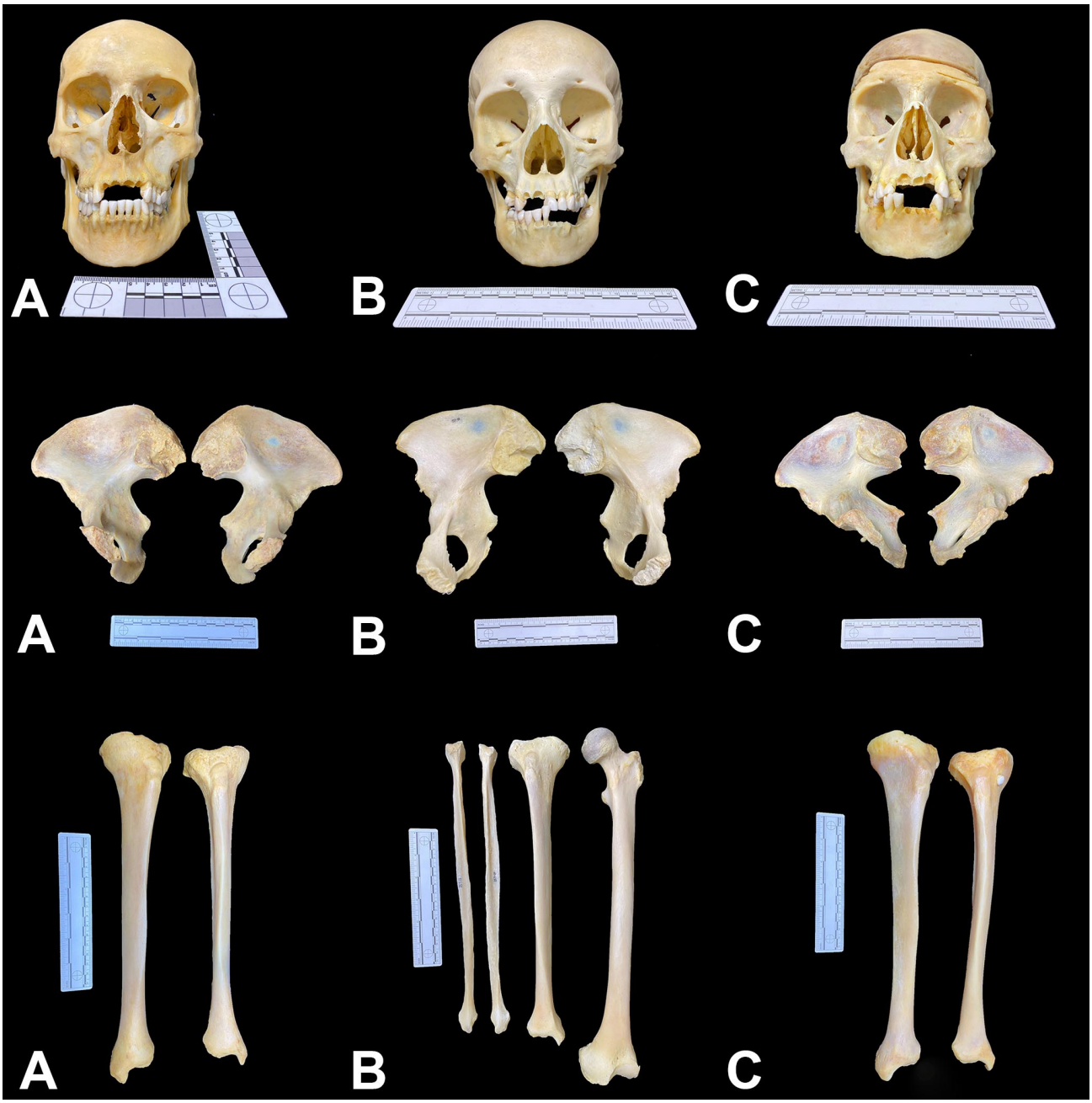

Fig. 2. A) Skull, iliac bones, right and left tibia, of the results of osteotechnics in bone material subjected to traditional preservation technique, traditional embalming, in which a darker coloration is observed compared to elements B and C. B) Skull, iliac bones, right tibia, right and left fibula, left femur. Osteotechnics in bone material from a fresh cadaver that was not subjected to any preservation technique, in which a more natural and whitish coloration is observed compared to elements A and C. C) Skull, iliac bones, right and left tibia, of the results of the osteotechnics in bone material submitted to the UNAM preservation technique, embalming like CarbowaxÒ , in which a darker coloration is observed compared to elements B and C.

preserved indefinitely if each one of the bone elements is properly stored and their conservation status is pending.

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RESUMEN: La osteotecnia es una de las técnicas diferentes de conservación anatómica y puede definirse como la técnica destinada a preparar, limpiar, obtener y conservar estructuras óseas que pueden ser utilizadas en el ámbito docente, museográfico o de investigación. El procedimiento de la técnica osteotécnica consta de las siguientes fases: descarnado y desarticulado, maceración, cocción, limpieza, desengrase, blanqueo y marcaje para la obtención de material óseo. Se explicarán en detalle siete fases, así como los materiales, instrumentos, cantidades de las sustancias utilizadas y el tiempo necesario para obtener material óseo humano. Consideramos que este artículo puede servir de guía, dado que toda la experimentación se realizó con material biológico humano. Esta propuesta metodológica pudo consolidarse y establecerse a partir de la experiencia adquirida durante la creación de la colección esquelética contemporánea del Departamento de Innovación en Material Biológico Humano (DIMBIH). Por lo tanto, el propósito de nuestra propuesta es brindar herramientas que faciliten el trabajo de quienes realizan este trabajo y fundamentalmente evitar daños irreversibles o irreparables en el material osteológico, ya que es de gran valor y difícil adquisición para las disciplinas como la anatomía, veterinaria, antropología física y forense, medicina, odontología y biología.

PALABRAS CLAVE: Osteotecnia; Restauracion; Técnicas de preservación anatómica; Anatomía; Antropología física.

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