

THE GENERAL PROTOCOL FOR THE S10 TECHNIQUE

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ABSTRACT

Plastination is a process of preservation of anatomical specimens by a delicate method of forced impregnation with curable polymers like silicone, epoxy or polyester resins, with vast applications in medical fields of study. In this process, water and lipids in biological tissues are replaced by curable polymers (silicone, epoxy, polyester) which are hardened, resulting in dry, odorless and durable specimens. Today, after more than 30 years of its development, plastination is being applied in more than 400 departments of anatomy, pathology, forensic sciences and biology all over the world. The standard S10 silicone technique produces flexible, resilient and opaque specimens. After fixation and dehydration, the specimens are impregnated with silicone S10 and in the end the specimens are cured. The key element in plastination is the impregnation step and therefore depending on the polymer used the optical quality of the specimens differ. The S10 silicone technique is the most common technique used in plastination. It is used worldwide for beginners, but also for experimented plastinators. The S10 plastinated specimens can be easily stored at room temperature, are non-toxic and odorless. The S10 specimens can be successfully used, especially in teaching, as they are easy to be handled and display a realistic topography. Plastinated specimens are also used for displaying whole bodies, or body parts in exhibition.

Key Words: *plastination, S10, anatomy, silicon*

INTRODUCTION

Anatomy remains one of the core courses of medical school. Anatomy is where students learn the basic language of medicine, learn to develop understanding through experimentation, and develop skills in solving problems in a three-dimensional space [1]. The nature of anatomy education has changed substantially over the past decade due to both a new generation of students who learn differently from those of past years and the enormity of advances in anatomical imaging and viewing [2]. Significant advancements in the teaching and study of anatomy have been made, thanks to a new method of preserving human organs through plastination. Water and fats in tissues are replaced with silicone in a process which takes about one month. After plastination, the resulting tissue is safe to handle (i.e., toxic fixatives are eliminated), the tissue has no odor and it is extremely durable, and do not disintegrate. The end product is an anatomical specimen practically indistinguishable from the original. Plastination is the most important recently developed technique for the preservation of biological specimens. Plastination is carried out in many institutions worldwide and has obtained great acceptance particularly because of the durability of the plastinated specimens, the possibility for direct comparison to CT- and MR-images, and the high teaching value plastinated specimens have.

Plastination has been developed for teaching as well as for research. Back in 1977, at the department of Anatomy of Heidelberg University, Dr. von Hagens invented plastination as a ground-breaking technology for preserving anatomical specimens with reactive polymers [3]. The process was patented between 1978 and 1982. This method has proved to be the superior method for preservation of gross specimen. Nowadays, the method is applied in more than 300 institutes for Human Anatomy, Clinical Pathology, Biology and Zoology worldwide [4-7]. Optical properties - opaque or transparent - and mechanical properties - smooth and flexible or hard - can be chosen by appropriate composition of plastination resins.

The S10 technique is the standard technique in Plastination. Specimen impregnation with BIODUR® S10 results in opaque, more or less flexible, and natural looking specimens. The procedure consists of the four main steps of plastination, besides the specimen preparation and dissection before.

FIXATION

Biological material must be fixed before plastination to prevent putrefaction and stop the action of other enzymes. Proper fixation of an organ or tissue sample is very important to the final quality of a plastinated specimen. The usual fixatives, such as 5-20% formalin,

may be used; however, exposure must be kept to an absolute minimum if natural color is to be preserved. All embalming fluids containing long chain alcohols (e.g., glycerol) have to be removed before dehydration, as they easily spoil the final specimens. Hollow organs must be dilated during fixation as well as during dehydration and gas curing. Fixation can be achieved by all usual fixatives as formaldehyde solution, Kayserling solution etc. Hollow organs must be dilated during fixation as well as during dehydration and gas curing.

Types of fixation: immersion, infiltration, injection, perfusion, dilatation. The best for plastination is the use of formaldehyde solution (at decreased concentrations). If one desire, he could use other fixation agents as Kaiserling's solution that yields a red-brown color to the specimens, while McCormick's, Klotz, or Jore's solutions may help preserve color.

Immersion: This is the easiest of them all; you just put the specimens into the fixative. A 5% solution of formalin is used most often. Occasionally 1% or 2% solution can be used, but a concentration higher than 5% is not advisable. Immersion is especially suitable for thin specimens such as aortas. A lower temperature (+1 to +5 °C) will retard putrefaction and hardly affect the rate of penetration of the fixative. Furthermore, it favors color preservation.

Injection: This term is used to denote the introduction of fixative fluid via vessels. In most cases the arteries are used, seldom the veins. A plastic cannula or piece of flexible tubing is inserted into the vessel and serves as its extension. Injection is especially suitable for whole bodies or extremities.

Perfusion: This is defined as the continuous flushing of the vascular system of whole bodies, organs or extremities with fluid. Perfusion liquid is injected into a main artery and flows through the capillaries and veins. Perfusion with tap water is used to rinse blood out of the vessels. Tap water perfusion must always precede fixative perfusion or else the blood will clot in the vessels and block further perfusion. In plastination, tap water perfusion is very commonly used to remove blood from organs such as kidney, heart and liver.

Infiltration: In plastination, this is defined as "direct injection of fixative into tissue." The vessels are not used. Infiltration is employed for specimens that are too thick for immersion and cannot be injected. It is also handy for specimens whose vascular system has been damaged or injected with colored material.

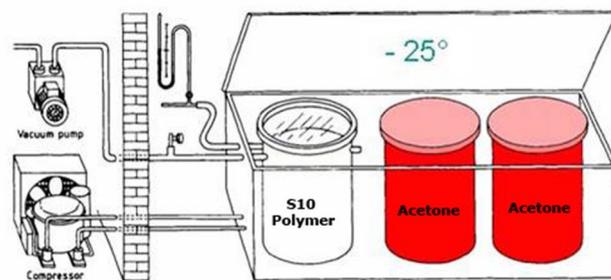
Optional: Bleach if necessary to brighten stained or darkened tissue, by using a 1-2% peroxide solution for 3 days.

DEHYDRATION

Dehydration removes the specimen fluid, as well as some fat. In this step, tissue fluid is replaced with an organic solvent. Either alcohol [8] or acetone may

be used as dehydrating agent for plastination. Acetone is used in most cases because acetone also serves as the intermediary solvent during the next step - forced impregnation. To minimize specimen shrinkage, dehydration is done in cold (-15 °C to 25 °C) acetone. The standard dehydration procedure for plastination is freeze substitution at -25 °C. This method saves time, requires less labor compared with ethanol dehydration, and causes less shrinkage. During freeze substitution, the specimens freeze immediately on immersion into the cold acetone, thereby stabilizing the specimens shape instantly. Within the next 3-5 weeks, the specimens become dehydrated.

Fig. 1. Freezer with dehydration container



If the removal of fat is also desired the dehydrated specimen must be kept in acetone (or methylene chloride) at room temperature for some time (this however, must not be done with nervous tissue, particularly with brains). An acetone amount of 10 times the specimen weight is best for good results. Dehydration is finished when the water content is less than 1%.

Equipment: deep freezer (explosion proof or motor and compressor removed and placed in a different room), acetonometer (to measure the content of water).

Dehydrate until you reach 99% - 100% acetone (3-6 weeks):

- Cold acetone;
- Ethanol.

Protocol:

- a. - Pre-cool the specimen (5 °C, 12h in water);
- b. - Acetone baths (-25 °C):
 - Acetone 97% (2-3 weeks);
 - Acetone 100% (1 week).

After two or three days in each acetone bath, the purity is checked with an acetonometer after stirring the acetone bath. Make the acetone changes at -25 °C, it will produce less evaporation and you are working under inflammation point (-19 °C).

FORCED IMPREGNATION

Forced impregnation is the central step of plastination and it's done under vacuum in a vacuum chamber [9]. In this step, the intermediary solvent (acetone) is replaced with a curable polymer (Biodur® S10). The silicone polymer S 10 is mixed with a curing agent Biodur® S3 (1 part S3 and 100 parts S10), which

commences the process of end-to-end linkage of the molecules. This linking is enhanced at room temperature; however, it is very slow when kept in the freezer at -15°C to -25°C .

Fig. 2. S10 silicon and S3 hardener



The dehydrated specimen is submerged in the cold (-15°C to -25°C) polymer mixture. After some days of immersion, vacuum is applied to it. Vacuum is applied slowly, as determined by the rate of gas bubble, building at the surface of the polymer. Vacuum is increased gradually to boil the intermediary solvent (acetone), which has a lower boiling point, out of the specimen.

Fig. 3. Vacuum chamber



Impregnation is monitored by watching the bubble formation on the surface of the mixture and by a vacuum gauge. Vacuum is complete when the pressure is around 4 mm Hg [10].

Fig. 4. Normal impregnation speed, slow bubble formation

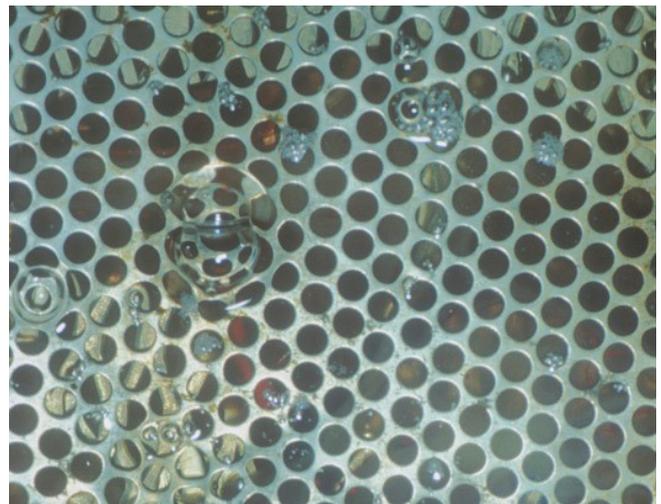
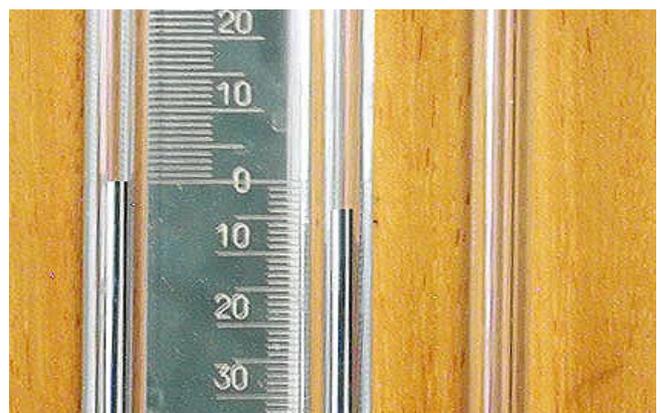


Fig. 5. Vacuum manometer



Fig. 6. Final vacuum is reached (4 mmHg)



Equipment: deep freezer (explosion proof or motor and compressor removed and placed in a different room), vacuum chamber (e.g. Heidelberg plastination kettle), vacuum pump.

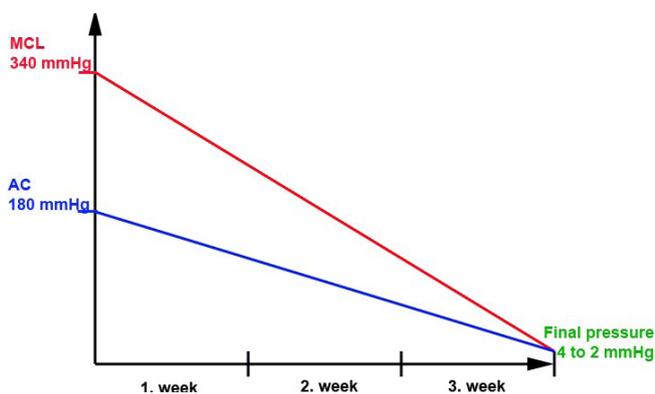
Fig. 7. Standard Heidelberg vacuum kettle



Protocol:

- a. Impregnation: Silicone polymer (Biodur S10) plus catalyst and chain extender (Biodur S3) (100:1 ratio) In a deep freezer at -15 °C is preferred.
 - Gradually decrease absolute pressure (increase vacuum) from one atmosphere (3-5 week period) until 5 mmHg is reached;
 - Room temperature impregnation is possible and is completed much faster (2-3 weeks).
- b. When impregnation is finished:
 - Drain polymer excess from impregnated specimens, while in deep freezer, back into plastination chamber.

Fig.8. Impregnation timetable



Impregnation time for S10: after the first week the impregnation pressure should reach 1/3 of the starting pressure (different for acetone and methylene chloride), after 2 weeks it should reach 2/3 of the initial pressure and after 3 weeks it should reach at least 4 mmHg.

GAS - CURING (HARDENING)

Finally, the polymer inside the specimen has to

be cured (hardened). This is achieved by exposing the impregnated specimen to a gaseous hardener (Biodur® S6). S6 is a liquid that vaporizes at room temperature [9]. The impregnated specimen and a flask filled with S6 is placed in a tightly closed chamber for several weeks. To keep the environment for curing dehumidified, a flask with a desiccant (e.g. anhydrous calcium chloride) is also placed in the curing chamber. To enhance the curing procedure, air may be bubbled through the fluid S6. For complete curing inside the specimen, this should be kept in a plastic bag for several weeks [11].

Equipment: (plastic or stainless steel) box, stretch foil, membrane (aquarium) pump.

Protocol:

- a. Slow Curing:
 - Room temperature (1 - 6 months) then use gas cure (Biodur S6).
- b. Fast Curing:
 - Gas cure (3-7 days) vaporize S6. Enhance volatilization by using an aquarium pump to bubble air through the S6 or a fan to circulate the S6;
 - Wipe excess polymer daily;
 - Bag specimen for 1-3 months.

Each of the curing methods has advantages and disadvantages. The slow curing gives you a more flexible specimen, but it take more time, the fast curing produces stiffer specimens, but is more quick.

Fig. 9. Gas curing unit.



After curing, the specimens are finished and can be stored at room temperature for a long time. Impregnated Biodur S10 silicone plastinated specimens have the same ultimate quality. Surface clarity is perfect and some flexibility is associated with thinner specimens. All specimens are durable, free of offensive odor and dry. They make excellent teaching aids both in the classrooms and the clinical setting [12]. As they are accumulated, they can become a fantastic library of specimens for normal, exotic and pathological anatomy. They may be used as teaching aids both in the class room and the clinical setting [12-15].

Fig. 10. Dissected brain plastinated with S10



Fig. 11. Head & neck

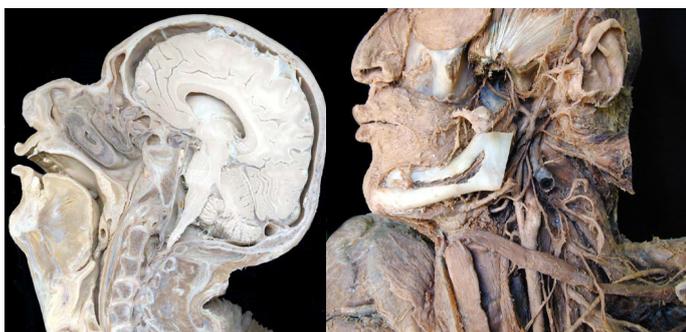


Fig. 12. Abdominal cavity



Fig. 13. Kidney



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