

Preservation of Tissue by Plastination: A Review

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ABSTRACT

For generations, formalin has been used as a fixative and preservative of specimens. Formalin has always suffered from various drawbacks. There is now a better alternative to formalin as a preservative and storage for specimens. The technique of “plastination” was invented in 1978 by Dr. Gunther Von Hagens, and involves four basic steps-fixation, dehydration, forced impregnation using a vacuum chamber, and hardening/curing. Plastinates thus obtained can be used in the museum display, teaching, research, and molecular studies. This review gives an overview of the types of plastination, its uses, advantages, disadvantages and contamination, and also toxicity of the chemicals.

Keywords: Curing, Dehydration, Silicone, Vacuum chamber

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INTRODUCTION

There has always been a quest for knowledge about the interior of the human body.¹ After death, biological tissue decomposes by autolysis or putrefaction. Fixation and preservation prevent this decomposition and deterioration and make biological tissues insoluble, firm, and keeps it in a specific lifelike state. Preservation is done for the indefinite maintenance of the specimens obtained by fixation.²

When we visit a museum, we can see specimens preserved in jars of formalin, containing specimens of abnormal growth of human beings and animals. Though the various features can be appreciated, the specimens appear bleached, and a pungent odor emanates if the liquid surrounding the specimen oozes out. We cannot touch the specimen as the chemical in which the specimen is preserved, that is formalin, is a health hazard.

The main drawbacks of fixation and preservation of tissues in formalin are that they suffer from easy breakage when handled (brittle), and transportation is a tedious process with problems of spillage. Toxicity of formalin is also a major health concern. However, formalin is still the main preservative of tissues because of its properties.

Dr. Gunther Von Hagens, began experimenting on a new technique of preservation of specimens. Though a few articles earlier to his study mentioned about plastics, it was Dr. Gunther who experimented voraciously on diffusing various plastics into large specimens, and ultimately succeeded and coined the term “plastination” in 1977.^{3,4}

Plastination is a process wherein a curable polymer is pulled or drawn into the cellular structure of the specimen using a vacuum chamber and is an interface between anatomy and polymer chemistry.⁴

This procedure utilizes polymers which are forcefully impregnated into the tissues to make them stable and free from deterioration. They can also be handled with ease and are not brittle like the formalin preserved tissues.

DEFINITION

Plastination is a technique which uses polymers to permit the preservation of bodies, body parts, anatomical specimens and surgical specimens in a physical state approaching that of the living condition, keeping it fulsome, lifelike, indefinitely antiseptic without surface morphological modification.⁵⁻⁷

DESIRABLE PROPERTIES OF THE POLYMER FOR PLASTINATION⁸

It is desirable for the polymer to have the following properties to obtain the best results.

1. It should be easy to handle and also have a low viscosity in the uncured state.
2. The base and catalyst/resin activator mixture must have a long working time or be in a liquid phase for a long time to allow for impregnation into the tissues.
3. Curing should not be inhibited by the presence of tissue.
4. After curing, it should have appropriate mechanical properties, that is;
 - a. Firm- to permit its surface to be ground or
 - b. Rubber-like- to simulate a natural state.
5. It should be affordable.

The polymer that has a majority of the above considerations and has the widest acceptance in preparation of specimens is silicone rubber.

Limitations/Disadvantages of Plastination⁹

There are a few limitations/ disadvantages that prevent plastination from being commonly used worldwide at present. They are as follows:

1. Process is technique sensitive and time-consuming. Hence, it may require a dedicated pathologist.
2. It is expensive and requires more equipment than the conventional laboratory method.
3. A beginner has to do a lot of trial and error to achieve the desired outcome which might lead to wastage/ loss of rare and unusual specimens.
4. Requires a lot of post-curing work such as trimming, polishing, coloring, and mounting to achieve a good display specimen.
5. Learning anatomy on only plastinated specimens is a compromise in terms of tactile and emotional experience that is achieved in wet cadavers.

Uses of Plastination⁸⁻¹²

The plastinates that are obtained after the plastination procedure can be used in the following:

1. Preservation of autopsy or surgical tissue samples for teaching purposes.
2. Storage of autopsy or surgical tissue samples for histologic examination.
3. Museum display of historically important or unusual material.
4. Preparation of surgically removed facial organs like the nose or ear, for use as the patient's own prosthetic replacement.
5. Preparation of tissue samples to use as evidence.
6. As spotters in undergraduate practical examinations.

7. As an adjunct to problem-based type curriculum for postgraduate training.
8. As patient educative tools; to explain a pathology or anomaly that the patient is suffering from, with the help of a plastinated specimen of such an anomaly.
9. Can be pinned and/or photographed and labeled to highlight certain landmarks which the student will easily identify.
10. Unusual or rare specimens can be made available for study when no longer seen in clinical practice.
11. Preservation of the whole organisms such as parasites, insects, snakes, or plants for instructional use.
12. In teaching of regional anesthetic techniques.
13. Comparison of palatal rugae pattern in the plastinated post-mortem specimen with the plaster model from the antemortem dental record can be made for odontologic identification.
14. Practice of photography of specimens by students without the risk of damaging them.
15. As documentary evidence in forensic medicine.
16. To review the anatomy and demonstrate the relationship of soft tissue hemorrhage to fractures of the laryngeal cartilages or hyoid bone.
17. Tool-mark and bite mark comparison techniques can be taught.

Advantages of Plastination

1. Can be stored in simple plastic bags, along with appropriate documentation.
2. Require little storage and no maintenance.
3. Can be easily carried to lecture halls/classrooms and also can be easily passed to each student without gloves, appreciating features, which are impossible in jar specimens.
4. Superior to their counterparts in formalin both in terms of aesthetic superiority and in their demonstration of specific features.
5. Generally easier to interpret; therefore students are more interested in examining plastinated specimens than those preserved in formalin jars.
6. Plastinated bone specimens bear a remarkable resemblance to the original resection specimens, minus the putrid odor.
7. Wet fragile tissues such as intracerebral hematoma can be permanently preserved and rendered durable.
8. Complex and fragmented fractures can also be protected by plastination. The small particles of bone remain adherent, because a thin layer of clear silicone coats the specimen.
9. Charred specimens, including calcined bone, can be plastinated with other wet tissue specimens. The plastinated surface inhibits chalking off of the calcined fragments, and therefore protects the integrity of the specimen.

10. Insect larvae within the soft tissue of putrid specimens can be preserved.

TYPES OF PLASTINATION¹³

There are basically four types of plastination providing four different kinds of specimens as the end result:

1. Silicone impregnated specimens - resilient and flexible and mainly used in teaching.
Example: S10, from Biodur Company.
2. Specimens produced with polymerizing emulsions - are as opaque as the silicone specimens, but are rigid and to some extent breakable. This technique is used in thick body slices.
3. Transparent body or organ slices - produced with epoxy resins. Used for research purposes wherein, these slices allow the study of the topography of body structures in an uncollapsed and non-dislocated state. The specimens are also useful in advanced training programs.
Example: E12 of Biodur Company
4. Opaque brain slices - impregnated with polyester resin; they allow a unique discrimination between fiber and nuclear areas.
Example: P35, of Biodur Company.

PROCEDURE OF PLASTINATION

The specimen/body part to be plastinated is first fixed in a chemical like formalin. It is then placed in an acetone bath. The acetone is absorbed through diffusion and dissolves fat. After the specimen/body part has been thoroughly impregnated with acetone, it is placed in a polymer such as silicone. The acetone is extracted with a steady vacuum, while the polymer is drawn into the body, replacing the acetone. The specimen/body part is then hardened by gas or by heat.¹³

In this process, water and lipids in biological tissues are replaced by curable polymers which are subsequently hardened.

The procedure consists of the following steps - Fixation, Dehydration, Forced impregnation in a vacuum, and Hardening.¹³

Fixation

Formalin can be used in concentrations between 5 and 20%. To enhance color preservation, Kaiserling solution is suitable. Hollow organs have to be dilated during fixation.

Fixation may be omitted when epoxy resins are used (because epoxy resins have fixing properties), resulting in better color preservation.¹³

Dehydration/Defatting

This step is compulsory because polymers cannot directly replace lipids and water. When dehydration is done properly, excessive shrinkage can be avoided. This can be done in a stepwise manner using graded ethanol or freeze substitution with acetone.

Ethanol dehydration is used when a histological examination of the plastinated specimen is intended. The standard dehydration procedure for plastination is freeze substitution in acetone at -25°C .¹³

Forced Impregnation

Replacement of the intermediary solvent by curable polymers is the most important and central step in plastination, and is achieved by forced impregnation using a vacuum chamber. The specimen, soaked within a volatile intermediary solvent (acetone or methylene chloride), is placed into the polymer solution. The intermediary solvent has a high vapor pressure and a low boiling point (acetone: $+56^{\circ}\text{C}$, methylene chloride: $+40^{\circ}\text{C}$), while the polymer solution has a low vapor pressure and a high boiling point. Thus, on application of vacuum, the intermediary solvent is continuously extracted out of the specimen as gaseous bubbles. As the solvent leaves the tissue, a void is produced in the specimen and polymer is drawn into the specimen.¹⁴

The rate of extraction of solvent (pumping speed) depends on the property of the specimen to be impregnated. We can visualize the ideal rate of extraction of the intermediary solvent when bubbles gently rise to the surface and burst. To monitor the changes in vacuum, initially a vacuum gauge or an Hg column is used and later a manometer is used.

The impregnation is said to be complete when the absolute pressure has stabilized to around 2-10 mm Hg for a few days. Furthermore, on visualization of the bubbles, the small bubbles measuring about 1-1.5 cm are now large 4-5 cm, which is water vapor. It is imperative to leave the specimen in the impregnation bath at atmospheric pressure for a day, to allow the equilibration of pressure of the polymer in the specimen and in the impregnation bath. After this step, the specimen can be removed.

Curing (Hardening)¹⁴

This is the last step in the plastination procedure. There are three specific techniques of curing the specimen depending upon the resin used.

Gas curing is specially developed for plastination using silicone resin. In this technique, the decisive

crosslinking curing agent is applied in a gaseous form to the specimen. The impregnated specimens which used silicone are kept in a closed chamber and are exposed to a gaseous hardener which, on evaporation from a stock solution, is continuously circulating in the atmosphere of the chamber. A small membrane pump helps in the evaporation and circulation of the gas, leading to faster curing.

The curing of epoxy resin impregnated specimens or polymerizing emulsion impregnated specimens uses the tissue amines present within the specimens for curing. These amines are effective accelerators and together with anhydrides, they are sufficient to fully cure the specimens.

Different Types of Silicone Available

1. Biodur S-10
2. Su-Yi Chinese silicone
3. COR-TECH PR-10 silicone (Corcoran company)

COLOURED PLASTINATES¹⁵

Hanno Steinke developed colored plastinates in 2005. He achieved this by using different chemical reagents and coloring the anatomical structures in yellow, blue, and red. During dehydration, degreasing, and silicone resin impregnation of the specimen, the colors remained stable and thus these colors appeared to be a part of the plastination process. The colors remained stable even on exposure to light and heat for more than 5 years.

He observed that in order to get the maximum coloration, the specimens have to be preserved in the alcohol solution for as long as possible.

FUNGAL CONTAMINATION AND PREVENTION¹⁶

The plastinates can sometimes get contaminated due to humidity or exposure to water, as in leakages at the storage facility or in the rainy season.

A fungicide protocol was developed by Rafael Augusto *et al* in 1999 and is as follows:

1. Immerse the plastinated specimens in 10% formalin solution at room temperature for 5 min.
2. Rinse and manual surface brushing, in cold running tap water, for 5 min.
3. Immersion for 20 min, in an alcohol-chlorine solution, at room temperature (100 mg granular chlorine, dissolved in 100 ml absolute alcohol).
4. This solution must be stirred/shaken every 5 min.
5. Rinsing under cold running tap water and surface brushing manually, for 5 min.

6. Rinsing in distilled water for about 5 min.
7. Drying in a clean and clear environment, with low humidity.

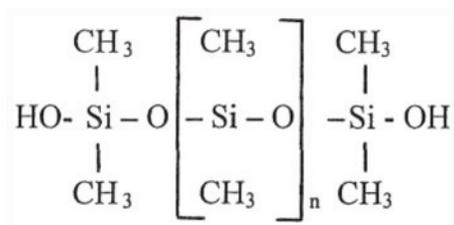
The drying process may be quickened using an electric fan.

TOXICITY OF PLASTINATION CHEMICALS¹⁷

The chemicals used in the plastination technique are toxic ranging from mild to severe. Some can be toxic either in the liquid or gaseous form. Care should be taken while being exposed to these chemicals.

In 2001, Holladay *et al.* did a study and review on the chemicals such as silicone, epoxy, and polyester that is used in the plastination process.

Plastination requires the handling of chemical agents not normally encountered by anatomic preparators. The silicone products commonly used contain poly-alkyl-siloxane. A variety of adverse effects including skin hypersensitivity type responses have been associated with these poly-alkyl-siloxanes. Epoxy resins are well-known mucous membrane, skin and eye irritants and are also associated with allergic skin response.



Structure of hydroxyl-terminated polydimethylsiloxane with (Si-O) representing a basic silicone molecule (Source: Holladay *et al.*¹⁷ Risk Factors Associated with Plastination: I. Chemical Toxicity Considerations).

The following first aid measures are indicated in case of contact with the polymers:

- Eye contact - rinse with water. Contact doctor immediately.
- Skin contact - mechanically remove product.
- On swallowing - seek medical assistance.

These chemicals should always be handled using proper protection, like industrial grade gloves, eye protection, and face protection. It is always safe to work in an area having proper ventilation.

CONCLUSION

Plastinated specimens are an excellent alternative to formalin-fixed specimens. The advantages are many

with the only hindrance being the high initial cost of equipment. With expanding popularity of this procedure, a day might come when plastination will be a standard part of the museum, classroom, and research activity.

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