Review

www.anatomy.org.tr
Received: December 30, 2014; Accepted: January 13, 2015
doi:10.2399/ana.14.040

anatomy
International Journal of Experimental and Clinical Anatomy

Plastination: basic principles and methodology

Mustafa F. Sargon, İlkan Tatar

Department of Anatomy, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Abstract

Plastination is the method of long term preservation of the biological tissues with completely visible surface and high durability. The plastinates are devoid of harmful effects of formalin and they serve as excellent teaching tools in education. Additionally, plastination is an outstanding tool to study cross-sectional anatomy. The three major methods used in plastination are silicone plastination, sheet plastination with epoxy method and sheet plastination with polyester method. Silicone plastination is the most versatile technique which can be used for the cadavers, organs, portions and slices. Fresh or formalin-fixed (embalmed) specimens can be plastinated with this technique. More flexible specimens can be obtained if fresh tissues are preferred. Silicone plastination consists of five main steps. These are the preparation of the specimen (dissection and fixation if necessary), dehydration, defatting (degreasing), forced impregnation and curing (hardening). Epoxy plastination preserves 2-5 mm slices of biological tissues by using epoxy resins. In this technique, all tissue fluid and a significant amount of fatty tissue is replaced with a curable epoxy resin mixture. Epoxy plastination method provides precise semi-transparent sectional specimens and in these preparations; gross anatomical structures can be examined with the naked eye in a superb quality down to a sub macroscopic level. Polyester plastination and classic silicone plastination techniques utilize the similar basic principles. In polyester plastination method, the tissue fluid is removed and is replaced with a curable polyester resin. This method can be used for head brain and body slices.

Keywords: epoxy method; plastination, polyester method; silicone method

Anatomy 2014;8:13–18, © 2014 TSACA

Introduction

Plastination is the method of long term preservation of the biological tissues with completely visible surface and high durability. It was developed by Dr. Gunther von Hagens in 1978 at the Heidelberg University in Germany. In this process, the water and lipids in biological tissues are replaced by curable polymers and the anatomical specimens are preserved by impregnation with these polymers which are subsequently hardened.[1] Most often used polymers are the silicone, epoxy or polyester resins which keep the anatomical specimens in a dry, odourless state and they require minimal aftercare.[2,3] All of these plastinates are devoid of harmful effects of formalin and they serve as excellent teaching tools in medical education and as great artistic museum specimens. Additionally, plastination is an outstanding tool to study cross-sectional anatomy as utilization of sectional imaging modalities in medicine has heightened the need to understand sectional anatomy.[4,5] In plastination, gross specimens, dissected specimens and cross-sectional slices can thus be preserved permanently into specimens, which are clean and dry.[6] Three major methods used in plastination are silicone plastination, sheet plastination with epoxy method and sheet plastination with polyester method. Many different techniques can be used in plastination, and below is described some favourable methods among these procedures.

Silicone Plastination

Silicone plastination is the most versatile technique which can be used for cadavers, organs, portions and slices (**Figures 1-9**). The chemicals used in this technique include fixatives (mostly formalin, if fixation is necessary), acetone, methylene chloride (if necessary), silicone polymer, catalyst to prepare silicone molecules for elongation and cross-linkage, chain extender to promote the formation of longer chains of silicone molecules and cross-linker to form a 3-D meshwork of elongated silicone molecules by side to side linkage.[2]

Silicone plastination consists of five main steps. These are the preparation of the specimen (dissection and fixation if necessary), dehydration, defatting (degreasing), forced impregnation and curing (hardening).

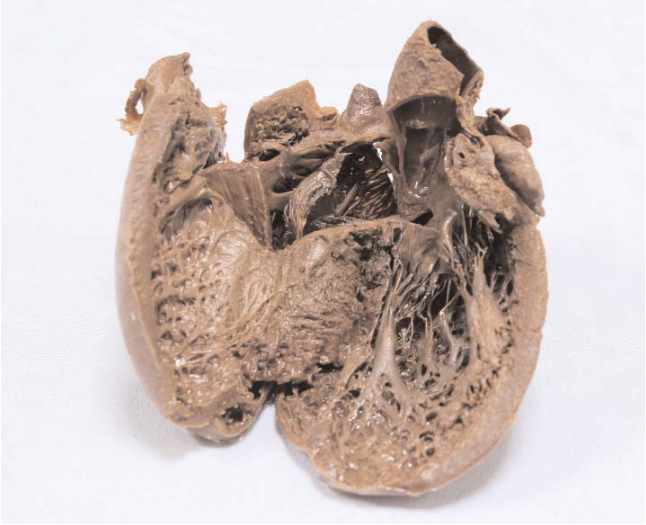

Figure 1. The heart of a formalin-fixed human cadaver. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon).

Figure 2. The lung of a formalin-fixed human cadaver. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon).

Fresh or formalin-fixed (embalmed) specimens can be plastinated with this technique. More flexible specimens can be obtained if fresh tissues are preferred. Therefore, the specimens may or may not be fixed with formalin or other fixative agents. However, the tissues can be fixed in order to prevent any type of potential biohazard risk associated with the handling of tissues.[7,8] Hollow organs must be dilated during fixation in order to obtain better specimens. Before the dehydration step, all embalming fluids must be removed from the specimen. The principle of dehydration is to replace tissue water and tissue fluid with the dehydrating agent. The standard dehydration procedure for silicone plastination is freeze substitution in acetone at -25 °C. Freeze substitution causes only minor tissue shrinkage and within the next 3-5 weeks, the specimens become completely dehydrated. During this procedure, at least three changes of the acetone are necessary. Acetone is a very successful dehydration agent, a defatting agent and an intermediary solvent which mixes with all type of resins used in plastination. Ethanol may also be used in dehydration, but not very preferable in routine procedure. Defatting is the removal off excess fat (lipid) from the specimen and it is done by removing the dehydrated specimens from -25 °C to room temperature for

Figure 3. The liver of a formalin-fixed human cadaver. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon).

Figure 4. The spleen of a formalin-fixed human cadaver. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon).

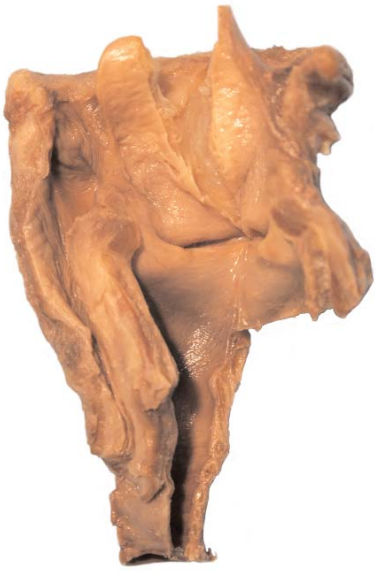

Figure 5. Interior of the larynx taken from a fresh frozen head and neck specimen. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon).

Figure 6. The larynx of a fresh frozen head and neck specimen. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon).

several days to weeks. In this procedure, the colour change of acetone from clear to yellow must be controlled as the lipid turns acetone into yellow. When the lipid on the specimen begins to lose its white colour and it becomes slightly opaque, this step can be finished. During defatting, related to the lipid content of the specimen, weekly acetone changes may be necessary. The next step in silicone plastination is forced impregnation. The principle of forced impregnation is to replace the volatile intermediary solvent (acetone or methylene chloride) with the reaction mixture of silicone. As the reaction mixture of silicone is too viscous for becoming into equilibrium with the dehydrating agent, a force (vacuum) is necessary. The vacuum gets the reaction mixture of silicone inside the specimen and the adjustment of this vacuum value is very important for preventing the shrinkage. The last step in silicone plastination is curing (hardening) which is performed after the removal of specimen from the impregnation bath. In silicone plastination, a gas curing procedure is mostly preferred. In this technique, the crosslinking curing agent is applied to the specimen in the gaseous form in a closed chamber. The continuous evaporation and circulation of the curing agent inside the closed chamber can be achieved by a small membrane pump for speeding up the process. Curing is a double step process which consists of chain extension and cross linkage of polymer. After a few days, depending upon to the surface concentration of curing agent and the degree of chain extension, the surface of

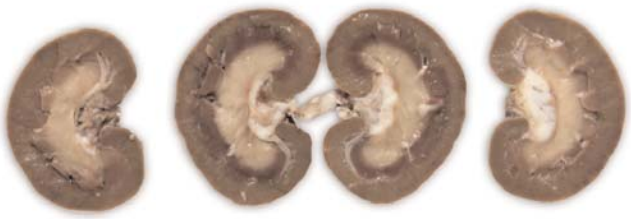

Figure 7. Sheep kidney sections. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon).

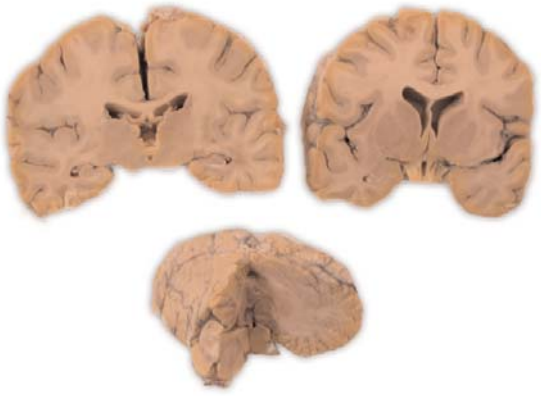

Figure 8. Cerebrum and cerebellum sections taken from a formalin-fixed human brain. Silicone plastination. (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon and Ilkan Tatar)

the specimen will become dry. Then, the specimen must be stored in an airtight bag or in a closed chamber for obtaining complete internal hardening of the polymer. This last storage step is also very important for preventing the leakage of polymer in later period.[2,6,7]

Sheet Plastination with Epoxy Method

Epoxy plastination preserves 2-5 mm slices of biological tissues by using epoxy resins. In this technique, all tissue fluid and a significant amount of fatty tissue is replaced with a curable epoxy resin mixture. Epoxy plastination method provides precise semi-transparent sectional specimens and in these preparations gross anatomical structures can be examined with naked eye in a superb quality down to a submacroscopic level.

In epoxy plastination, 2-5 mm thick slices taken from biological specimens are plastinated using a flat chamber technique or a sandwich technique.

The chemicals used in epoxy plastination include fixatives (if necessary), acetone, epoxy resin, epoxy hardeners and epoxy plasticiser.

The standard steps of epoxy plastination are specimen preparation, fixation (if necessary), slicing, cold dehydration, defatting (degreasing), impregnation and curing (hardening). In this method, the selected specimens may or may not be fixed with formalin or other fixatives. The specimens may be fixed in order to prevent any potential biohazard risk. Freezing the biological specimens chosen for sectioning at -70 °C or -75 °C is very important. Specimens must be kept in the deep freezer at this temperature at least for five days (7-10 days) to assure complete freezing. Sawing of the specimens is the next step and the slices can be taken on a butcher band saw. After sawing, the saw dusts must be removed in order to prevent the appearance of artefacts on the plastinated slices. The cleaned slices are then placed on acetone resistant grids and they are submerged in -25 °C acetone for freeze substitution. Then, the dehydration bath which has a final concentration of at least 98.5% of acetone is put into room temperature for defatting. For producing high quality transparent slices, defatting is a very important step in epoxy plastination. For the slices having high amounts of lipid, a stronger lipid remover is necessary after bath three of defatting, and this agent is the methylene chloride. The next important step in epoxy plastination is forced impregnation. In forced impregnation, the solvent is extracted from the cellular and interstitial space of the slice and is replaced with the resin impregnation mixture. The level of the epoxy resin impregnation mixture must

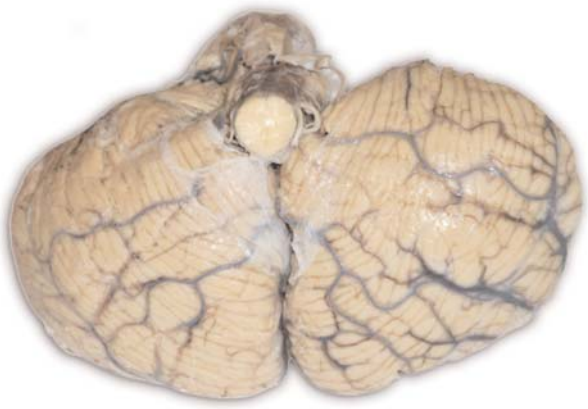

Figure 9. Cerebellum of a formalin-fixed human brain. Silicone plastination (Specimen prepared at the Plastination Laboratory of Hacettepe University, Department of Anatomy by Mustafa F. Sargon and İlkan Tatar).

be at least 3-5 cm over the top of the slices. The best indicator which shows the completion of impregnation is the increase of reaction-mixture temperature. At +5 °C, standard impregnation takes 36-48 hours and at room temperature, it is about 32 hours. At the end of impregnation, the chamber is returned into atmospheric pressure, the glass port is opened and the box containing slices is removed. After the drainage of excessive epoxy resin mixture, two methods - flat chamber method and sandwich method - can be used for curing. In flat chamber method, the flat chambers which are assembled from 3-4 mm thick safety glass plates, appropriate diameter gasket or silicone tubing and fold-back clamps can be used. The flat chambers containing the impregnated slices are filled with the epoxy resin casting mixture, the small air bubbles in the resin are removed and the chamber is placed at +15 °C or room temperature for at least 24 hours and finally they are placed in a 45 °C oven for four days. After removal from the oven and cooling down to room temperature, the glass plates are removed and the sheets are cut to desired size. In sandwich method, heavy foil (plastic) sheets and glass plates are used. This method is faster than the flat chamber method and safety glass is not required. In this method, a foil sheet overlaps the glass plate and fresh, deaerated casting mixture is poured onto the foil. Impregnated slices are placed on the pool of casting mixture on the foil and then, the casting mixture is spooned on top of the slices. The slices are covered with another foil and by using a spatula; pressure is applied on the foil in order to remove air bubbles. Then, the foil sandwich model (foil – slice – foil) is turned over and with a spatula the foil is squeezed to remove air on this side of the slice.

In the presence of more slices for curing, every three foil sandwiches are placed together and a glass plate is put on the top of them. If more than three slices are prepared for curing, the same process starts over again on the top of the glass. Then, a weight is placed upon the top glass plate and sandwich block remains at room temperature for one day and at the end of one day, it is placed into a 45 °C oven for four days. After removal from the oven and cooling down to room temperature, the foil sheets are removed and the sheets are cut to the desired size.[5,9,10]

Sheet Plastination with Polyester Method

Polyester plastination and the classic silicone plastination techniques utilize the similar basic principles. In polyester plastination method, the tissue fluid is removed and it is replaced with a curable polyester resin. This method can be used for head slices, brain slices and body slices.

The chemicals used in polyester plastination include fixatives (if necessary), acetone, methylene chloride (if necessary) and polyester resin.

The basic steps of polyester plastination include preparation of the specimen, fixation (if necessary), slicing, dehydration, impregnation and curing. The preparation of the specimen, fixation, slicing and dehydration steps are similar with the epoxy method. Fixation is necessary for brain specimens to bring the brain firm enough for slicing. Perfusion, immersion and injection fixations can be done for the brain tissue. However, perfusion fixation is the ideal method if it is possible. In the sheet plastination of the body slices with polyester method, fixation is not preferred and the fresh tissue is the ideal one. The tissue may be fixed in formalin in order to decrease the potential for exposure to biohazards that may be associated with routine handling and sawing of the biological specimen. If formalin is not used, the tissue colour will be preserved best. In order to obtain the best body slices, ultra-cold freezing is necessary (7-10 days at -70 °C or -75 °C). The next step in polyester plastination is dehydration which is done by freeze substitution in -25 °C acetone. The dehydrated slices, especially the brain specimens are easily breakable and therefore must be handled very carefully. During polyester plastination of body slices, defatting is essential in order to obtain the best resolution of the tissues. However, defatting should not be done on brain slices; because defatting will cause excess shrinkage on these slices. Defatting procedure for body slices is similar with the silicone and epoxy plastination methods. The next step in sheet plastination with polyester method is the impregnation of the specimen. The slices may stay in the polyester resin overnight for equilibration before applying vacuum for forced impregnation. The vacuum chamber must be kept darkened during the whole impregnation process. At the end of forced impregnation, the vacuum chamber is returned to atmospheric pressure and the box containing slices is removed from the polyester resin impregnation bath. The specimens must also be kept in a dark environment during this step. The last step in polyester technique is curing (hardening) of the resin. The equipments used in curing include 2 mm window glass or 2-3 mm tempered glass, silicone gasket, large fold back clamps, ball bearings, magnet, 1 mm wire with small hooked tip, 1.2 mm hypodermic needle and UVA light. Additionally, the slices may also be cured in a heated water bath at 40 °C instead of UVA light in some polyester resins.

During the preparation of casting chambers for slices, two different techniques can be used. These techniques are building the casting chamber around a slice and building the casting chamber for later insertion of a slice into the chamber. Then, the flat casting chamber containing the specimen is filled with fresh polyester resin using a flat funnel. Air bubbles are poured with the resin into the flat chamber, and they must be allowed to rise to the surface. A 1 mm wire can be used for raising the bubble to the surface. At the end of this stage, the top of the flat chamber must be closed with the remaining longer length of the silicone gasket and fold back clamps. The air bubbles can be removed by inserting a 1.2 mm hypodermic needle between the glass and the silicone gasket. Before closing the top of the casting chamber, one or two 3 mm ball bearings can be inserted into the chamber. These ball bearings can be used for centrally positioning and aligning the slice by the help of a heavy duty magnet. After this step, the glass chamber containing the slice and polyester resin is exposed to UVA light. During the exposure of UVA light, it is important to cool the glass chamber on both sides either by using a ventilator or blowing compressed air over both sides of the chamber. If cooling is not done, the UVA light which is the catalyst commences an exothermic reaction and this will damage to the specimen. On the other hand, the natural daylight outside is an effective light for curing the cast slices. Especially shadow is recommended during this procedure. The glass chamber must be turned at 15 minutes intervals to assure uniform exposure to the UVA light of the sun in the shadow. At the end of curing and during cooling, cracking sounds may be heard as the cured resin releases from the glass plate. Then, the glass plates are released and the specimen is wrapped in order to prevent any uncured resin. At the end of wrapping step, the excess amount of cured resin can be trimmed by using a band saw.[11-14]

References

1. Weiglein AH. Plastination in the neurosciences. Keynote lecture. Acta Anat (Basel) 1997;158:6–9.
2. DeJong K, Henry RW. Silicone plastination of biological tissue: Cold-temperature technique BiodurTM S10/S15 technique and products. J Int Soc Plastination 2007;22:2–14.
3. Steinke H, Rabi S, Saito T, et al. Light-weight plastination. Ann Anat 2008;190:428–31.
4. Magiros M, Kekic M, Doran GA. Learning relational anatomy by correlating thin plastinated sections and magnetic resonance images: preparation of specimens. Acta Anat (Basel) 1997;158: 37–43.
5. Latorre RM, Reed RB, Gil F, et al. Epoxy impregnation without hardener: To decrease yellowing, to delay casting and to aid bubble removal. J Int Soc Plastination 2002;17:17–22.
6. Baptista CAC, Skie M, Yeasting RA, Ebraheim N, Jackson WT. Plastination of wrist: Potential uses in education and clinical medicine. J Int Soc Plastination 1989;3:18–21.
7. Ulmer D. Fixation: the key to good tissue preservation. J Int Soc Plastination 1994;8:7–10.
8. Smith BJ, Holladay SD. Risk factors associated with plastination. II. Infectious agent considerations. J Int Soc Plastination 2001;16: 14–8.
9. Weber W, Henry RW. Sheet plastination of body slices – E12 technique, filling method. J Int Soc Plastination 1993;7:16–22.
10. Sora MC, Cook P. Epoxy plastination of biological tissue: E12 technique. J Int Soc Plastination 2007;22:31–9.
11. Latorre R, Arencibia A, Gil F, et al. Sheet plastination with polyester: An alternative for all tissues. J Int Soc Plastination 2004;19: 33–9.
12. Gao H, Liu J, Yu S, Sui H. A new polyester technique for sheet plastination. J Int Soc Plastination 2006;21:7–10.
13. Sui HJ, Henry RW. Polyester plastination of biological tissue: Hoffen P45 technique. J Int Soc Plastination 2007;22:78–81.
14. Latorre R, Henry RW. Polyester plastination of biological tissue: P40 technique for body slices. J Int Soc Plastination 2007;22: 69–77.

Correspondence to: Mustafa F. Sargon, MD, PhD
Department of Anatomy, Faculty of Medicine,
Hacettepe University, Ankara, Turkey
Phone: +90 312 305 21 11 Fax: +90 312 310 71 69
e-mail: mfsargon@hacettepe.edu.tr

Conflict of interest statement: No conflicts declared.