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TECHNICAL REPORT

Hoffen P45: A Modified Polyester Plastination Technique for Both Brain and Body Slices

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ABSTRACT:
Plastination is the premier methodology for preservation of biological specimens, and is applicable to many allied areas: anatomy, biology, pathology, embryology, and clinical medicine, as well as art. This polymer technique produces 2-3 mm semi-transparent to translucent slices which display anatomy within its normal relationships and anatomical environs. Polyester slices are an excellent modality for understanding modern diagnostic images: computed tomography, magnetic resonance and ultrasound. Polyester plastination was developed for the preservation and study of brain tissue. In recent years, polyester has also been used for presentation of numerous tissues. The Hoffen P45 technique was developed near the turn of the century for both brain tissue and body slices. Both the resin and the curing method are different from classic polyester techniques. The Hoffen P45 technique uses a water bath for curing of the polymer rather than UVA light.

KEY WORDS: P45, Polyester, Cross Section, Sheet Plastination, Body Slice, Brain Slice.

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Introduction

Polyester sheet plastination was developed to manufacture and preserve 4-8 mm brain slices. To promote curing and smooth surfaces, impregnated slices are placed between pieces of glass. This glass apparatus is called a flat chamber and the slices are used for both anatomical study and research (Barnett, 1997; Henry and Weiglein, 1999; Sora et al., 1999; Latorre et al., 2002; Henry and Latorre, 2007).

Polyester plastination incorporates the general principles of classic plastination techniques (von Hagens 1979; 1986; von Hagens et al., 1987) wherein tissue fluid is removed from the slices and replaced with a curable polyester resin. The P35 resin, developed in the late 1980's, is the gold standard for brain slice production, and produces translucent brain slices with exquisite differentiation of white and gray matter. Head slices have also been produced with P35 resin (de Boer-van et al., 1993).

P40 brain slices yield good white/gray differentiation. Generally, the P40 process utilizes thinner slices (2-3 mm) (von Hagens, 1994; Henry and Latorre, 2007). More recently, P40 polyester resin has also been used for body slices (Latorre et al., 2004) and to plastinate gross anatomical structures (Sora, 1998). A newer polyester technique (P45) has been developed which utilizes both brain and body slices (Gao et al., 2006; Sui and Henry, 2007). This new polyester plastination technique is easy to use and well-suited for research (Zheng et al., 2014). Each of these resins utilizes forced impregnation and casting between glass plates.

Chemicals used in polyester plastination include acetone and polyester resin components. The Hoffen products for polyester plastination are:

P45 polyester resin, P45A & P45C polyester plasticizers, and P45B hardener.

Materials and Methods

The basic steps of plastination are similar for all plastination techniques: specimen preparation, dehydration & degreasing, impregnation and curing. These steps, with modifications, are used for the P45 resin technique.

Specimen preparation:

Two types of specimen preparation will be described: brain slices and body slices. Brain slices are prepared from well-fixed brains on a deli-slicer. Slices 2-3 mm thick are prepared, rinsed of saw dust and debris with running tap water, and placed on grids to allow water, dehydrant and impregnation-mix to flow between and around the slices. More details may also be found in the P35 and P40 techniques described in the Journal of the International Society for Plastination, Vol 22 (Henry and Latorre, 2007; Weber, et al, 2007).

Production of P45 body slices

Specimen preparation equipment:

- Band saw
- Grids
- Specimen basket

The non-fixed specimen is positioned for proper anatomical alignment and frozen, preferably in an ultra-cold (-70° C) deep freeze for two days (longer for larger specimens) for best slice production. To decrease biohazard exposure, tissue should be fixed in formalin (Smith and Holladay, 2001). Hairy specimens should be clipped.

Slicing

Large specimens should be divided into smaller, manageable portions which will also prevent thawing during prolonged slice production. Set the guide stop at the desired specimen thickness (2-3 mm) and saw serial sections. Cooling the guide stop and saw table with ice, dry ice, or liquid nitrogen prevents premature thawing of the specimen and slices. Square the end of the tissue block and commence sawing serial sections. Place produced slices on an acetone resistant grid. Remove sawdust by scraping with a knife and/or running a small, brisk stream of tap water or cold acetone across the surface. Caution: do not thaw the slice. After cleaning, the grids with their cleaned slices are stacked, tied together with twine/string and placed in either the first cold acetone (-25° C) bath or in a fixative bath.

Fixation and bleaching (optional): depending on the specimen, it may be necessary to fix the slices, as well as bleach them. For fixation, slices can be submerged in 10% formalin for one or two weeks. Once fixed, fixative should be rinsed from the specimens in running tap water for one to two days. If brightening of the slices is desirable, immerse them in 2% dioxogen (bleach) overnight or until the desired brightening is completed. Flush with running water for one hour and pre-cool (5° C) to prevent ice crystal formation before submerging in the cold acetone.

Note: for production of P45 body slices, the steps of specimen preparation, slicing and dehydration are similar to the “Biodur E12 Epoxy Technique”. Please refer to that section of the E12 epoxy process for a more-detailed description (Sora and Cook, 2007).

Dehydration and degreasing of body slices

Freeze substitution in -25° C acetone is the recommended dehydration procedure for all plastination techniques and is also the case for the Hoffen P45 plastination technique. Methylene chloride may be used for faster or more thorough degreasing.

Dehydration equipment:

- Acetonometer and cylinder
- Specimen/slice basket
- Chemical resistant acetone reservoirs

The precooled, cleaned stack of slices is placed into the first cold (-25° C) acetone bath for one week. Next the stack of slices is placed into the next fresh acetone bath at -15° C for seven days. The third change is into 100% acetone at room temperature for at least one week for degreasing.

When the body slices are appropriately degreased, either in prolonged room temperature acetone or methylene chloride, transfer the slices from their bath into the impregnation resin-mix.

Forced impregnation of body slices

Forced impregnation (replacement of the solvent with curable resin), is based on the difference of vapor pressure of the solvent and the resin and is carried out in flat-chambers inside the vacuum chamber.

Impregnation equipment

- Vacuum chamber with a transparent glass cover

- Vacuum pump (oil pump is preferred)
- Vacuum tubing and fine adjustment needle-valves
- Vacuum gauge
- Bennert mercury or digital manometer
- Flat chambers

Preparing flat (glass) casting chambers for forced impregnation of slices

Casting chambers are built for casting of the slices prior to impregnation. Flat chambers are constructed of two appropriate sized 5mm tempered glass plates, an appropriate length of 4mm flexible latex/silicone tubing and large fold-back clamps. A glass plate is placed on a glass support and tubing is placed around the perimeter of three sides of that plate. One end of the gasket is left longer to close the end of the glass chamber after impregnation is complete. The 2nd plate is placed on the tubing. Clamps are placed around 3 sides of the perimeter with clamp contact surface positioned over and parallel to the sandwiched gasket. The gasket end, which is left longer, will be used to close the chamber prior to curing. Once the casts are assembled, the impregnation resin-mixture is prepared.

Preparing the impregnation-mixture

The polyester resin impregnation-bath is made by thoroughly mixing: 1000ml Hoffen polyester P45 resin with 10g of P45A, 30ml P45B, and 5g of P45C. P45A and P45C are plasticizers and P45B is the hardener. The glass chambers are then partially filled with the impregnation bath-mix.

Immersion of dehydrated/degreased body slices into impregnation resin-mix After preparation of impregnation reaction-mixture, a dehydrated slice is removed from the acetone and placed in the chamber. Using a funnel, the chamber is then slowly filled with the impregnation-mixture. The filled chamber is placed upright in the room-temperature vacuum chamber for impregnation. Manually remove large bubbles trapped in the casting chamber and slice using a 1 mm stainless steel wire. Turn on the vacuum pump and allow it to become hot. Place the glass lid on the vacuum chamber and seal the chamber. As pressure is slowly lowered by closure of the by-pass valve, bubbles will form as the acetone vaporizes. Slow closure of the valve will assure slow bubble formation. Maintain slow bubbling by incremental closure of the by-pass valves as needed. The pressure should drop to 20 mm Hg over four or five hours. Similarly, the pressure is lowered incrementally and slowly through 10 mm Hg, 5 mm Hg and finally to near 0 mm Hg while maintaining slow bubble production. Pressure is maintained at 0 mm Hg until bubbling ceases. Duration of impregnation is eight+ hours depending on the volume of slices.

Table 1: General protocol for P45-polyester body slices (2-3mm)

Day 0 - Prepare and freeze specimen in anatomical position.
Day 2 - Slice, rinse, clean and cool slices.
Day 3 or X - Immerse in first cold (-25° C) acetone bath (100%).
Day 10 - Immerse in second cold (-15° C) acetone bath (100%).
Day 17 - Immerse in room temperature acetone bath (100%).
Day 24 or X - Build flat chambers; prepare P45 impregnation-mix; insert dehydrated /degreased slices into flat chamber and fill with resin-mix.
Day 25 - Insert filled flat-chambers into vacuum chamber; impregnate with P45-mix.
Day 26 - Remove flat-chambers after impregnation; inspect for bubbles and align slices.
Day 26 - Cure: Place in 40° C water bath.
Day 29 - Remove from water bath and cool.
Day 30 - Open flat chamber, cover slice with foil, saw and sand.

Heat curing of body slices

After impregnation is complete, the vacuum chamber is returned to atmospheric pressure and the flat chambers checked for trapped bubbles. Any remaining bubbles should be removed with the aid of a wire. Slice alignment is checked and corrected using the stainless steel wire. The top gasket is closed across the top of the flat chamber and clamped in place in preparation for curing (Table1).

Curing

Slices in their casting chambers are placed upright in a 40° C water bath for three days. A circulation pump is used to circulate warm (40° C) water around the chambers to maintain a constant 40° C temperature.

Completion

After 3 days of curing at 40° C, the sheets in their flat chambers are removed from the water bath and cooled to room temperature. Flat chambers are dismantled by removing the clamps, gasket and finally the glass. Avoid smearing uncured resin-mix onto the cured surface of the slice. The specimen is wrapped in light-weight foil to prevent uncured resin around the edges and debris from contaminating the surface of the slice over the specimen.

After curing, release and wrapping is complete, the excess cured and sticky resin around the perimeter is cut off with a band saw. The edges may be smoothed using a wood sander and new foil is placed on the slice which is now ready for use.

Results

The P45 sections are semi-transparent (Fig. 1), durable, and correlate well with radiographic, CT and MR images.

Discussion

The advantages of the P45 plastination techniques are as follows.

I. Save time

Placing the dehydrated slices directly into the open top flat chambers for impregnation is a potential time and mess saver. Also, heat curing in a water bath in the same chamber after closure of the top is unique, and a further time saver. As with the other polyester techniques, the impregnated slice is surrounded by polyester resin-mixture (P45) while it is curing. Hence, the plastinated slices are incorporated as a part of a single cured sheet of resin. They are not merely embedded in the resin, but a part of the resin sheet which makes the specimens in the slice durable. The slices also show good anatomical detail.

II. Less polyester resin is used

The main advantage of the P45 sheet plastination method is the decreased volume of resin used. As with P40, the impregnation resin is used as the casting resin. The process is not complicated and less equipment and time is needed.

III. Equipment is simple and quality control is easy

The P45 technique uses a heated water bath for curing. The curing process of polyester is an exothermic process. The released heat during the curing process should be removed quickly to prevent the temperature of the curing slice from going too high. Since water is a good conductor of heat, the temperature of slices during curing is readily controlled by the circulating water. In addition, a UV light curing apparatus, as well as a ventilator are not necessary in the P45 process.

With the equipment for P45 technique being simple and easy to use, this new polyester technique is easy for beginners to use and applicable for sectional anatomical research.

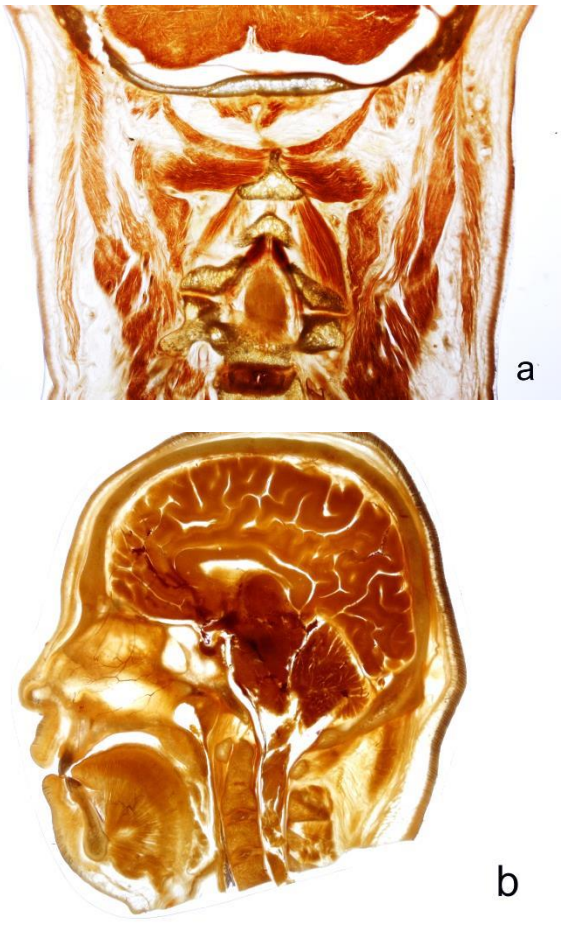

Figure 1: a) Coronal, b) sagittal head and neck sections, Hoffen P45 technique

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