

A Novel Dry Preservation Technique for Human Wet Bone and Cartilage Following Dissection using Waterproof Glue

Baneswar Baro¹, Gunamani Rabha², Ritu Saloi³, Sumitra Hagjer⁴, Mrinmoy Saikia⁵, Simanta Kalita⁶

¹Associate Professor, Department of Anatomy, Diphu Medical College and Hospital, Diphu, Karbi Anglong

²Assistant Professor, Department of Anatomy, Diphu Medical College and Hospital, Diphu, Karbi Anglong

³Professor, Department of Anatomy, Diphu Medical College and Hospital, Diphu, Karbi Anglong, 782460, Assam

⁴Principal-cum-chief-Superintendent, Diphu Medical College and Hospital, Diphu, Karbi Anglong, 782460, Assam

⁵Demonstrator, Department of Anatomy, Diphu Medical College and Hospital, Diphu, Karbi Anglong, 782460

⁶Research Scientist- B, Multi-Disciplinary Research Unit, Diphu Medical College and Hospital, Diphu, Karbi Anglong, 782460, Assam

Received: 18-04-2024 / Revised: 21-05-2024 / Accepted: 26-06-2024

Corresponding author: Mr. Simanta Kalita

Conflict of interest: Nil

Abstract:

Preservation of human wet bone and cartilage following dissection is critical for anatomical studies, medical education, and forensic investigations. Traditional preservation methods often involve the use of chemicals that may pose health and environmental risks. In this study, we propose a novel dry preservation technique utilizing waterproof glue, offering a safer and more effective alternative. This study investigated the feasibility and efficacy of waterproof adhesive glue for preservation of bones and cartilages. The results showed effective binding of glue to the bones and tissues. The results showed promising efficacy by which the preserved specimen can be easily transported to different places and needs less maintenance. These preserved specimens can add value to academic teaching to a great extent as they can be taken to classrooms for teaching and also the students can take it to their hostels for self-study. Moreover these specimens can also be used in examination to assess the knowledge of the students.

Keywords: Dry Preservation, Wet Bone Preservation, Cartilage Preservation, Dissection.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

The preservation of human wet bone and cartilage after dissection plays an indispensable role in various academic as well as research activities across various scientific disciplines, including anatomy, anthropology, pathology, and forensic science [1–3]. The structural and biochemical integrity of these tissues is crucial for research, education, medical procedures, forensic investigations and for the development of therapeutic treatment [4,5]. The need of the hour is to develop effective preservation methods which not only maintain the structural integrity but also retain its biochemical composition, facilitating accurate analysis and assessments in future. During the 17th century only the wet preservation process was evident which was performed by submersing

the whole specimens in chemical agents [6]. Traditionally the use of chemical fixatives like formaldehyde and glutaraldehyde have been used for their ability to cross-link proteins which stabilizes the tissue structure [7]. The limitations of these chemical fixatives is that it alters the mechanical properties of bones and cartilages and poses health risk during exposure while handling the specimens [1,7]. Moreover, one the major challenges faced during preservation is maintaining the specimens for a longer period without decay, which if not prevented may be disadvantageous to morphological studies to a great extent. Another major drawback of the traditionally used preservation technique which uses formalin as a submersible agent for the specimens is attributed to

its difficulty in transportation and therefore hinders academic teaching. Previous studies have suggested that the use of 'Plastination' which have been extensively used but the disadvantages of it that it is very costly and needs trained manpower [8]. Therefore it is the need of the hour for a simple technique for preservation of specimens in a best possible way which still warrants further research. We in this manuscript define a simple yet effective

protocol for preservation of wet bone and cartilage after dissection wherein we use the property of waterproof adhesive glue that forms an imperable bond that helps protect tissues from various factors and maintains the structure for a longer time. This process will help to preserve the skeletal remains after dissection which can be used for preparation of museum specimen as well as for academic purposes for demonstration in classroom.

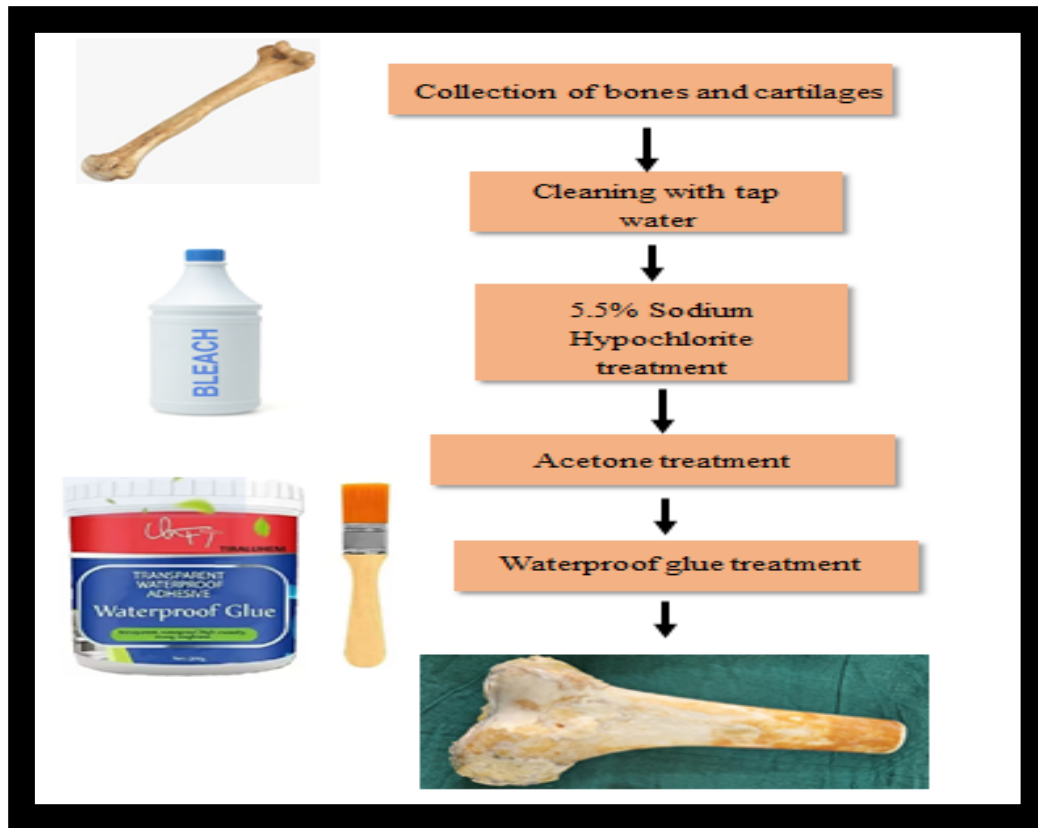


Figure 1: Graphical abstract

Materials and Methods

The study was carried out by using human skeletal remains with articular hyaline cartilage and fibrocartilage (meniscus) after dissection from cadavers in the Department of Anatomy, Diphu Medical College and Hospital, Diphu, Karbi Anglong. The study is approved by the institutional ethics committee of Diphu Medical College and Hospital, Diphu having ref no: DMCH/EC/2022/105/772. The study was done taking 2 (two) numbers of knee joints which constitute 6 cm part of femur and 4 cm part of tibia and fibula along with patella without having any pre-existing degradation or abnormalities. Initially the patella, femur and tibia were disarticulated cutting the cruciate ligament. The meniscus and capsular ligaments are not removed from the upper articular part of tibia. The muscles, ligaments and periosteum are cleaned with clean tap water to remove any blood spots and other unwanted

materials. The excess water was squeezed out and the surface was wiped dry with a sponge. Then it was cleaned by soaking the specimens in a household bleach solution (5.5% sodium hypochlorite) overnight to bleach. Again the specimens were washed with distilled water. It is then treated for dehydration with 95% acetone (Merck) for 3 hours. The specimens are then coated with a thin layer of commercially available waterproof glue evenly over the surface of the specimen using a brush or applicator. The specimens are then allowed to dry and then again re-coated with glue and the process is repeated several times until a smooth surface is achieved. The waterproof glue is then allowed to dry according to the manufacturer's instructions. Photographs were taken using a digital camera (Cannon).

Results

After processing of the specimens images were captured using a digital camera. The following figure depicts the smooth surface of the bones

which are now ready to be used in various academic teaching programs. The bones are enlisted in the Figure 1 and 2. The glue coating done can prevent microbial and moisture decomposition.

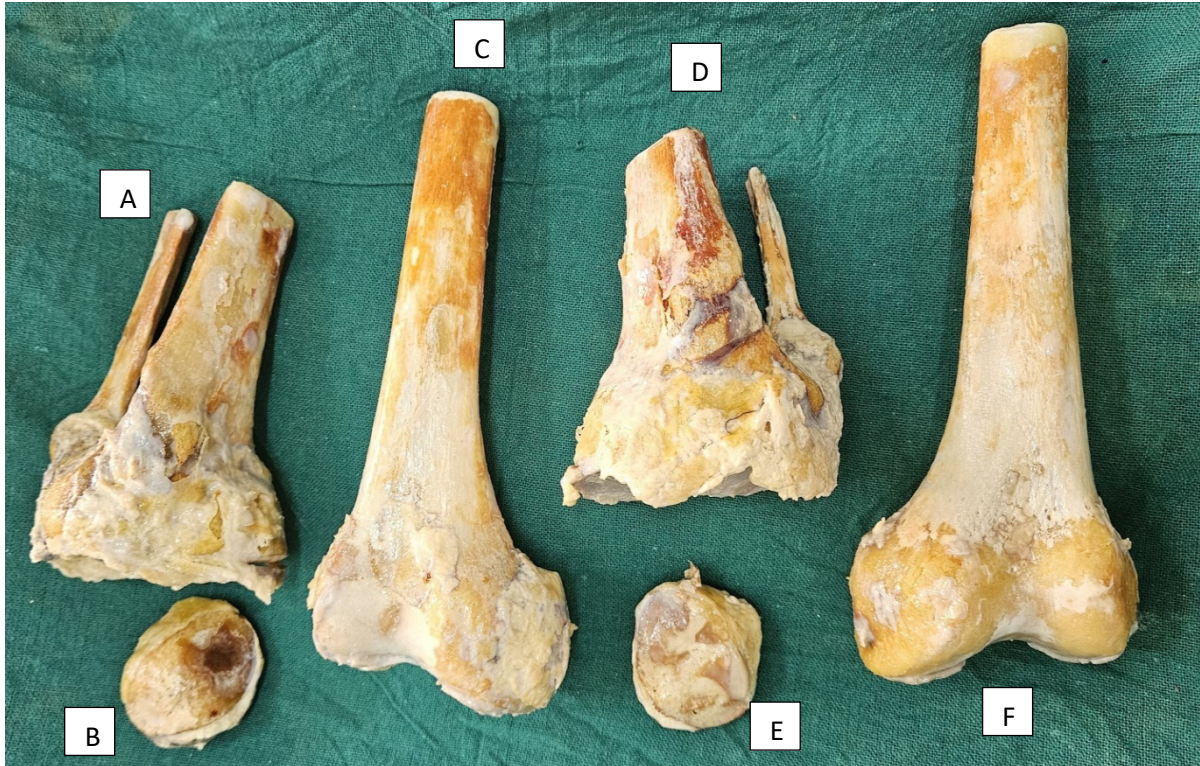


Figure 2: Top view of the human wet bones after processing. (A & D): Upper end of Tibia and Fibula , (B &E): Patella , (C &F):Femur

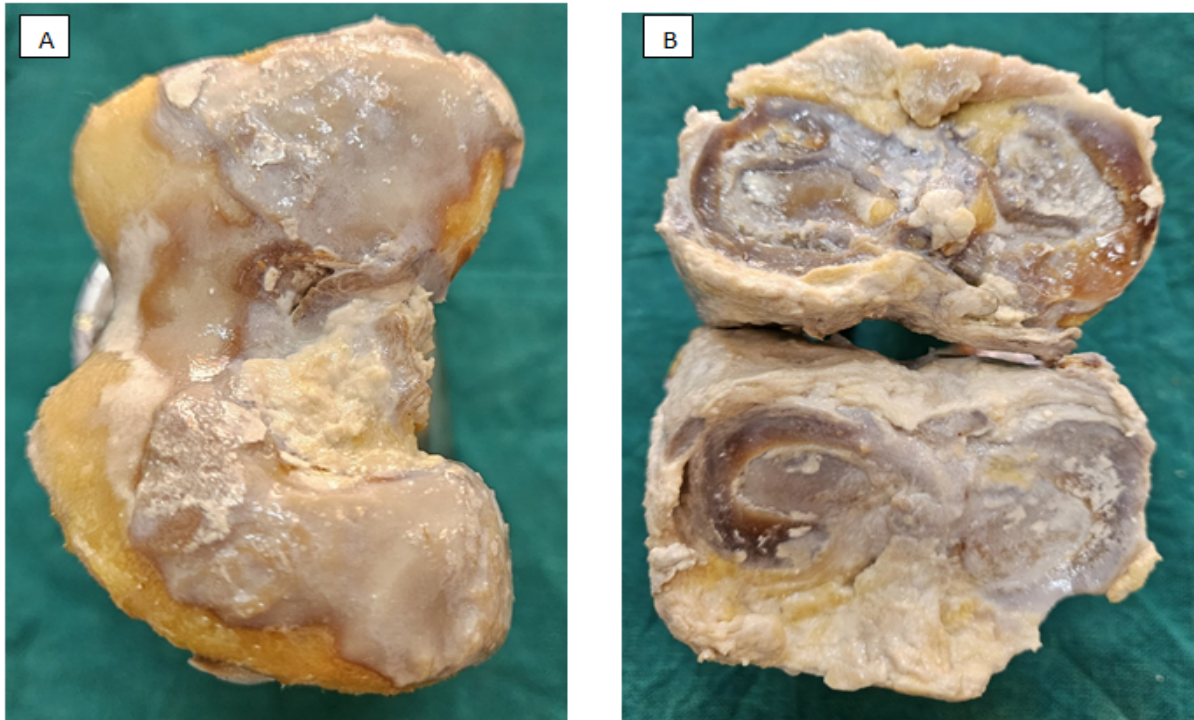


Figure 3: (A): Articular surface of femur, (B): Articular surface of upper end of tibia and fibula with meniscus

Discussion

Preservation of bones and cartilages serve as a pool for biological information which can be later used for understanding anatomical structure, its biomechanics and also to study associated diseases [1]. Considering the present scenario as cadaver procurement is very costly and it is extremely difficult to preserve due to different factors such as lack of man power, ethical and social issues [9–12], therefore the preservation of specimens in a cheap and easy to use way is the utmost need of the hour. This study originated from the need of overcoming challenges in preservation of specimens used in academic as well as research purposes. Despite the technological advances in preservation techniques, tissue shrinkage and alteration of tissue property by preservative agents are a major concern as it is evident in providing confounding effect which hinders the accuracy of the results [7,13–15]. Furthermore, some of the procedures requires specialized equipment and requires technically skilled manpower which is difficult in resource limited setting. A technique of preservation which involves freeze-drying utilizes vacuum chambers and lyophilizers which becomes a costly affair for small scale settings specially use in only academic activities [16–18]. Since many of the preservative agents are potentially hazardous substance such as formalin due to its carcinogenic properties it is therefore important to find the best possible way to preserve the specimens in an easy, cost effective way which is safer for the handlers and should be able to mitigate environmental concerns as well [19–22]. In the present scenario wet preservation using alcohol is being treated as the gold standard technique owing to its easy handling and preparation. But the health risks associated with the use of these techniques has been a major shortcoming [23,24].

Our study emphasizes the use of the inherent property of waterproof adhesive glues to be able to form chemical bonds with the surface of bones and cartilages. Previous studies have suggested various types of adhesives such as cyanoacrylate adhesives and epoxy resins as adhesive [25]. The glue used form strong chemical bonds and seals the holes and irregularities in the tissue surfaces thus stopping water, oxygen, and other reactive substances from penetrating and thereby preventing degradation. Waterproof adhesive glues are able to form chemical bonds with the surface of bones and cartilages as the glues contain reactive functional groups which interact with the organic component matrix of the collagen in bone and proteoglycans present in cartilages. Also when applied as a coating the glues undergo curing which involves crosslinking between the polymer chains which in turn lead to enhancement of stability of adhesive, also ensures high mechanical strength with

mobilizing the tissue structure effectively. These glues are waterproof and thereby form a layer that prevents moisture from entering the tissue which reduces the risk of degradation. Apart from providing mechanical support the glues also provides structural stability of the bones which further prevents any deformation or tissue damage during storage or handling. The glue also protects the specimens from environmental factors like moisture, microbes and thereby helps slowing down decomposition [25]. This process thus provides a good medium of preserved anatomical structure for the anatomists to use specimens for academic purposes for teaching in classrooms due to their ease in handling and non-toxic in nature. Furthermore, medical and paramedical students can transport it to their hostels for self-study due to the convenience in transport.

The use of dry preservation technique by the use of waterproof adhesive glue showed promising results when used for preservation of wet bones and cartilages. The procedure is of merit as compared to the gold standard techniques due to its convenience in transport, non-toxic in nature, and negligible risk during handling. Also the maintenance of the wet preserved specimens is very high which in contrast in our study has no maintenance once the process is completed. In terms of long term cost this dry preservation has advantages over the wet preservation as the chemicals in jars needs to be changed when stored for a longer duration and requires monitoring for any discoloration of the preservatives which in contrast to glue based method requires very minimal observation. Limitations of the study includes that there may be a comparative study with other methods for a longer duration and planning a response based study would further enhance the knowledge about the best way for preservation. Another major disadvantage of the method is that once the specimen is prepared it cannot be restored if needed, which might be not beneficial in some cases where the specimen is rare and needs to be reverted with its original properties. Moreover, detailed study to assess the long-term effects and efficacy of waterproof adhesive glues in bone preservation, which will consider factors such as biocompatibility, durability, and the preservation of histological structures is warranted in the field. In future to find further modified protocol for preservation there may be a need for interdisciplinary approach which may be possible with the help of the recent advances in the field of tissue engineering, 3D printing and microfluidics.

Funding: This study is funded by intramural research grant of Multi-Disciplinary Research Unit, Diphu Medical College and Hospital, Diphu, funded by DHR, Ministry of Health and Family.

Acknowledgements: The authors thank Department of Anatomy, Diphu Medical College and Hospital, Diphu and Multi-Disciplinary Research Unit, DHR, Ministry of Health and Family welfare for the infrastructure support.

References:

- Brenner E. Human body preservation – old and new techniques. *J Anat* 2014;224:316. <https://doi.org/10.1111/JOA.12160>.
- National Museum of Health and Medicine: The Micrograph - A Closer Look at NMHM n.d. https://medicalmuseum.health.mil/micrograph/index.cfm/posts/2020/preserving_specimens_wet_tissue_conservation (accessed May 3, 2024).
- Nakahama N. Museum specimens: An overlooked and valuable material for conservation genetics. *Ecol Res* 2021;36:13–23. <https://doi.org/10.1111/1440-1703.12181>.
- Ator GA, Andrews JC, Maxwell DS. Preparation of the Human Skull for Skull Base Anatomic Study. *Skull Base Surg* 1993;3:1. <https://doi.org/10.1055/S-2008-1060557>.
- AK N, S G. Bone Preparation from Embalmed Human Cadavers - ARetrieval and Curation Technique. *Austin J Anat* 2021.
- Rose, C.L. and de Torres AR. Storage of Natural History Collections: Ideas and Practical Solutions | The Society for the Preservation of Natural History Collections. *Soc Preserv Nat Hist Collect* 2009. <https://spnhc.org/resources/storage-of-natural-history-collections-ideas-and-practical-solutions/> (accessed May 3, 2024).
- Bancroft JD, Gamble M. Theory and Practice of Histological Techniques, Sixth Edition. *Theory Pract Histol Tech Sixth Ed* 2007:1–725. <https://doi.org/10.1097/nen.0b013e31817e2933>.
- Spoorthi BR, Bhat VM. Plastination: A novel, innovative teaching adjunct in oral pathology. *J Oral Maxillofac Pathol* 2011;15:133. <https://doi.org/10.4103/0973-029X.84475>.
- Stimec B V., Draskic M, Fasel JHD. Cadaver procurement for anatomy teaching: legislative challenges in a transition-related environment. [Http://DxDoiOrg/101258/Msl2009009008](http://DxDoiOrg/101258/Msl2009009008) 2010;50:45–9. <https://doi.org/10.1258/MSL.2009.009008>.
- Rokade SA, Gaikawad AP. Body donation in India: Social awareness, willingness, and associated factors. *Anat Sci Educ* 2012;5:83–9. <https://doi.org/10.1002/ase.1263>.
- Kramer B. Challenges to sourcing human bodies for teaching and research in Africa: Are the challenges insurmountable? *Ann Anat - Anat Anzeiger* 2024;252:152196. <https://doi.org/10.1016/J.AANAT.2023.152196>.
- EwonuBari EB, Watson JT, Amaza DS, Madueke NM, Donatus AA, Effiong OE. Problems and prospects of acquisition of human cadaver for medical education in Nigeria. *J Pak Med Assoc* 2012;62:1134–6.
- Sompuram SR, Vani K, Messana E, Bogen SA. A Molecular Mechanism of Formalin Fixation and Antigen Retrieval. *Am J Clin Pathol* 2004;121:190–9. <https://doi.org/10.1309/BRN7CTX1E84NWWPL>.
- Kiernan JA. Formaldehyde, Formalin, Paraformaldehyde And Glutaraldehyde: What They Are And What They Do. *Micros Today* 2000;8:8–13. <https://doi.org/10.1017/S1551929500057060>.
- Howat WJ, Wilson BA. Tissue fixation and the effect of molecular fixatives on downstream staining procedures. *Methods* 2014;70:12–9. <https://doi.org/10.1016/J.YMETH.2014.01.022>.
- Pappas AM. Current methods of bone storage by freezing and freeze-drying. *Cryobiology* 1968;4:358–75. [https://doi.org/10.1016/S0011-2240\(68\)80135-X](https://doi.org/10.1016/S0011-2240(68)80135-X).
- Draenert DRY, Draenert K. Freeze-drying of articular cartilage: Investigation of rat femoral heads by SEM. *Scanning* 1979;2:57–71. <https://doi.org/10.1002/SCA.4950020201>.
- KREUZ FP, HYATT GW, TURNER TC, BASSETT AL. The Preservation And Clinical Use Of Freeze-Dried Bone. *JBJS* 1951;33.
- Jalali M, Moghadam SR, Baziar M, Hesam G, Moradpour Z, Zakeri HR. Occupational exposure to formaldehyde, lifetime cancer probability, and hazard quotient in pathology lab employees in Iran: a quantitative risk assessment. *Environ Sci Pollut Res Int* 2021;28:1878–88. <https://doi.org/10.1007/S11356-020-10627-0>.
- Waschke J, Bergmann M, Bräuer L, Brenner E, Buchhorn A, Deutsch A, et al. Recommendations of the working group of the Anatomische Gesellschaft on reduction of formaldehyde exposure in anatomical curricula and institutes. *Ann Anat* 2019;221:179–85. <https://doi.org/10.1016/J.AANAT.2018.10.007>.
- Aung WY, Sakamoto H, Sato A, Yi EEPN, Thein ZL, Nwe MS, et al. Indoor Formaldehyde Concentration, Personal Formaldehyde Exposure and Clinical Symptoms during Anatomy Dissection Sessions, University of Medicine 1, Yangon. *Int J Environ Res Public Health* 2021;18:1–18. <https://doi.org/10.3390/IJERPH18020712>.
- Protano C, Buomprisco G, Cammalleri V, Pocino RN, Marotta D, Simonazzi S, et al. The carcinogenic effects of formaldehyde occupational exposure: A systematic review. *Cancers (Basel)* 2022;14. <https://doi.org/10.3390/CANCERS14010165/S1>.

23. Balta JY, Cronin M, Cryan JF, O'Mahony SM. Human preservation techniques in anatomy: A 21st century medical education perspective. *Clin Anat* 2015;28:725–34. <https://doi.org/10.1002/CA.22585>.
24. Domański J, Janczura A, Wanat M, Wiglusz K, Grajzer M, Simmons JE, et al. Preservation fluids of heritage anatomical specimens — a challenge for modern science. *Studies of the origin, composition and microbiological contamination of old museum collections. J Anat* 2023;243:148–66. <https://doi.org/10.1111/JOA.13854>.
25. Thamilselvan, Snega; Sherlin, Herald J.; Jayaraj, Gifrina; Don, K. R.; Santhanam A. Preservation of Museum Specimens with Epoxy Resin - A Dry Preservation Technique. *Oral Maxillofac Pathol J* 2021;12:14.