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Original Article

Comparative study of anatomical specimens using plastination by araldite HY103, polypropylene resin, 6170H19 Orthocryl and silicone – A qualitative study

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ABSTRACT

Background: 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. Plastination developed by western authorities is a labour and equipment intensive affair. Most common polymer used is S10, however this study uses easily available alternative polymers for plastination.

Method: Various polymers like Epoxy resins, Polypropylene resins, Orthocryl and silicone were used in plastinating the anatomical specimens. Specific methods were used for solid, hollow organs and brain specimens. The specimens were made to undergo stages of fixation, dehydration, impregnation and curing. The results were studied and interpreted under various parameters.

Results: The results were interpreted under various parameters like shrinkage, retention of colour, odour, pliability and retention of gross anatomy. The study concluded that Orthocryl and Epoxy resins retained maximum colour with minimal shrinkage while maximum discolouration was with polypropylene plastinates. Brain sections were best preserved in Orthocryl.

Conclusion: The study concluded that indigenous methods and materials can produce quality plastinates which can be an important adjunct to traditional methods of teaching however more studies need to be done for refinement.

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Introduction

Plastination was invented by German Anatomist Dr Gunther Von Hagens.^{1,2} It is a method of preserving tissue in a life like state in which the tissue, water and lipids are replaced with curable polymers which are subsequently hardened to form dry, odourless, and durable specimens circumventing the disadvantages of wet specimens.³ With ease of handling, the educational value of plastinates are immense as these are pleasant to touch with no respiratory irritation and allergic reaction.^{4–6}

Plastination involves Fixation, Dehydration, Impregnation and Curing. The specimen to be plastinated is fixed by a fixative^{2,7} and then dissected. It is then placed in acetone bath (preferably in low temperature) for dehydration, followed by impregnation (with a polymer) and curing.^{8,9} Fixation provides firmness and shape to specimens, reduces shrinkage and prevents putrefaction.¹⁰ Fixatives commonly used are 5–20% formalin. Special techniques like Freeze fixation done in –25° acetone with simultaneous fixation and dehydration is best for colour preservation.

In dehydration, the specimen is serially exposed to acetone or ethanol (causes more shrinkage).⁸ Specimens in dehydrating agents with shorter exposure time produces less bleaching.² Freeze substitution in –25° acetone is a better method of dehydration as it prevents formation of ice, causes less dimensional change and shrinkage of the specimen. End point of dehydration is reached in three months which also removes all the fat from the specimen.

In impregnation, a curable media replaces acetone and the most commonly used is S10, invented by Von Hagens. When impregnation is done using vacuum at 5 mm Hg, it causes acetone (BP 56 deg) to boil out of the specimen creating a pressure gradient which draws in the polymer mixture and releases acetone vapour as bubble.^{7,11} Rapid vacuum is avoided as low pressure is formed within and might crush the specimen by the incoming polymer, causing incomplete impregnation. Fire hazard and explosion from the acetone vapours may be avoided by simple modifications in the lab.

Curing involves hardening and drying of specimen. Hardening causes polymerization and cross linking where the constituent molecules become firm and hard. Curing can also be hastened by accelerators like gas, UV light and heat and involves stages of precure, gas cure and after-cure. The specimen are placed in an air tight container for the purpose.^{2,8,12} Precure causes the polymer to polymerize, gas cure causes polymerization and cross linking while after-cure forms a hard barrier and prevents further diffusion of uncured polymer from interior of specimen, thus preventing shrinkage. Other variants of curing are fast and slow cures. In fast cure, curing vapour produces less shrinkage with retention of colour but the plastinates are less durable. It is ideal for brain specimens and extremity sections. Slow cure makes the specimen dry, flexible and resilient, requires more time and causes more shrinkage. It is ideal for bone specimens. Hollow organs are cured in dilated state. Curing may also be done with heat or at room temperature. Use of a desiccant prevents whitish discolouration on the surface.¹²

Plastination can be done for the whole body, slices of tissue as in sheet plastination and luminal plastination for hollow organs like GIT, lungs, cerebral ventricles, intravascular patterns of kidney, coronaries etc.¹³ Hollow specimens are inflated prior to fixation and maintained throughout the period of fixation. Intravascular injection of latex, gelatin or silicone may be used to highlight the vessels.²

Plastination has its own disadvantages, as it may cause undesirable changes in bulk and colour with other visible defects (e.g. deformations, shrinkage, spots on the organ surface etc) in the specimen. The frequent reasons for these defects may be unsuitable temperature, use of old or poorly fixed specimens and longer dehydration. Retention of colour is best possible by decreased exposure to fixatives and quick dehydration. Use of fresh tissue, fixative baths at low temperature, use of special agents like Kaiserling's fluid and intravascular colouring dyes may produce specimens with natural colour. Shrinkage may be decreased by avoiding ethanol as dehydrating agent and use of freeze substitution.¹⁴

Plastination is done in a Plastination lab.^{15,16} Plastination lab can be established in any place with adequate space and ventilation. The requirements are, a deep freezer, vacuum chamber with pump and vacuum control unit, gas curing chamber, open and airtight stainless steel containers of appropriate volume for holding specimens. The specimens are immersed in the freezer containing acetone and polymer. The vapours of acetone are flammable hence, all efforts are made to prevent sparks from igniting the vapours by keeping the motor, compressor and vacuum pump in another room or near a fume hood. Specimens kept inside the freezer should be in air tight containers and the temperature tightly controlled. In freeze substitution when used, the vacuum chamber is kept inside the freezer and exhaust pipe which comes out of the freezer is properly sealed to prevent leakage of acetone vapours.

Plastination with the standard S10 method with all its advantages may not always be a viable option, however alternative methods using easily available local resources may be explored. There has been only few studies on alternative methods of plastination in India¹³ and hence keeping in view a perceptible void in plastination using alternative means, this study was envisaged to explore the possibility of plastinating specimens using locally available materials. This study endures to plastinate anatomical tissues and organs by the standard methods described using local resources and collect qualitative data from the plastinated specimens.

The aim of the study is to plastinate anatomical specimens and understand its strengths and weakness using local resources after analysing the qualitative data. This study endeavours to prove that plastination can be attempted using locally available materials and explore the feasibility of using plastinated specimens in teaching.

Materials and methods

A lab was established within the available resources. The lab satisfied most of the protocols required for the process. The room was adequately ventilated and adjacent to the verandah. It ensured proper diffusion of acetone vapour. The

refrigerator of the department was used as a freezer and it was placed away from the flammable gases. Most of the electrical circuitry was checked for loss of insulation. Freeze substitution was not used and curing was done by heat outside the lab.

Materials

Stainless containers and polypropylene containers were used for fixation and storage. Glass panes 6"X6", OHP sheets, rubber tubes and steel clips were procured for sheet plastination of brain slices. Dissection instruments were used for dissecting the parts for plastination.

Polymers and chemicals

The various types of materials in this study are.

- i. Epoxy adhesives – Araldite HY 103 with AY 951 as hardener.
- ii. Resin – Polypropylene resins with catalyst and accelerator
- iii. Acrylics – 6170H19 Orthocryl
- iv. Silicone rubber

AY 103 araldite and AY 951 hardener (epoxy resins)

Araldite AY103 and Araldite AY 951 (hardener) have been used with mixing ratio of 100:9. The epoxy is transparent, colourless and has medium viscosity. It has low odour and chemically resistant. It is stable from –60 to +60 deg Celsius and requires 24–25 h for curing at room temperature. The chemical must be handled with care when uncured and use of gloves is mandatory.

Polypropylene resin

These are thermoplastic polymers resistant to fatigue and heat. These are translucent, durable, tough, flexible and light with a low density. The resin is used with a catalyst (5%), and accelerator (0.01%). Curing takes 12–14 h for completion in presence of heat.

617H19 orthocryl with 617P37 hardener

These are PMMA based cold curing polymers mainly used in orthodontic appliances and artificial prostheses. It is slightly flammable, and may cause irritation of eyes, skin and respiratory organs. Use of facial masks and gloves are recommended. It is stored in well ventilated space, away from sources of ignition and electrostatic charges and in a cool environment to prevent premature polymerization.

Silicone rubber

Silicone rubber is a polymer containing silicone with carbon. These are nonreactive, stable and resistant to extremes of temperature. These are used in various industrial applications from electronics to sealants. Heat is required for curing process.

Dehydrating agent

Commercial Acetone (99%) has been used for dehydration and defatting. Acetone is highly flammable. Complete dehydration and defatting is accomplished once specific gravity of Acetone has reached 0.89 and when it does not turn yellow anymore with the transfer of specimen to fresh acetone. Ten times the volume of acetone is used for dehydration and it is changed every few days to few weeks.

Specimens

Specimens of brain, kidneys, heart, spleen, hand etc were collected from embalmed bodies. The specimens were cleaned and dissected for the process. The physical features of the specimens in respect to their colour, size and shape were noted.

Methodology

A. Plastination using epoxy araldite HY 103

For specimens of kidneys, duramater and hand, the dehydration was done in acetone for three months till endpoint was reached. Impregnation was carried out for specified duration by mixing equal quantity of HY103 Epoxy and Acetone where the acetone is replaced by the epoxy. Vacuum was maintained at 5 mm Hg till no bubbles were seen. The specimen were removed from the solution and put in 10:1 solution of HY103 epoxy-HY951 hardener. It was subsequently cured with gravity method and at room temperature.

Dehydration for brain slices were done in Acetone for three months (Fig. 1). Thin slices of 5 mm thickness were made using a warm slicing knife after complete dehydration and freezing of brain at –5 to –6 °C for two days. Impregnation was done using equal quantity of HY103 Epoxy and Acetone mixed together in a flat bath (Fig. 2). Vacuum was maintained at 5 mm Hg spread over 10 Hrs till no more bubbles were observed. The specimens were removed from the solution and put in 10:1 solution of HY103 epoxy::HY951 hardener. To ensure shape, the slices were put between glass panes with OHP sheet intervening between the specimen and the panes. Curing was done on a flat wire grid at room temperature. The excess polymer mixture was removed by gravity.

Fig. 1 – Brain specimens being dehydrated in acetone.

Fig. 2 – Brain sections impregnated with Epoxy HY103. Impregnation was done using equal quantity of HY103 Epoxy and Acetone.

B. Plastination using polypropylene resins

Previously fixed specimens of heart, ileocaecal junction, testis, spleen were dehydrated as previously described. Impregnation is carried out in polypropylene resin for 2 weeks and then transferred to a mixture of resin, catalyst (5%) and accelerator (0.1%). The ratio used is resin:catalyst:accelerator::100:5:1 parts. Impregnation was completed using vacuum at 5 mm Hg. Specimen is cured in room temperature, positioned on a suitable base with proper orientation.

Cerebellum and brain sections were dehydrated in Acetone for three months. Thin slices of 5 mm thickness were obtained and impregnated with the media using vacuum at 5 mm Hg.

The specimen is then transferred to a mixture of Resin, Catalyst (5%) and accelerator (0.1%) and cured.

C. Plastination with orthocryl

Specimen of kidney, testis, hand and parotid were used. After dehydration, impregnation was carried out with 617H19 Orthocryl Resin and mixed with equal quantity of acetone and kept overnight. The specimen was then transferred to pure orthocryl and impregnated over few days under vacuum. The excess polymer was removed from the specimen by gravity and subsequently brushed with 100 parts resin: 3 parts 617P37 hardener and left for drying at room temperature.

Specimen of brain and cerebellum was dehydrated, sliced and impregnated similar to the process followed with polypropylene resins. The brain slices were then transferred to a mixture of resin and hardener and cured between glass panes.

D. Plastination with silicone

Specimen of ileocaecal junction, stomach were plastinated using similar procedures. Complete curing was attained in 7 days.

Results

The various methodologies used for plastination was analysed and a comparative study was made. The specimens were studied under the following parameters:

Parameters observed in 24 specimens are for duration of fixation, dehydration, impregnation, curing and media used.

Table 1 – Retention of colour and gross anatomy of plastinated specimens.

	Number of plastinates with retention of colour and anatomy	Number of plastinates with no retention of colour and anatomy	Number of plastinates with retention of colour and anatomy	Number of plastinates with no retention of colour and anatomy
Duration of fixation	Less than 100 days		More than 100 days	
N = 24 specimens	15 Araldite: 5 Ppr: 3 Orthocryl: 5 Silicone: 2	1 Ppr: 1	4 Araldite: 2 Ppr: 2	4 Ppr: 2 Orthocryl: 2
Duration of dehydration	Less than 100 days		More than 100 days	
N = 24 specimens	17 Araldite: 7 Ppr: 3 Orthocryl: 5 Silicone: 2	1 Ppr: 1	2 Ppr: 2	4 Ppr: 2 Orthocryl: 2
Duration of Impregnation	Less than 10 days		More than 10 days	
N = 24 specimens	14 Araldite: 5 Ppr: 3 Orthocryl: 4 Silicone: 2	3 Ppr: 2 Orthocryl: 1	5 Araldite: 2 Ppr: 2 Orthocryl: 1	2 Ppr: 1 Orthocryl : 1
N = 24 plastinated specimens, Total number of Plastination with Araldite = 7, Polypropylene resin (Ppr) = 8, Orthocryl = 7, Silicone = 2.				

Table 2 – Plastination.

S No	Specimen type	Duration of fixation	Duration of dehydration	Duration of impregnation in days	Media used	Duration of curing in days	Colour retention- R,N,L,D	Shrinkage	Odour	Retention of gross anatomy	Pliability
1	Brain sec	69	31	4	aral	33	R–D	Minimal	Nil	Distorted	Brittle
2	Brain sec	69	31	7	aral	26	R–N	Minimal	Nil	Distorted	No
3	Brain sec	69	31	8	aral	25	R–D	Minimal	Nil	Distorted	No
4	Brain sec	69	31	9	aral	24	R–N	Minimal	Nil	Yes	No
5	Hand	130	90	17	aral	30	R–N	Minimal	Nil	Yes	No
6	Kidney	130	90	17	aral	30	R–N	Minimal	Nil	Yes	No
7	Duramater	69	31	9	aral	24	R–N	None	Nil	Yes	Yes
8	Heart	254	106	3	ppr	15	L–D	None	Nil	Yes	Yes
9	Ileo-cecal junction	271	100	24	ppr	22	L–D	None	Nil	Yes	Yes
10	Testis	271	100	27	ppr	26	R–D	None	Nil	Yes	Yes
11	Spleen	271	100	28	ppr	26	R–D	Minimal	Nil	Yes	No
12	Cerebellum	69	31	3	ppr	21	R–N	Minimal	Nil	Yes	No
13	Cerebellum	69	31	4	ppr	21	R–N	Minimal	Nil	Yes	No
14	Brain sec	69	31	3	ppr	23	R–N	Minimal	Nil	Yes	No
15	Brain sec	69	31	6	ppr	10	L–D	Minimal	Nil	Distorted	Brittle
16	Kidney	254	106	3	or	15	L–D	More	Nil	Distorted	No
17	Testis	271	100	26	or	25	L–D	Minimal	Nil	Yes	No
18	Cerebellum	69	31	2	or	21	R–N	Minimal	Nil	Yes	No
19	Brain sec	69	31	5	or	7	R–N	Minimal	Slight	Yes	No
20	Brain sec	69	31	6	or	8	R–N	Minimal	Nil	Yes	No
21	Hand	92	56	21	or	31	R–N	Minimal	Slight	Yes	No
22	Parotid	69	31	2	or	21	R–N	None	Nil	Yes	Yes
23	Ileocaecal junction	92	63	6	sil	7	R–N*	None	Nil	Yes	Yes
24	Stomach	92	63	7	sil	8	R–N*	None	Nil	Yes	Yes

The specimen arranged as per the media used. ppr-polypropylene resin, or-orthocryl, aral-epoxy araldite, sil-silicone, Colour: R-retained, N-Normal, L-lost, D-discoloured or dark, *- signs of putrefaction after 2 months.

Fig. 3 – Parotid gland after impregnation with orthocryl. The colour is retained with minimal shrinkage with retention of gross anatomy.

The specimens were fixed in formalin ranging from 69 days to 271 days. It has been observed that the specimens fixed for more number of days (100 days and above) became discoloured however the specimens retained gross anatomy. Fixation for less than 100 days, 15 specimens out of 24 exhibited retention of colour while only 04 retained colour with a duration of more than 100 days (Table 1 and Table 2). Results were satisfactory with Araldite, Orthocryl and Silicone while with Polypropylene resin it was inconclusive. In dehydration with less than 100 days, 17 specimens retained

Fig. 4 – Duramater plastinated with Epoxy (Araldite). Illustrates the meningeal vessels on the dura.

Fig. 5 – Duramater plastinated with Epoxy (Araldite) Duramater showing falx cerebri, tentorium and dural sinuses.

colour with minimum shrinkage, however were less pliable. Satisfactory results were observed with Araldite HY103 and Orthocryl, while only 02 specimens fixed for more than 100 days retained colour. Retention of colour and gross anatomy was associated with less number of changes in Acetone (3–4 days). 14 specimens with less than 10 days of impregnation showed the best results with only 05 retaining colour with more than 10 days of impregnation. Araldite and Orthocryl exhibited the best results. Curing, done at room temperature was the maximum for specimens impregnated with Epoxy resins (Araldite) .

The specimens were studied for colour, shrinkage, odour, pliability and gross anatomy. Orthocryl and Araldite displayed maximum retention of colour with brain sections, cerebellum, hand and parotid (Fig. 3). Two specimens plastinated with silicone also retained colour. Polypropylene resin discoloured

Fig. 6 – Ileocaecal junction plastinated with silicone. Retains the colour and gross anatomy.

the spleen, testis, heart, ileocaecal junction and one brain section from slight loss of colour to gross darkening. Orthocryl, Araldite and Silicone produced minimal shrinkage. The shrinkage was assessed visually by a scale comparing the original specimen with the plastinated specimen. Most of the plastinated specimens are odourless except the ones using orthocryl. Three specimens of brain plastinated with Epoxy resin, one by Polypropylene resin and a kidney plastinated by Orthocryl displayed gross distortion. Epoxy resin and Orthocryl maintained good anatomical relationship in general shape, visibility of the neuromuscular structures, muscles, tendons etc. Hollow organs like heart, ileocaecal junction and stomach plastinated with resin and silicone displays pliability.

Discussion

Formalin has been used since last century for preservation of specimens and embalming but it is also well known that it is a carcinogenic, irritant and allergenic.^{15,16} Plastination was invented by Dr Gunther Van Hagens using S 10 silicone to circumvent the side effects of formalin. Keeping in view the constraints on economics and availability of S10 polymer, some alternate curable polymers for Plastination were studied under the parameters mentioned. The specimens were collected from old formalin fixed cadavers¹⁷ and plastinated using standard methods. Colour retention was observed to be maximum in Orthocryl and Epoxy specimens. The plastinated specimen of parotid (Fig. 3) using orthocryl, duramater using Epoxy resin (Figs. 4 and 5) and Ileocaecal junction using silicone (Fig. 6) retained colour and spatial relationships with less than 100 days of fixation and dehydration and less than 10 days of impregnation, however the hand plastinated with epoxy (Fig. 7) exhibited minimal shrinkage with a reddish tinge with normal gross anatomy. Orthocryl and Epoxy was easily miscible with acetone during dehydration but the mixture containing Epoxy produced a reddish tinge which may explain the reddish hue. The section of brain and cerebellum showed best results with Orthocryl (Fig. 8). There was no loss of colour but the pliability was lost. The relatively higher viscosity of Epoxy resin may have distorted the brain section due to imperfect impregnation when compared with specimens with more number of days of impregnation. The plastinates from Polypropylene with higher number of days in impregnation had lost colour. This may be explained by adverse reaction between the tissues and the media which needs further investigations. The study could only plastinate two specimens with silicone. The specimens exhibited surface spots and signs of putrefaction after few months. The future study will use impregnation for more number of days and use of desiccants.¹² As the specimens were sourced from embalmed cadavers and it's quite likely that the long fixation time may have produced some discolouration however, use of fresh organs in future may produce better results. Methods not utilized in this study includes use of Kaiserling's fluid and freeze fixation.² Most of the specimens had minimal shrinkage however, use of freeze substitution and gas cure decrease the probability of shrinkage even further. Freeze fixation and freeze substitution will be attempted with deep

Fig. 7 – Hand plastinated with Epoxy resin (Araldite). Gross anatomy well maintained. The tendons, vessels and nerves well visualized.

freezers capable of -25° in future. Expensive equipments were averted by using accelerators and hardeners with curing at room temperature. With this project, our aim to attempt use of alternative polymers for plastination has been achieved. Most of the media used are easily available and the most basic of equipments were used however, more studies will be required to refine the methodology over the conventional ones till perfection is reached.

Summary and conclusion

Plastination of various human tissues were done using alternative media. Orthocryl and Epoxy resins retained colour and

Fig. 8 – Cerebellum plastinated with Orthocryl. The section of cerebellum has retained its colour and the folia are well visualized.

gross anatomy. The best results were observed in plastinates with less than hundred days of fixation and dehydration and less than ten days of impregnation.

Limitations of the study

This study was performed on old embalmed specimens from donated cadavers.

Conflicts of interest

All authors have none to declare.

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