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Plastination of Brain Using Orthocryl: A Viable Alternative?

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ABSTRACT
Tissue preservation is an important aspect in teaching Anatomy. Most of the organs and tissues are preserved in formalin with its own set of disadvantages. Plastination is a unique method of permanently preserving tissue in a life like state.
The classical way of Plastination developed by western authorities is a labour and equipment intensive affair. The expensive polymers like S10 are not readily available. Only few studies using locally available polymers have been done and this study attempts to bridge this gap.
Orthocryl which is a PMMA based polymer was used to plastinate brain specimens. The specimens were made to undergo stages of dehydration, impregnation (with polymer) and curing. The results were interpreted under various parameters like shrinkage, retention of colour, odour, pliability and retention of gross anatomy. The study concluded that results with Orthocryl were satisfactory in the various parameters studied.
The study was able to prove the hypothesis that indigenous methods and materials can produce quality plastinates which may be an important adjunct to traditional methods of teaching.
Key words: *Plastination, Orthocryl, Brain*

INTRODUCTION

Plastination is a process of replacing the body tissues with curable polymers. It was invented by a German Anatomist Dr Gunther Von Hagens.[1,2] Plastination as a mode of preservation has become very popular throughout the world in contrast to formalin fixed specimens, which are wet, carcinogenic and irritable to the respiratory tract. The plastinates embedded with cured resins are dry, odourless, durable and inert to the skin.[3,4,5,6] The process involves four steps : fixation, dehydration, impregnation and curing. In fixation the specimen is kept in 5-20% formalin for a particular duration. Fixation induces firmness and gives shape to the specimen while reducing shrinkage and putrefaction.[7,8,9,10] Dehydration is done by exposing the specimen to Acetone.[8] The specimen releases water and takes in Acetone. The duration of dehydration is important as a shorter time frame produces less bleaching.[2] Use of Acetone at minus 25 degree (Freeze Substitution) produces less of ice and minimal morphological changes. Dehydration is carried out for 1-2 months depending upon the type of specimen. In addition to water, there is removal of fat at the end of dehydration. Acetone which is used for dehydration is miscible with the curable polymer and helps in its impregnation. In this stage the Acetone is

replaced by the polymer which may be accelerated by using vacuum at 5mmHg. The pressure gradient draws in the polymer and releases the Acetone as vapour, which boils out as bubble.[7,11] Curing of the specimen is a delicate step which may be carried out by gas, UV light or heat. The process of curing hastens the drying and hardening of the specimen due to polymerization and cross linking of the molecules. The outer hard barrier formed during curing prevents shrinkage by stopping the outward diffusion of the polymer.[2,8,12]

Plastination is a very versatile procedure which may be used on whole body, slices of tissues and hollow organs.[13] Inspite of its advantages, a plastinated specimen does not feel the same as the original and there may be undesirable changes to its colour, size and bulk. Anatomy is best learnt by having a feel of the organs and tissues where formalin fixation may present few advantages but that does not take away the fact that in today's truncated medical curriculum, the student has the liberty to study the plastinated specimen in its entirety in the place and time of his choice.

Plastination has been done by using the standard S10 method,[1,2] however only a handful of workers have tried to do Plastination with locally available resins often with variable results.[13,14] Keeping in view the scarcity of Plastination using locally available polymers and the difficult handling of the naturally friable brain specimens in the traditional method of preservation, a study was initiated to explore the feasibility of plastinating brain specimens and perform a comparative qualitative data analysis. The aim of the study is to produce brain plastinates which could be used as an additional method of teaching and learning. It is hypothesized that the brain with its unique characteristics may be plastinated using local means using the standard procedures.

MATERIAL AND METHODS

The process of plastination is simple and may be carried out in any laboratory with a deep freezer, vacuum chamber, wire grids, glass panes of 6X6 inches size, OHP sheets, rubber tube, steel clips, steel or polypropylene containers for the specimen and adequate ventilation. The laboratory needs to be modified and insulated to prevent any ignition by the flammable vapours of Acetone.

The material used for the Plastination is 617H19 Orthocryl with 617P37 hardener. It is a PMMA based polymers used in the manufacture of orthodontic and artificial prostheses. The polymer has a mildly irritant vapour for which proper precautions with face mask and gloves is taken. The polymer is stored away from potential sources of ignition in a cool well ventilated space.

For the study, 15 brain specimen were studied, sourced from the cadavers from the Department of Anatomy. The cadavers were embalmed with formalin between December 2014 to May 2015. Any degenerated brain due to lesions or otherwise were excluded from the study. The whole brain specimen were sectioned sagittally (Fig 1). The other halves were used as control for comparison after Plastination. Three cerebral hemisphere sectioned sagittally were kept for whole Plastination.

Commercial grade Acetone (99%) was used for dehydration. The brain was kept in Acetone containing polypropylene containers for one month followed by transferring the specimen with the container in an air sealed double wrapper of plastic into the deep freezer (Freeze substitution). The brain was extracted and reintroduced in the freezer at 5 degree celsius for 24 hours. A warm brain knife was used to cut sections in various planes with a maximum thickness of 5mm (Fig 1) The sections were subsequently transferred to a mixture of an equal quantity of the Orthocryl and acetone and kept for 10 hours. In the next step, the sections were put in pure Orthocryl for 7 days followed by vacuum at 5 mm Hg till no bubbles of acetone were seen. At this stage the specimen of brain has been fully impregnated and subsequently it was kept in a bath to drain out the excess polymer. The section are thereafter brushed with 100 parts resin::3 parts 617P37 hardener. The slices were put between two layers of glass panes with the OHP sheet intervening and put on a wire grid for curing. This ensured retention of shape. Curing was completed at room temperature.

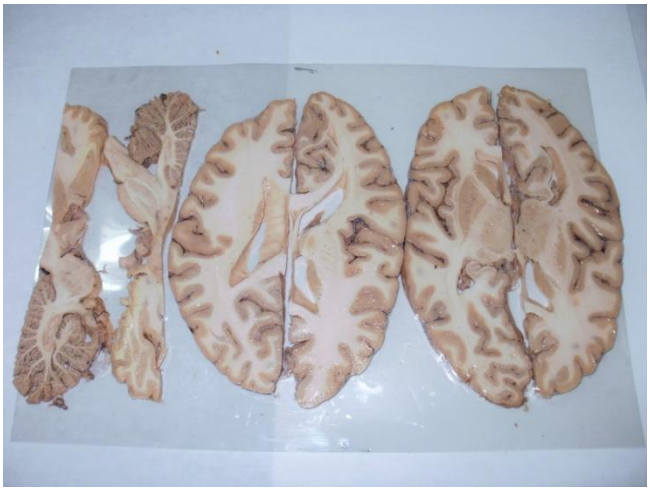

Fig 1. Shows section of Brain. The section were obtained after dehydration with acetone.

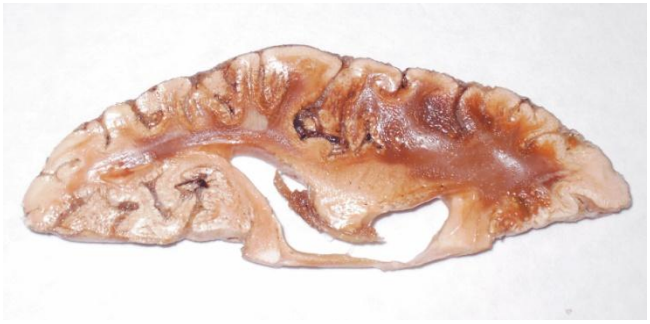

Fig 2 Shows a horizontal section of Brain. There is a focus of discolouration after plastination. The discolouration could be due improper impregnation of polymer or presence of a preexisting focal lesion which affected the stages of plastination.

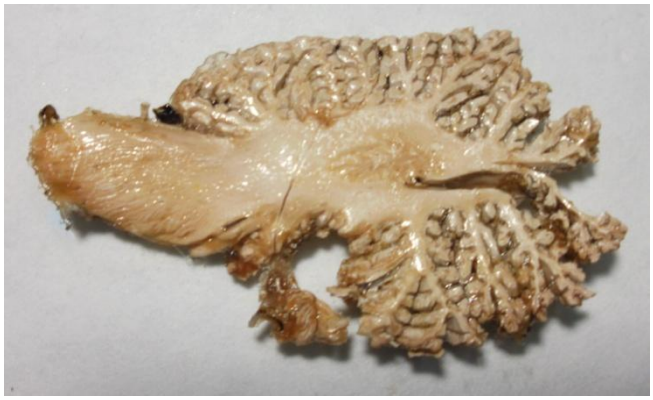

Fig 3. A specimen of Cerebellum after Plastination. The folia of the cerebellum is appreciated. The colour and gross morphology has been retained. The loss of some part of the Cerebellum is due to processing.

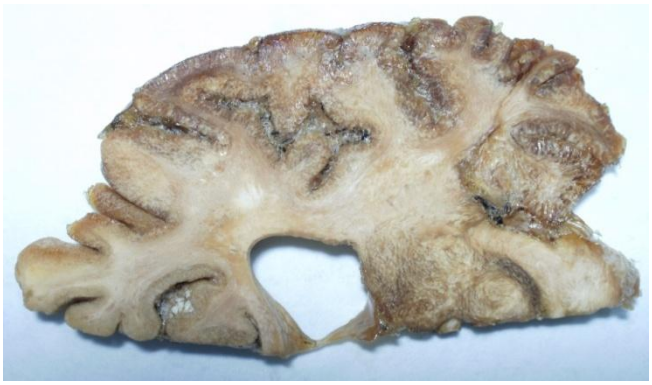

Fig 4. The section of a part of the Cerebral Hemisphere. The white matter is clearly visualized with differentiation of the white and grey matter. The sulci shows some accumulated remains of the cured polymer.

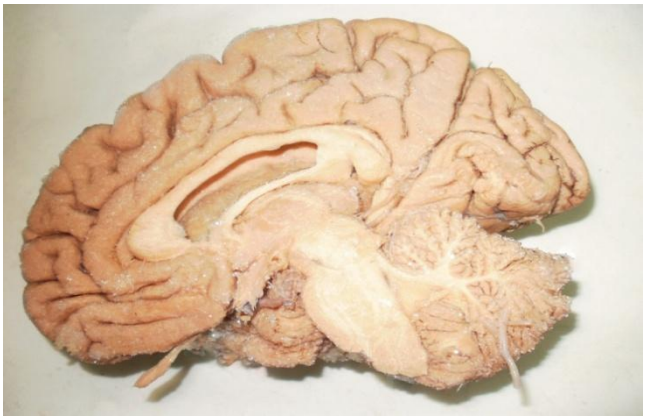

Fig 5 Shows a sagittal section of the Brain. The anatomy is well preserved. All structures can be clearly visualized. There is some accumulated remains of the cured polymer which were cleaned at a later stage.

RESULTS

The methods used for Plastination was analyzed interpreted and compared with the control. The specimens were used from cadavers which were embalmed for a duration ranging from 30 to 180 days. There were five cadavers which were procured in the last two months. The dehydration was done for 30 days followed by impregnation for 7 days. The specimens were analyzed with regards to retention of colour, friablity, pliability, change of shape and size (shrinkage), odour, surface characteristics and gross anatomy. Gross changes in morphology was assessed by comparing with the non plastinated half

The study elicited that the five specimens with a lesser number of days in fixatives were better plastinated,

especially the ones with a duration less than 60 days. Four specimens were lost in the various processes. The rest of the specimen exhibited intermediate grades of Plastination with regards to the parameters studied (Table 1).

TABLE NO 1. Showing the results of Plastination of Brain

Month of embalming of the specimen	No of specimen used	Specimen remaining for plastination*	Result of the brain Plastination under various parameters					
			Colour retention	pliability	Presence of shrinkage	Odour**	Gross anatomy	Remarks
Dec 2014	02	01	discoloured	absent	absent	mild	retained	The discoloured specimen was probably with a focal lesion which was not detected during early stages of Plastination.
Jan 2015	02	nil	--	--	--	--	--	Both the specimen were lost
Feb 2015	03	02	Specimen 1 : retained colour Specimen 2: discoloured	Absent in both	Absent in both	Mild in both specimens	Retained in both specimens	One specimen was discoloured. Cause unknown.
Mar 2015	03	03	Retained in all specimens	Absent in all specimens	Absent in all specimens	Mild in all specimens	Retained in all specimens	Two specimens were of the whole Cerebral hemisphere
April 2015	03	03	Retained in all specimens	Absent in all specimens	Absent in all specimens	Mild in all specimens	Retained in all specimens	Included one specimen of Cerebellum
May 2015	02	02	Retained in all specimens	Absent in all specimens	Absent in all specimens	Mild in all specimens	Retained in all specimens	One specimen was of the whole Cerebral hemisphere.

DISCUSSION

Plastination as a method of preservation of specimens where the biological tissue is replaced with curable polymers.[1] It produces specimens which are easy to handle, dry and odour less. It does not have the adverse qualities of formalin fixed wet specimens. The process carried out by Dr Gunther Van Hagens used S10 silicone as the polymer [1] however, some indigenous studies have been done on plastination using locally available polymers like Polypropylene resins, [13] Epoxy resins and Orthocryl. [14] A previous study was done on brain using Orthocryl, which was pilot in nature and needed refining. [14] There is scarcely any literature on plastination of brain using indigenous polymers.

In this present study, brains from embalmed cadavers were plastinated. [15] Out of the fifteen specimens, four were lost during processing. The lost specimens were from the lot which were fixed for the maximum duration of time. There is a possibility that the long fixation time had an adverse effect on plastination. Two brain specimen, one from the December batch and one from February batch (Table No 1) was discoloured and become brown (Fig 2). The patchy discolouration may have been attributed to the longer fixation time or due to a brain lesion which may have been overlooked during the processing. Certain procedures which are specific to colour preservation like Freeze fixation with --25 degree Acetone and use of Kaiserling's fluid [2,17] have not been tried here due to economical constraints. Nine specimens of brain with fixation time less than ninety days with thirty days of dehydration and impregnation of less than seven days showed retention of colour and gross anatomy, which commensurates with a previous study.[14,2]

The pliability of the tissue were lost but it is not of much significance because the specimen still retained the normal gross anatomy for studies, however there was an increased firmness observed primarily due to the impregnation by the polymer. This reduced any any chance of sectioning of the brain at this stage. There was no gross change in the size or shape of the sections nor shrinkage of any significant degree was observed. In this study a modified version of Freeze substitution was carried out to retain the morphology of the specimens.[2,17] The brain sections presented with an odour which decreased in intensity after a

month.

There is very little literature available on plastination of brain by alternative means and this preliminary study proves the hypothesis that it may be attempted within available means. It is also to be noted that plastination is best done on fresh biological tissues while this study used old embalmed cadavers. Many of the advanced procedures for plastination like using S10 polymer and gas cure have not been used intentionally, primarily to remain within the aim of this study, i.e to plastinate within the locally available resources. But more research into better polymers and methodologies will be required before a refined and reliable procedure can be accepted for implementation.

CONCLUSION

Plastination of brain using Orthocryl was used to see its efficacy. The brain was sectioned and plastinated. The sections were studied for it's colour and gross morphology. The sections retained colour and consistency in its morphology.

REFERENCES

1. Dr. Gunther von Hagens' frühere Tätigkeit als Wissenschaftler an der Universität Heidelberg" (in German) (Press release). University of Heidelberg. 24 January 2004. Retrieved 7 May 2009.
2. Robert W. Henry, Larry Janick, Carol Henry. Specimen preparation for silicone Plastination. J Int Soc Plastination. 1997 Dec; 12(1):13-17
3. O'Sullivan E, Mitchell BS. Plastination for gross anatomy teaching using low cost equipment. Surg Radiol Anat 1995;17(3): 277-81.
4. Bickley HC, Von Hagens G, Townsend FM. An improved method for the preservation of teaching specimens. Arch Pathology Lab Med. 1981 Dec;105(12):674-676.
5. Holladay SD, Hudson LC. Use of plastinated brains in teaching neuroanatomy at the North Carolina State University, College of Veterinary Medicine. J Int Soc Plastination 1989;3.1: 15-17.
6. Tiedemann K, von Hagens G. The technique of heartplastination. Anat Rec 1982; 204: 295-9
7. Robert W Henry, Nel PPC. Forced impregnation for the standard S10 method. J Int Soc Plastination 1993; 7.1: 27-31
8. Bickley, Harmon C, Robert S Conner, Anna N. Walker and R. Lamar Jackson. Preservation of tissue by silicone rubber impregnation. Journal of the International Society for Plastination 2009 Nov; 1(1):30–39
9. Von Hagens G. Impregnation of soft biological specimens with thermosetting resins and elastomers. Anat Rec 1979;194:247-256
10. Bickley HC, Conner RS, Walker AN, Jackson L. Preservation of tissues by silicone rubber. J Int Soc Plastination 1987;1:30–1
11. Grondin G, Grondin GG, Talbot BG. A study of criteria permitting the use of plastinated specimens for light and electron microscopy. Biotech Histochem. 1994 Jul; 69(4):219-34.
12. Dawson TP, James RS, Williams GT .Silicone plastinated pathology specimens and their teaching potential.J Pathol 1990 Nov; 162(3):265-72
13. Shama Sundar NM. Plastination – a new art in science. Journal of Anatomical Soc of India 1999 Dec;3:15-18
14. Pandit S, Kumar S, Mishra BK. Comparative study of anatomical specimens using plastination by araldite HY103, polypropylene resin, 6170H19 Orthocryl and silicone - A qualitative study. Medical journal, Armed Forces India. 2015;71(3):246-53.
15. Cannas M, Fuda P. Plastination of old formalin-fixed specimens.J Int Soc Plastination. 1991;5:11–5.
16. Miklosova M, Miklos V. Plastination with silicone method S 10--monitoring and analysis causes of failure, Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2004 Dec;148(2):237-8.
17. Dawson TP, James RS, Williams GT .Silicone plastinated pathology specimens and their teaching potential.J Pathol 1990 Nov; 162(3):265-72.