Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 13 (1)

Preservation of Heart Using Standard Silicone S10 Plastination Technique Under Cold Condition

[1]Oghenemavwe LE, [2]Uahomo PO, [1,2]David LK

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria

[2]Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

Corresponding Author: Uahomo PO
E-mail: uahomprecious1@gmail.com; +234-7035313368

ABSRACT

Due to the drawbacks of employing formalin in tissue preservation and the related health risks, the plastination method of preserving specimens that are more flexible, resilient, and life-like utilizing liquid polymers was developed. In this investigation, the heart of a goat was acquired from the Ijanikin Slaughterhouse in Lagos State, Nigeria. In this experiment, the usual Silicone S10 process was employed for plastination under cold conditions. The end result was a heart plastinate that was dry, robust, flexible, odourless, lifelike, and non-toxic, with 52.2% shrinkage. The plastinate in the heart specimen had some morphological deformation but no significant distortion in shape or structure. When comparing plastination to formalin preservation, plastination has a greater benefit in terms of durability, lifelikeness, and flexibility.

Keywords: Plastination, Goat Heart, Silicone S10 Technique, Formalin

INTRODUCTION

There has always been a desire to learn more about the human body's interior[1]. Biological tissue decomposes after death by autolysis or putrefaction. Fixation and preservation stop biological tissues from decomposing and deteriorating by making them insoluble, hard, and in a lifelike form. The preservation of specimens obtained through fixation is done to ensure their long-term preservation[2]. When we go to a museum, we can find exhibits kept in formalin jars, which contain specimens of abnormal human and animal growth. Though the numerous features can be recognized, the specimens appear bleached, and if the liquid surrounding the specimen leaks out, a foul odor emits. We are unable to touch the specimen since the chemical used to preserve it, formalin, is a health threat. The main disadvantages of formalin fixation and preservation of tissues are that they are easily broken when handled (brittle), and transportation is a time-consuming operation with spillage hazards. Formalin toxicity is also a big health hazard. Formalin, on the other hand, is still the most common tissue preservative due to its characteristics[3].

Dr. Gunther von Hagens began experimenting with a new method of specimen preservation. Though a few articles prior to his study mentioned plastics[4][5][6][7], it was Dr. Gunther von Hagens who experimented voraciously on diffusing various plastics into large specimens, eventually succeeding and coining the term "plastination" in 1977[3].

Plastination is a preservation technique that employs polymers to retain bodies, body parts, anatomical specimens, and surgical specimens in a physical state that is similar to that of a living person, while also keeping them fulsome, lifelike, and indefinitely sterile[5]. It is an interaction between anatomy and polymer chemistry in which a curable polymer is pushed or sucked into the cellular structure of the specimen using a vacuum chamber[5]. Polymers are forcefully implanted into the tissues during this technique to keep them stable and free of degradation. They are also easy to handle and are not as brittle as formalin-preserved tissues[3]. Currently, plastination is not widely used in Nigeria; this could be due to a lack of knowledge or the high cost of materials. The goal of this study is to build a local plastination competence utilizing a goat heart.

MATERIALS AND METHOD

One fresh goat heart was used for this study. The goat heart was harvested from Ijanikin Slaughterhouse in Ijanikin, Lagos State following proper dissection process to avoided deformation of the organ. The goat heart was fixed using 10% formalin, dehydrated by freeze substitution using 100% acetone and force impregnated with liquid polymer using vacuum pump and hardened with a curing agent in a curing chamber. The standard silicone S10 technique under cold condition was used.

Fixation of the Heart: This is the process by which tissues and organs are preserved by chemicals which prevent autolysis and petrefaction, making the tissue hard and reduce their shrinkage. The dissected heart specimen was placed on a stainless sample tray in the laboratory. A graduated measuring cylinder was used to

measure the 10% formalin solution and poured into a plastic container with 0.1% phosphate buffer added to it. The specimen was immersed into the formalin solution in the plastic container and covered. It was ensured that the fixative was 10 times the volume of the specimen. Fixation was allowed for one week to avoided deformation of the specimen.

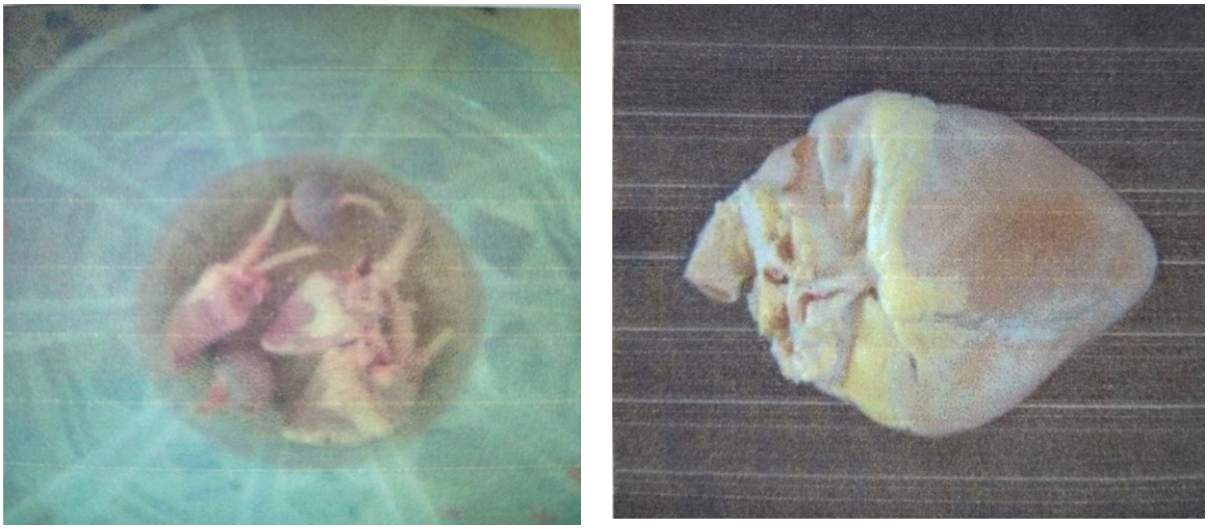

Figure. 1: Fixation of heart specimen in 10% formalin solution

Dehydration of Heart: In this step, the tissue is replaced with a dehydrating agent/organic solvent (acetone). Acetone is ideal for plastination as it acts as a dehydrating, defatting and intermediary solvent. The fixed hearted specimen was washed in a running tap water and placed in a stainless steel drum containing acetone solution and was placed in a deep freezer connected to a power source. After one week, the sample was transferred into a fresh bath of acetone in a stainless steel drum using a stainless steel basket and placed in a deep freezer connected to a power source. Hence; dehydration was done by freeze substitution by immersing the specimen in three changes of acetone bath at -25^{0}C. A digital thermometer was used to monitor the temperature within the deep freezer. The volume of the acetone was ten times that of the heart specimen for proper dehydration process. The concentration of the water in the tissue did not exceed 1% at the end of dehydration process. If it exceeds 1%, dehydration is said to be incomplete. Dehydration was done for 3 weeks.

Determination of Residual Water: A calibrated acetonometer with inbuilt thermometer was used to determine the residual water in the last acetone bath. 50ml mixture from the last acetone bath was measured using a measuring cylinder and the acetonometer dipped into the mixture in the measuring cylinder to determine the volume of the residual water in the acetone. It is important to note that water and dehydrating liquid have different density. This fact makes it possible to measure the residual water content in a specimen using acetonometer to know if dehydration is complete.

Defatting (Degreasing) of Heart Specimen: The specimen was transferred from the last acetone bath in deep freezer to a stainless steel drum containing 100% acetone at room temperature using a stainless steel basket for defatting process. Defatting was done for 48 hours during which lipids were dissolved out of the heart sample. After 48 hours of defatting, the heart sample became firmer. Defatting was complete when dehydration liquid stained or turned yellow in the course of defatting which is an indication that excess fats have been dissolved out of the tissue.

Forced Impregnation: Forced impregnation is the central step in plastination process. It involves the replacement of the intermediary solvent (acetone) occupying tissue space by curable polymer (BIODUR ® S10), achieved by applied vacuum. A polymer mixture of 100% per body weight of silicone S10 and 10% per body weight of silicone S3 (i.e. 100 PBW of S10 + 10% PBW S3) was mixed together with the aid of a mixing rod/stirring rod in the silicone S10 container. The mixing was thorough to ensure adequate mixture of the silicone S10 and S3. S10 is the silicone (liquid plastic) while S3 is a catalyst which causes the elongation of S10 molecules. The fixed and dehydrated specimen was kept in an air tight standard Heidelberg plastination kettle (vacuum chamber) fitted with vacuum pump by vacuum tubing with a Bennert and Digital Manometer connected to the vacuum tubing in a deep freezer. The S10 mixture was poured into the vacuum chamber and the vacuum pressure of 5mmHg was applied to the chamber by the vacuum pump which generated a negative (suction) pressure causing the acetone to vaporize out of the specimen and draws polymer mixture into the specimen through the vacuum

tubing. The forced penetration of the polymer (S10) into the tissues of the specimen and the evaporation of the acetone out of the specimen was indicated by bubble formation at the top of the solution in the plastination kettle (vacuum chamber). The extraction of acetone creates a vacuum inside the heart specimen which forces the penetration of the polymer into the heart and the evaporation of the acetone out of the plastination kettle. Vacuum was increased gradually through the vacuum adjustment valve to boil the intermediary solvent (acetone) which has a lower boiling point ($+56^0$C) out of the specimen. Impregnation was monitored by watching the readings in the Bennert and Digital Manometer to ensure that a steady vacuum pressure was maintained at the required point. Absence of bubbles indicates completion of the procedure.

Curing (Hardening of Heart): Hardening is the process where polymer molecules join to one another (polymerization) and cross link (curing) to form hardened specimen. The specimen impregnated with the polymer mixture reacts with hardener such as S6 wherein the polymerization (but no curing) occurs. Hardening was achieved by exposing the impregnated heart sample to gaseous hardener (BIODUR ® S6) in a gas curing box affixed with a membrane pump and fan. S6 is a liquid that vapourizes at room temperature. The impregnated heart specimen and a glass jar filled with S6 were placed in a tightly closed curing box. The glass jar containing the liquid S6 was connected to the membrane pump by means of air tubing causing the membrane pump to generate pressure into the S6 solution making it to generate vapour. The vapour generated was circulated round the curing box by the fan to harden the heart specimen. A stainless steel collecting tram helps to collect the excess S10 liquid from the specimen. To keep the environment for curing dehumidified, a Petri dish with a desiccant (calcium chloride) was placed in the curing chamber. Curing was done for 4 weeks.

Weighing of Specimen: The weight of the heart was determined by placing the heart specimen on a Cammry Electronic Weighing balance calibrated in gram and kilogram. The weighing balance was connected to as power source and put on. The weighing balance was calibrated to zero and the specimen was placed on the scale. The weight of the specimen was recorded before and after plastination.

Specimen Percentage Shrinkage: The weight of the heart specimen measured before and after plastination was used to determine the relative shrinkage. The shrinkage was expressed in percentage and calculated using the formula below;

$$Percentage\ Shrinkage = \frac{Weight\ before\ plastination - Weight\ after\ plastination}{Weight\ before\ plastination} \times 100$$

Precautions Taken: Curing method has potential problems and/or disadvantages such as;

- A white precipitate may appear on the specimen
- The specimen may shrink
- Oozing polymer may coat the specimen

To avoid precipitations;

- A desiccant was used e.g. calcium chloride
- The liquid gas cure was poured into the dish and the specimen was placed in the gas curing chamber.
- Slow curing was used as precipitates hardly ever form.
- Excess S6 vapour which might be toxic was allowed to evaporate from the cured specimen.

To avoid excess shrinkage;

- The specimen was wrapped with thin foil which adhered to the surface of the specimen.
- Slow cure was used on the specimen since the specimen has been formalin-fixed for a long period to avoid excess shrinkage.

The following general precautions were taken in the cause of the plastination process;

- Laboratory coat was put on at all times
- Disposable latex hand gloves were put on while working in the laboratory.
- Nose mask and face shield was put on while working in the laboratory as the chemicals used for plastination can be toxic and carcinogenic.
- It was ensured that the vacuum pressure was properly monitored to avoid too much influx of the liquid polymer.
- A digital thermometer was used to monitor the temperature of the acetone during freeze substitution to avoid fire outbreak as acetone is flammable at -19^0C.

RESULTS

As the primary objective of this study is to preserve goat heart using plastination technique, the following were also checked for;

- Weight of the heart before plastination and after plastination.
- Specimen percentage shrinkage.
- Quality of plastinated specimen.

The table below shows the weight of the heart specimen before and after plastination process.

Table 1: Weight of Plastinated heart

Weight (g) before plastination	138g
Weight (g) after plastination	66g
Difference in weight (g)	72g

The shrinkage of the heart specimen is as given in the table below;

Table 2: Percentage shrinkage of heart plastinate

Weight (g) before plastination	138
Weight (g) after plastination	66
% Shrinkage before plastination	0
% Shrinkage after plastination	52.2

Heart Specimen Evaluation: The quality of the heart specimen after plastination was dry, flexible, durable, odourless and non-toxic plastinate. The surface of the specimen appeared as it would on a fresh tissue specimen with minimal shrinkage and no gross distortion of morphology as observed. The internal structures of the plastinate were also retained.

Figure. 2: Heart Plastinate

DISCUSSION

A slightly above average percentage shrinkage (52.2%) was obtained from this study with no gross morphological distortion and with an aesthetically pleasing heart plastinate. The plastinate was dry, flexible, odourless, life-like and non-toxic. The finding from this study is in agreement with that of [8] who plastinated human brain using standard S10 technique under cold conditions to obtain a brain plastinate with a sound structural integrity that is aesthetically pleasing. It is also in conformity with that of [9] who described cold-temperature BIODUR S10/S15 techniques. They plastinated specimens following the standard S10 technique under cold conditions to conclude that the specimens plastinated were durable with no odour and flexible, depending on the thickness of the specimens.

Similarly, the results from this study also aligns with that of [10] who preserved body parts using silicone S10 technique under cold temperature to yield a dry, odourless and durable plastinates which were useful as an adjunct for demonstration of prosected and as excellent museum specimens. The heart plastinate had shrinkage of 52.2% which is not infirmity with that of Asadi [11] and [12] who plastinated fish specimens and liver, lungs, heart and stomach respectively under cold conditions using the standard S10 technique. No noticeable shrinkage was observed on any of the specimens while shrinkage of 10 to 30% was observed in the liver, lungs, and heart and stomach samples. The fish plastinates as well as the liver, lungs, heart and stomach plastinates appeared more natural, more flexible while retaining all of their internal structures which are in agreement with the findings of this study.

CONCLUSION

The plastinated heart specimen was dry, odourless, flexible, durable, non-toxic and easy to demonstrate the gross morphological details of heart. There was a slightly above average shrinkage of the plastinate with no gross morphological distortion. The plastinate can be utilized as teaching aids and anatomical museum models than formalin fumed dripping wet specimens. Hence, the study proves that plastinated specimen is better than formalin fixed specimen. Also, preservation of specimens by plastination technique using locally

available materials is achievable.

ACKNOWLEDGEMENT
The authors express their thanks to the museum curator of National Postgraduate Medical College, Ijanikin, Lagos and also the Museum curator of University of Port Harcourt Teaching Hospital (UPTH) for providing every necessary support for this study.

CONFLICT OF INTEREST
The authors reported no conflict of interest and no funding was received for this study.

REFERENCES

1. Moore, C.M., Brown, C.M., Gunther von Hagens & Body Worlds Part 1. The Anatomist as prosektor and proplastiker. *Anat Rec B New Anat.,* 2004; 276:8-14.
2. Weiglein, A.H. Preservation of biological tissue: Yesterday-today-tomorrow. *Journal of International Society for Plastination*, 2001; 16:31-41.
3. Prasad, G., Karkera, B., Pandit, S., Desai, D., and Tonse, R.G. Preservation of Tissue by Plastination: A Review. *International Journal of Advanced Health Sciences*, 2015; 1(11):27-31
4. Bennett, H.S., Wyrick, A.D., Lee, S.W., and McNeil, J.H. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technology*, 1976; 51(2):71-97
5. Henry, R.W. and Nel, P.P. Forced impregnation for the standard s10 method. *Journal of International Society for Plastination,* 1993; 7:27-31.
6. Weiglein, A.H. Preservation and Plastination. *Journal of International Society for Plastination*, 2002; 15(6):445
7. Pashaei, S. A brief Review on the History, Methods and Applications of Plastination. *International Journal of Morphology*, 2010; 28:1075-1079.
8. Cannas, M. and Fuda, P. Plastination of Old-formalin fixed specimens. *Journal of International Society of Plastination*, 1991; 5:11-15
9. DeJong, K. and Henry, R.W. Silicone plastination of Biological tissue: Cold temperature technique Biodur™ S10/S15 technique and products. *Journal of International Society of Plastination*, 2007; 22:2-11
10. Suganthy, J. and Francis, D.V. Plastination using standard S10 Technique, Our Experience in Christian Medical College, Vellore. *Journal of Anatomical Society of India*, 2012; 61(1): 44-47
11. Asadi, M.H. Plastination of Sturgeons with the S10 technique in Iran: The first trials. *Journal of International Society for Plastination,* 1988; 13(1):15-16
12. Farihad, S., Faizah, O, Fairuz, A.R, Hairi, G and Azian, A.L. Paper Published in 12th International Conference on Plastination held 11th July, 2004 at Murcia, Spain. Available at: https://isp.plastination.org/meetings.html