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Plastination of Human Limb Sections in Bangladesh: Entering a New Era of Body Preservation

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Abstract

Introduction*: Plastination becoming popular worldwide in teaching-learning of Anatomy. Scarcity of donated bodies in the medical colleges of Bangladesh is a major problem which may be solved to some extent with the plastinates.*

Materials and Methods*: Fifteen sections of human limbs were plastinated in Bangabandhu Sheikh Mujib Medical University (BSMMU). The previously embalmed sections were prepared by 50% ethanol and dehydrated with graded series of acetone at -25°C in a deep freezer. Forced-impregnation was done with S10 silicone at -25°C and gas-curing was done within a curing chamber by S6.*

Results and Discussion*: The specimens were dry, odorless, fumeless and easy to handle. Some sort of shrinkage and distortion was observed in the specimens.*

Conclusion*: Current attempt may be a crucial point in the history of body preservation and teaching-learning of Anatomy in Bangladesh.*

Keywords*: Plastination, S10 method, limb section.*

Introduction

Human body preservation is an important prerequisite for studying Anatomy. From the ancient time, people are trying to preserve the deceased one by various means. Traditionally, preservation of the body or viscera with different concentrations of formalin is used throughout the world. In traditional formalin preservation technique, the human body or body parts preserved are needed to be handled with great care to avoid some unexpected problems like contact dermatitis, pneumonia, and even cancer.[1] Moreover, their wetness and irritant fumes are most likely discouraging to handle the specimens.[2,3,4] Furthermore, the cadaver donation is not so common in third-world countries like Bangladesh. Therefore, the growing scarcity of the donated cadavers and their decay after a considerable period even after formalin preservation are the major problems in teaching-learning of Anatomy in Bangladesh as well as throughout the world. However, Plastination, a new preservation technique, is gaining attention to the scientific community nowadays for some advantageous attributes. Many universities have been adopted a curriculum with the plastinated specimens with or without the wet specimens.[5,6,7,8] To keep pace with other centers of the world it is therefore a valuable option for our country to produce a collection of plastinated specimens to combat those problems in Anatomy education.

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Materials and Methods

The limb sections were plastinated from a donated cadaver, as a part of a thesis work of MS, Anatomy in plastination laboratory at Department of Anatomy in BSMMU. The cadaver was previously embalmed, as a routine practice, just after its arrival to our Department, five months prior to collecting the sections.

Procedure of specimen collection: Plastination has four basic steps: Fixation, Dehydration, Forced impregnation, and Curing. For plastinating the limb sections S 10 cold temperature plastination method was adopted.

Fixation and sectioning: To prevent putrefaction of the tissues, fixation is must. As the final quality of the plastinated specimens depends on proper fixation[9], the fixation is considered as the first step of plastination. The selected cadavers was previously embalmed, therefore it was already fixed. However, the cadaver was frozen for readiness of making sections. Following Ottone et al[10] cadaver was stored into a refrigerator (BIODUR^{TM} HK06A1.0 Plastination Deep-Freezer) for three weeks at -20°C; but, before placing the cadaver into the refrigerator, the cadaver was rinsed with tap water to remove excess formalin. Fifteen sections of about 5 cm thickness were collected from the limbs of the cadaver with the help of a high-speed meat-cutting band-saw (Hobart; USA). The 'bone-saw blade', having four (4) teeth per inch of its length, as well as the 'bone- saw guide stop' were also cooled at -20°C for 4 hours prior to the commencement of cutting to retard early thawing of the frozen tissue.[11]

The frozen cadaver was cut at transverse planes. The body was placed on a table, having same height as that of the saw machine, and the four limbs were cut to separate them from the body. During cutting, care was taken to ensure consistently even sectioning of an overall thickness of 5 cm (2 inches). 5 cm areas on each side of the elbow and knee joints were spared from sectioning. The arm was sectioned 10 cm beyond the tip of the acromion process. The wrist and the ankle joints with their proximal 5 cm area were also spared. The thigh was sectioned beyond the prominence of the greater trochanter. We have taken fifteen sections for plastination.

Preparation for dehydration: Just after sectioning, the sections were placed into a receptacle containing 50% ethanol and were allowed to thaw within the receptacle as per direction of Dejong and Henry.[12] The sections remained immersed into 50% ethanol solution for seven days. Then the sections were rinsed for five hours and again placed into a fresh 50% ethanol solution for another seven days. The intention for treating the sections with ethanol was to remove some chemical compounds like glycerin, phenol etc., those are present in the embalming solution along with formalin.

After the second session of ethanol bath, the sections were rinsed into running tap water and the ethanol concentration of the water sample of the receptacle was measured daily with an ethanolometer (i.e., acetonometer). This was done to remove ethanol, as because ethanol in the sections, if present, may give false reading of acetone during dehydration stage.[12,13] After four days, the acetonometer reading was found zero and on fifth day the sections were taken off the water bath.

Several researchers[12,14] had adopted precooling, as a preparation for dehydration stage, to cope the specimens with the subsequent cold temperature used in the next steps. the sections were precooled in a freezer, where they were placed into a box containing tap water, at 4°C for 12 hours.[15] Then the sections were placed over some stainless-steel grids to hold and support them in further stages of plastination.

Dehydration: To remove water from the tissues, the sections were gradually dehydrated by using graded concentration of acetone.[16] In this case, 80% acetone was used as the first bath. This 80% acetone was prepared by mixing previously used acetone with pure acetone[12,17] and the concentration was ensured by measuring it using an acetonometer at +20°C. The volume of acetone was five times than the volume of the sections[16,18,19,20,21] which was measured by the fluid displacement method.

A stainless-steel receptacle was filled with the acetone. The stainless-steel grids holding the sections were immersed into that receptacle and placed in a deep freezer at -25°C for dehydration (Figure 1A). Careful wrapping of the receptacle was done with plastic foil paper to prevent the evaporation of acetone. Regular stirring of acetone

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was done at morning and evening to enhance dehydration.[12]

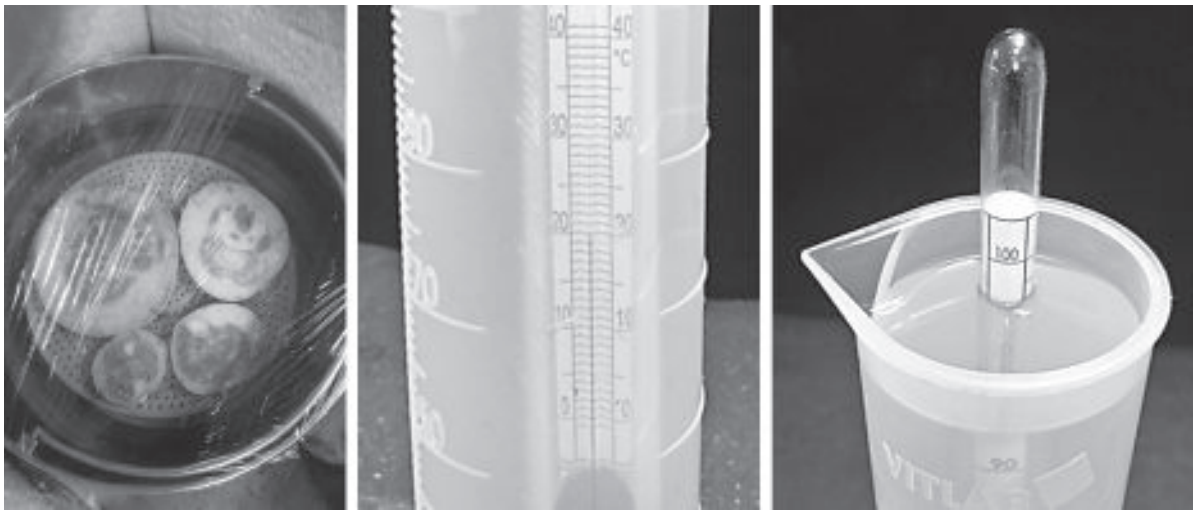
The status of the concentration of acetone or the purity of acetone in the receptacle was measured daily by an acetonometer at +20°C[12] after taking the solution within a graduated measuring cylinder (Figure 1B and C). Stirring of the acetone solution was done before measuring the concentration. The acetonometer reading was taken every day at 11 AM and at +20°C to maintain consistency of the reading. As the acetonometer was calibrated for +20°C, the temperature of the acetone solution, taken from the receptacle in the freeze, was adjusted to +20°C before the reading was taken.

When the acetonometer reading of consecutive three days became same, then the sections were transferred to another aliquot of acetone of higher concentration of 90% and the procedure was repeated for the next two bath of acetone concentration of 100%. During transfer of the sections from one aliquot to another, care was taken to prevent drying of the sections by keeping the surface moist and by rapid transfer. In the final (4^{th}) acetone bath when the acetone concentration was measured 99% for consecutive three days, the dehydration was considered completed.[22]

Degreasing: After completing dehydration, the receptacle containing the sections at the final acetone bath was placed in the room temperature (at +22°C to +26°C; measured by thermometer) for three days.[20,23] This was intended to remove the soluble fat from the tissues. 'Too much lipid may decrease specimen durability' as noted by Dejong et al[12] is the reason for defatting. In the meantime, the concentration of the acetone was measured daily and was found 99% in those days. After degreasing, the colour of acetone solution became yellow.

Forced impregnation: After degreasing, the grids holding the sections were placed into the reaction mixture of silicon polymer within the deep freezer at -25°C. The reaction mixture was prepared by mixing the silicone polymer (BIODUR® S10) with a catalyst and chain extender (BIODUR® S3). S3 causes end-to-end linkage of S10 molecules. The ratio of S10: S3 in the reaction mixture was 100: 1[12,16,18] therefore total 400 ml of S3 was mixed with 40 L of S10. The sections were placed into the reaction mixture after thorough stirring and proper removal of the air bubbles from the mixture.

The sections were allowed to remain overnight within the reaction-mixture without applying any vacuum



(A) (B) (C)

Fig.-1: *Placing the sections into the acetone-filled receptacle within the deep freezer (A) and measuring the purity of acetone with an acetonometer (B and C).*

to equilibrate the surface solvents (acetone) of the sections with the reaction mixture.[24] As the reaction mixture is too viscous to enter into the sections, a vacuum force was introduced from the following day to extract the acetone from the sections and to push the reaction mixture into the sections. Before applying the vacuum force, the kettle (Heidelberg Plastination Kettle; BIODUR® HI03A1.0), containing the receptacle having the sections within it, was covered with a 15 mm thick glass plate (Replacement glass plate; BIODUR® HI22A1.0) making it airtight by placing a silicon lid sealer (Replacement lid sealer; BIODUR® HI06A1.0) in between them.

The vacuum force was applied slowly to decrease the absolute pressure within the kettle. With the raising of the vacuum forces, the acetone within the sections began to boil (as the boiling point of acetone is +56°C) and was extracted as bubbles were seen outside from the glass lid. The extent of force applied was monitored by a vacuum gauge. The vacuum force was adjusted by the help of a vacuum adjustment unit that comprises two vacuum adjustment valves which controls the entry of external air into the kettle. The schedule of applying the force is shown in Table I. Forced impregnation was considered completed when no bubble was seen in the reaction mixture.

Table- I

Schedule of forced impregnation

Day- 1	The section-containing receptacle was loaded within the plastination kettle and was allowed to remain overnight within a deep freezer at -25°C without applying any vacuum force to equilibrate with the reaction mixture.
Day- 2	Vacuum pump was started and 50 mm Hg vacuum force was applied.No bubble was seen.
Day- 3 to Day- 6	The vacuum force was raised every day at the rate of 50 mm Hg. On day- 6, it reached at 300 mm Hg.No bubble was seen.
Day- 7	When the applied force reached at 350 mm Hg, the first bubble of acetone appeared. The rate of bubbling was monitored for next 24 hours and then the force was raised again if bubbling ceased.
Day- 8 to Day- 10	The vacuum force was raised after monitoring the rate of bubbling on the next three days at the rate of 50 mm Hg to reach up to 500 mm Hg.
Day- 11 to Day- 15	Vacuum force was raised every day at the rate of 30 mm Hg and the bubbling was monitored for the next 24 hours. On Day- 15, the pressure reached to 650 mm Hg.
Day- 16	As the bubbling continued, the vacuum force was not raised further on Day- 16.
Day- 17 to Day- 24	The vacuum force was raised at the rate of 20 mm Hg in every alternate day and the bubbling was monitored. On Day- 24, the pressure reached to 730 mm Hg.
Day- 25 to Day- 29	The vacuum force was raised at the rate of 10 mm Hg in every alternate day and the bubbling was monitored. On Day- 29, no bubble was seen and the impregnation was considered completed.

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After completion of the forced impregnation stage, the deep freezer was turned off and the sections were kept into the reaction mixture for another 24 hours within the freezer. Then the receptacle was taken off from the freezer and the sections were prepared for the gas-curing stage.

Gas-curing: Excess polymer of the sections was poured off. Finally, the polymer inside the sections was cured (hardened). This was achieved by exposing the impregnated sections to a gaseous hardener (BIODUR® S6). S6 is a liquid that vaporizes at room temperature. The impregnated sections and a flask filled with S6 were placed in a tightly closed gas-curing chamber (Figure 2). To keep the environment dehumidified for curing, a cup with a desiccant (e.g. calcium chloride) salt was also placed within the curing chamber. Then the chamber was closed and a small ventilator fan was run two times a day for twenty minutes. This fan was used to enhance vaporization of the liquid S6 as air bubble, thus accelerating the curing of the sections.[12] The surfaces of the sections were manicured every day. The position of the sections was regularly changed to ensure full exposure of all side of the sections to the gas-curing agent (S6) until their surfaces became dry. Once the sections became dry, desired plastinated specimens were considered ready to be used.

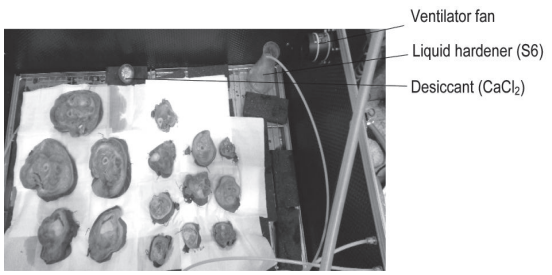

Fig.-2: *The gas-curing chamber with the sections into it.*

Results

Total 105 days were spent to prepare plastinated specimens, of those: 19 days for their preparation for dehydration stage, 18 days for dehydration in acetone, 3 days for defatting, 29 days for forced-impregnation with S10 silicone, and 36 days for gas-curing.

The specimens produced from the limb sections through S10 plastination in the laboratory was dry, odorless, fumeless, easy to hold and easy to handle. 10.41% shrinkage of the specimens and some sort of distortions were observed. Color of the specimens were changed to some extent. They can be transported from one place to another easily. Handling of the specimens does not cause any soiling as there is no dribbling of any kind of fluid.

Ethical clearance

This study was approved by Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Discussion

To prepare the specimens, S10 plastination method was used as it is considered as gold standard.[12] With a view to prepare the embalmed specimens for subsequent stages of plastination, they were run through two baths of 50% ethanol, as per recommendation of Dejong et al[12] to remove long chain alcohols like glycerin, phenol etc. of the specimens. They argued that, these chemicals may interfere with the curing process, or may precipitate on the specimen's surfaces years later, or may produce a brittle plastinated specimen. Oostrom[25] and Bickley et al[26] suggested to remove the glycol containing fixative fluids. Although Lischka et al[27] and Pashaei[28] submerged the sectioned specimens in 80% ethanol at room temperature for the same purpose, however in this study 50% ethanol was used following the protocol of Dejong et al.[12] Moreover, Cook and Dawson[29] used 50- 90% ethanol containing 5-20% hydrogen peroxide prior to dehydration to extract the embalming fluid ingredients to prevent the seepage of the greasy residues from the S10 plastinates that they experienced previously. During preparation of the specimens, the ethanol concentration was measured from water sample of the bucket during rinsing them into running tap water. The logic behind this was to ensure complete removal of ethanol, otherwise it would interfere the purity reading of acetone by giving a false increased purity reading during dehydration step as the specific gravity of the ethanol (0.79) and that of the acetone is same.[12,13]

Even though it is advantageous to dehydrate the embalmed specimens by ethanol as it can remove long chain alcohols[30] nevertheless acetone dehydration was chosen, because considerable shrinkage (roughly 50%) occurs in ethanol dehydration.[30]

Although most of the researchers use pure (100%) acetone as the standard procedure for dehydrating the specimens, however several researchers have used graded series of acetone from lower to higher concentration.[16,21,27,28,31] The specimens of this research work were dehydrated with graded series (80%, 90%, 100% and 100%) of acetone as it is cost-effective as stated by Asadi et al[16] On the top of that, Tiedemann et al[21] stated that 'proper use of a graded dilution series of cold acetone extracts more water than starting with pure acetone'. Therefore, the concentration of first acetone bath (80%) was adopted from the observation of Tiedemann and Ivic-Matijas[21] where they claimed that the concentrations of 60% to 80% do not lead to shrinkage and dilution of acetone below 70% is ineffective except as first bath.

Instead of using 1:10 ratio of specimens to acetone volume (as practiced by von Hagens et al[30] and Dejong et al[12]) and following several other researchers (Asadi et al[16]; Baptista et al[18] ; Oostrom et al[19]), 1:5 ratio is adopted in this research work as the experiment by Tiedemann et al[21] showed that 1:5 ratio is enough to dehydrate the specimens.

Considering the previous reports of shrinkages, the specimens were dehydrated by freeze substitution method at - 25°C as majority of the researchers had used and some including von Hagens had recommended this[32] even though some of the researchers stated that there is no need to use deep freezers.[16]

According to von Hagens et al[30] , dehydration and defatting are mandatory for plastination. In line with their directions, defatting was done by placing the specimen containing receptacles filled with final bath of (100%) acetone at room temperature. It was seemed that, with the progression of defatting the concentration of acetone might be changed and when it would be found unchanged that is the ending point of defatting. For this reason, acetonometer readings were recorded also to find out change in the acetone concentration if any during defatting stage.

Conclusion

In Bangladesh, plastination is somewhat a new technique for body preservation. Several centers of the world have already adopted plastination in their curriculum. Consequently, current procedure may contribute to enter a new history of body preservation in Bangladesh. Along with advantages, considerable shrinkage and distortion was observed in the plastinated specimens. However, the extent of shrinkage and distortion and their effect on teaching-learning is needed to be explored further.

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