

## PRESERVATION OF TISSUE BY SILICONE RUBBER IMPREGNATION

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### INTRODUCTION

In recent years a process called plastination has been introduced that permits excellent preservation of biological material by impregnation with a variety of curable polymers (1). In effect, tissue water (and some lipid) is removed and replaced with plastic. Although this process is protected by several patents (U.S. patents 4205059, 4244992, 4278701 and others), the production of specimens for education or museum display is not restricted, providing written authorization is obtained in advance (2) and such specimens are not sold or traded for profit.

Uses of plastination in anatomic pathology and forensic science include at least the following:

1. Preservation of autopsy or surgical tissue samples in a form useful for teaching
2. Long-term storage of autopsy or surgical tissue samples for later histologic examination
3. Preparation of unusual or historically important material for museum display
4. Preparation of tissue samples for use as evidence
5. Preservation of whole organisms, such as parasites, insects, snakes or plants, for instructional use
6. Preparation of surgically removed facial organs (nose and ear) for use as their own prosthetic replacement (3)
7. Serial sectioning of whole organisms, organs or extremities for detailed examination

### PRINCIPLES OF PLASTINATION

Plastination has been performed with a number of different polymers, the most common being epoxy, silicone rubber and polyester. The polymer that has enjoyed widest acceptance in the preparation of specimens for teaching is silicone rubber.

Regardless of the polymer used, the process involves four basic steps: (1) fixation, (2) dehydration (freeze substitution with acetone is recommended), (3) forced impregnation, and (4) curing. Other treatment such as vessel injection is often used for enhancement of specific detail.

Forced impregnation (Step 3) is the key to the entire process and is the one part protected by patent. It is carried out by placing the acetone-saturated specimen (from Step 2) in a reaction mixture of special polymer and slowly reducing the ambient pressure. This causes the acetone to boil out of the specimen, creating a difference in pressure between its interior and the surrounding fluid plastic. Under the influence of this gradient, polymer is drawn into the specimen, filling all intracellular and interstitial space occupied by water in the natural state.

Given the unusual requirements of the process, particularly the need for a polymer of low viscosity that affords a long-enough working time, only the specially formulated plastics developed by the Biodur Company of Heidelberg, West Germany (4) will give uniformly satisfactory results. In fact, these are the only polymers that can be used in compliance with patent restrictions.

We will first discuss each of the above four steps in some detail, particularly as they apply to the Standard Technique for impregnation with Biodur S 10 silicone rubber. Further information regarding the S 10 Standard Technique is available as a leaflet from Biodur. Information presented in this paper will revise and update that presented in an earlier publication (5).

## FIXATION

Biological material must be fixed before plastination to prevent putrefaction and stop the action of other enzymes. Plastination of freeze-dried material has been attempted but such specimens tend to develop an offensive odor. Thus, fixation is presently considered an essential step in the process.

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. Care must be taken to avoid fixative fluids containing glycol since this will interfere with the later curing of the S 10 silicone. Color-conserving fixatives, such as Kaiserling's fluid (6) are recommended where appropriate. Color retention is also enhanced by maintaining the fixative bath at +5°C.

Best of all for color preservation, is the practice of freeze fixation. This is defined as the fixation and simultaneous dehydration of specimens in  $-25^{\circ}\text{C}$  acetone (7). It is carried out by mixing 5 parts (by volume) of methanol-stabilized formalin (available commercially) with 95 parts of acetone, chilling the mixture to  $-25^{\circ}\text{C}$  and using it to fix specimens which have been precooled to  $+5^{\circ}\text{C}$ . Fixation time should not exceed two weeks. Maintenance of color, size and shape is excellent and the specimen becomes partially dehydrated as well. In fact, the only drawback to freeze fixation is that the acetone used cannot be easily reclaimed since formaldehyde will precipitate as paraformaldehyde in the condenser of the distillation apparatus. Even so, this can be dissolved by occasional distillation of methylene chloride.

For the most natural-appearing product one should start with fresh tissue; however, if a specimen has been rendered colorless by long exposure to formalin it can still be successfully plastinated and some color restored by staining. In working with fresh or frozen tissue, however, overexposure to fixative fluid must be scrupulously avoided if retention of natural color is important. Assuming reasonable ease of penetration, no more than 48 hours at room temperature should be necessary. If longer exposure is required, the specimen should be sliced to a more easily penetrated thickness or infiltrated with the fixative via a fine needle.

Because fixation imparts some rigidity, the specimen should be fixed in the form that it will exhibit when finished. Lungs, for example, should be fixed by inflation with fixative under hydrostatic pressure introduced through the airway. Smaller solid organs, such as the tongue, should be infiltrated with fixative in addition to being immersed in the same solution. Fixation of the heart should be done by introducing fixative under pressure through one of the great vessels while the organ is suspended in a fixative bath. Bulky organs such as liver should be sliced to an easily penetrated thickness and the slices kept flat by pressing onto a porous surface such as a fine screen or filter paper supported by a grid.

## DEHYDRATION

Once fixed, a specimen must be thoroughly dehydrated before impregnation. Serial exposure to solutions of gradually increasing ethanol concentration is a familiar and convenient method, however it often results in an intolerable amount of shrinkage.

Far preferable is dehydration by freeze substitution with acetone. In this technique, the specimen is first precooled to  $+5^{\circ}\text{C}$  in the fixative bath, removed, blotted and transferred to a container of acetone at  $-20$  to  $-30^{\circ}\text{C}$ . Precooling discourages the formation of ice crystals and is particularly important in the dehydration of delicate structures.

The volume of acetone used must be at least 10 times that of the specimen to be dehydrated. Three aliquots of this 10X volume of anhydrous acetone are employed. Specimens the size of a heart or larger should be left in the first for three weeks, in the second for two weeks and in the third for one week. The final aliquot is monitored with a hydrometer to assure that its concentration of water does not exceed 1%. If a higher concentration is detected, a fourth change is used.

Freeze substitution with acetone takes a shorter time than ethanol dehydration and results in a specimen with less dimensional change. In addition, the tissue emerges completely saturated with a solvent that is appropriate for use in the next step. If ethanol dehydration is used, an additional step is required in which the final saturation of absolute alcohol is replaced with a suitable intermediary solvent such as acetone or methylene chloride. Anhydrous acetone will enhance dehydration but methylene chloride is immiscible with water.

#### FORCED IMPREGNATION

Forced impregnation is carried out by transferring the acetone-saturated specimen to a reaction mixture of polymer (S 10 base material containing 1% S 3 Hardener) and placing the entire assembly under vacuum at freezer temperature. As explained, this causes the acetone to boil out of the specimen, creating a pressure gradient which draws the reaction mixture in.

Vacuum is applied slowly, as determined by the rate at which the mixture bubbles. Rapid boiling must not be permitted because the pressure within the specimen will become too low and it may be compressed or crushed by the force of the intruding polymer. Also, rapid boiling is liable to result in incomplete impregnation.

Pressure can be regulated by adjustment of a shutoff valve in the line between the pump and chamber or, better, by a bypass valve that admits air to the chamber at a controlled rate. This permits stabilization of the pressure at any level, a capability that becomes important in a polymer-attenuation technique to be described later.

The use of acetone as a volatile intermediary solvent is highly recommended because it boils readily under vacuum (even at low temperature) and is a good solvent for most uncured resin materials. In some laboratories, dichloromethane (methylene chloride) is used for this purpose because it is more volatile than acetone, non-inflammable and mixes even more readily with uncured resin. Although the use of acetone usually requires a longer impregnation time than dichloromethane (three weeks as compared to two), it is now used more widely than the latter because it is less toxic in vapor form and, with minimal precaution, constitutes no more of an explosion or fire hazard. Many plastinators prefer dichloromethane, however, and have learned to avoid its irritant properties while taking advantage of its superior qualities as a solvent.

Another feature of acetone that would encourage its use as both a dehydrating agent and intermediary solvent is that it can be relatively easily recycled by distillation, thereby reducing solvent waste, environmental contamination and expense.

An additional point to be made regarding reduction of expense is that in carrying out impregnation at -20 to -30°C, the same polymer reaction mixture can be used indefinitely. Following impregnation, the volume of polymer absorbed is restored by simply adding a freshly mixed aliquot. Temperature this low inhibits the slow polymerization (end-to-end joining) of molecules within the reaction mixture that occurs at room temperature, and prevents the viscosity of the mixture from increasing to a degree that renders it unusable.

## CURING

The curing of Biodur S 10 consists of two separate events: polymerization and crosslinking. The process is quite complicated and terminology in general use can be confusing if certain definitions are not established at the outset:

### 1. PRECURE:

At room temperature (or more rapidly in a 50 °C oven) S 10 polymer molecules, react with molecules of S 3 Hardener and join end-to-end. This elongation of its constituent molecules renders the reaction mixture first viscous, then sticky. End-to-end joining (polymerization) imparts toughness and flexibility—both desirable qualities of a finished specimen — but will not result in hardness. If it is allowed to happen as the FIRST step after impregnation it is termed the "precure" stage of the whole curing process.

### 2. GAS CURE:

The complete curing process consists of both polymerization (as described above) and crosslinking. Crosslinking of its constituent molecules causes S 1 to become firm and hard, also a desirable quality (if not exaggerated). It is accomplished by exposure to a weak acid vapor that acts as a crosslinking agent. This curing vapor is released from a preparation called Biodur Gas Cure 86. Exposure to S6 and the resulting crosslinking is called simply "gas cure."

### 3. AFTERCURE:

As S6 gas contacts the specimen it hardens the polymer at the surface and sets up a barrier that slows its own further diffusion. Also, formation of this cured surface "crust" stops continued leakage of uncured polymer from the interior of the specimen and prevents shrinkage.

In effect, it seals the specimen and creates a hardened superficial layer in which the concentration of curing gas is quite high. If the specimen is now placed in a plastic bag, 86 gas within the surface layer will diffuse slowly toward the center and complete the curing. This phase is called "aftercure."

Two variations of the curing process are presently employed, the difference being determined by whether a significant precure phase is used. These two variations are called fast cure and slow cure. In the former, a specimen impregnated with S 10 reaction mixture is wiped free of excess polymer and placed directly into curing vapor in a sealed chamber (kept very dry by the inclusion of open containers of calcium chloride). In the latter, the specimen is allowed to precure on the bench top for a length of time (up to 4 weeks) and then placed in a 50°C oven for an additional interval (again, up to 4 weeks), following which it is placed in curing vapor. Advantages and disadvantages of both methods are summarized in Table 1.

In the fast cure method, the surface is cured first and the interior of the specimen hardens during aftercure. The most conspicuous advantage of this method is that shrinkage is held to an absolute minimum. This is extremely important when one starts with fresh tissue and uses short fixation time to conserve color.

Specimens prepared by the fast cure method have proven somewhat less tough and flexible than those that are slow cured. Also, control of the moisture within the curing chamber is very critical since an excess concentration of water vapor will result in the appearance of white spots on the finished specimen. Despite its need for close attention to detail, however, the fast cure method is recommended when starting with fresh tissue, particularly lipid-rich tissues such as brain and extremity sections because it serves to limit the characteristically severe shrinkage of this kind of material that will result from the oozing out of interstitial polymer during precure.

In the slow cure method, the specimen is allowed a rather long interval for end-to-end polymerization (precure), during which almost all excess polymer drains out of its internal space. Exposure to curing vapor then hardens the remaining polymer thoroughly, resulting in a dry, flexible, resilient specimen. The principal objection is the time required before the specimen becomes useful and its potential for shrinkage. This method is particularly adaptable to bone specimens and those that have been fixed for a long time and will not undergo further contraction.

As experience is gained, most plastinators come to use some variation of the fast cure method because of the shorter time that it takes to produce a useful specimen and because it discourages shrinkage and permits the use of a color-preserving regimen of fixation.

## VARIATIONS OF THE STANDARD TECHNIQUE BASED ON POLYMER ATTENUATION

In the S 10 Standard Technique described above, forced impregnation is carried out using a reaction mixture consisting of S 10 silicone base material mixed with 1% S 3 hardener. Undiluted reaction mixture flows into the specimen and fills all available space. For the purpose of distinguishing it from a method to be explained below, this is designated "complete polymer impregnation" with S 10 silicone.

If the reaction mixture, rather than used uncut, is diluted with a solvent that remains essentially nonvolatile during impregnation, but is evaporated after curing, the result is somewhat different. Under these circumstances, a sponge-like microstructure is formed in which the polymer permeates the solid components of the tissue but does not completely fill its interstitial spaces. Because the ultimate result is a specimen only partly suffused with polymer this technique is termed "incomplete polymer impregnation" with S 10 silicone.

Incomplete polymer impregnation with S 10 silicone is particularly useful for thin-walled, flexible specimens such as gut or lung because it permits retention of flexibility and simulation of a natural surface. A segment of intestine, for example, when incompletely impregnated with S 10 silicone, will be quite flexible and will exhibit a villous substructure at its mucosal surface that can be appreciated by examination with a hand lens. Lung tissue incompletely impregnated with S 10 silicone remains palpably spongy, while areas of consolidation can be detected by their comparative induration.

Incomplete polymer impregnation is carried out by diluting the standard S 10 reaction mixture with xylene before using it in Step 3 (forced impregnation). The recommended solution consists of the following (in parts by weight):

S 10 Silicone Base Material: 100  
S 3 Hardener: 1  
xylene (reagent grade): 30

Forced impregnation is carried out in much the same manner as in the standard technique, except that the ambient pressure is not allowed to drop below 15 mm Hg, a pressure at which xylene will boil. The use of xylene as a diluent has the added advantage of lowering the viscosity of the reaction mixture and decreasing the time needed for impregnation to less than one week.

Following impregnation, the specimen is wiped clean and exposed to gas cure in a dry, sealed chamber for one or two days. It is then stored for at least one week in a plastic bag to permit aftercure. To reduce shrinkage to an absolute minimum, the polymer must be allowed to cure rather thoroughly before the xylene is permitted to evaporate.

## HISTOLOGIC EXAMINATION OF S 10-IMPREGNATED SPECIMENS

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One of the most interesting and potentially useful qualities of tissue plastinated by the standard S 10 technique is that its microscopic structure remains intact (8). This, of course, means that it can be preserved almost indefinitely in a form that is easily stored while still retaining full potential for histologic examination.

Given the current state of the art, plastination could be used for routine preservation of tissue samples obtained through surgery and autopsy. Indeed, there is no doubt that it would prove more versatile than the paraffin and formalin storage now practiced.

The key to an appreciation of this facility is an understanding of "deplastination". Deplastination takes advantage of the unusual ability of the sodium ion to depolymerize silicone rubber. Tissue samples to be deplastinated are exposed to a saturated solution of sodium methylate in anhydrous methanol until free of polymerized silicone rubber and then subjected to standard histologic techniques. Although some staining procedures are slightly prolonged, the results are comparable to those obtained with more conventional methods.

## USES AND ADVANTAGES OF PLASTINATED SPECIMENS IN TEACHING

As previously reported (9) , plastinated specimens have been introduced into the pathology teaching laboratories at the University of Texas Health Science Center at San Antonio, and compared with the same types of specimens preserved in formalin. The educational value of plastinated specimens was judged equal or superior to the latter, and their ease of handling was deemed greatly improved.

Also, plastinated specimens have been in use for some time at Mercer University School of Medicine where they have proven successful as an adjunct to a new, problem-based type of curriculum.

Further experience at both San Antonio and Mercer has shown that, given the availability of a collection of plastinated specimens, they will be employed in circumstances where the use of human tissue samples would not have been considered. Such specimens are now used as an adjunct to lectures and seminar sessions, both within these institutions and with lay audiences ranging from Rotary Clubs to high school classes.

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Table 1. COMPARISON OF THE FAST AND SLOW CURE METHODS FOR S10

	FAST CURE	SLOW CURE
Duration of Process	Comparatively short. No precure, short gas cure, long aftercure (but specimen may be used during this phase)	Lengthy, long precure, short gas cure, no aftercure Specimen cannot be used until end of process
Shrinkage of Specimen	Minimal, surface hardens immediately preventing further loss of polymer	may be severe as a result of polymer drainage during precure
Toughness of Finished Specimen	Acceptable but not as tough and flexible as slow-cured specimen	very tough and flexible
Sensitivity of Process to Moisture*	Highly sensitive, curing chamber must be kept very dry with calcium chloride	Relatively insensitive, no need to use calcium chloride
Need for Attention during Process	Polymer is expressed onto surface of specimen and free of polymer must be removed every hour or two during the first day of cure	specimen is wiped once per day during precure but only once or twice during gas cure

Newer techniques in which curing gas is bubbled out of solution with an aquarium pump have reduced moisture sensitivity of the fast cure method at this step. Details are available from Dr. von Hagens.