IN THIS ISSUE:

Hoffen P45: A Modified Polyester Plastination Technique for Both Brain and Body Slices. – p 4

Coloring Muscles and Vessels Of Plastinated Limbs with Colored Silicone to Supplement Teaching. - p 9

Comparison of Cold and Room Temperature Silicone Plastination Techniques Using Tissue Core Samples and a Variety of Plastinates. – p 13

Non-Perishable Museum Specimens: Redefined Plastination Technique. – p 20

The 18th International Conference on Plastination Announcement– p 25
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Cover: Coronal head and neck sections, Hoffen P45 Technique in Hoffen P45: A Modified Polyester Plastination Technique for Both Brain and Body Slices, H. J. Sui and R.W. Henry
## Contents

Letter from the President, *Carlos. A. C. Baptista*  
2

Letter from the Editor, *Philip J. Adds*  
3

4

Coloring Muscles and Vessels of Plastinated Limbs with Colored Silicone to Supplement Teaching, *J. Kang, S. Iliff, R.W. Henry and D. Hermey*  
9

Comparison of Cold and Room Temperature Silicone Plastination Techniques Using Tissue Core Samples and a Variety of Plastinates, *D. Starchik, R.W. Henry*  
13

Non-Perishable Museum Specimens: Redefined Plastination Technique, *N. N. Singh, A. Chaudhary, S. Nair, S. Kumar*  
20

The 18th International Conference on Plastination Announcement  
25

Instructions for Authors  
26
LETTER FROM 
THE PRESIDENT

Dear Fellow Plastinators,

Next year (2016) will be the 30th anniversary of the International Society for Plastination (ISP). I invite you to join me in the celebration of this milestone at the 18th International Conference on Plastination, which will be held in Toledo, Ohio June 27 – July 1, 2016. The Conference was originally scheduled to be held in Pereira, Columbia, but due to unforeseen circumstances the venue has been changed to Toledo. A copy of the poster for the Conference can be found on page 25.

I would like to announce that after 8 years as president of the ISP, I do not intend to continue as such after July 2016. Elections for the new Council (the principal governing body of the ISP) will be held in May, with nominations accepted in mid-March, 2016. For the first time we will be electing 5 councilors in addition to the officers of president, vice-president, treasurer and secretary. This is according to the amended Bylaws that occurred in St. Petersburg, Russia, in 2014. Nominations for officers and councilors should be submitted to Anthony J Weinhaus, chairperson of the Nominations Committee. More information about the election process will be sent to all members in good standing as part of an email message early in 2016. I encourage you to become actively involved in the election process and exercise your right to vote.

We are still awaiting a notice from the National Library of Medicine (NLM) regarding the indexation of the Journal of Plastination in MEDLINE. I commend the efforts of PhilAdds, editor of the Journal, in submitting the application to the NLM and in the steps he is taking to make the Journal a first-rate publication.

In closing, I would like to say that I am somewhat surprised at the low number of responses I receive when sending out correspondence for the Society. It is in stark contrast to the number of members we currently have in the ISP. The health and growth of our society depends on an actively involved membership. I ask that you seriously consider becoming more involved by participating in interim meetings and conferences, and submitting articles for the Journal. I would also encourage you to nominate students to become members of the ISP. They are the future of our organization and we should involve them as early as possible in their professional development.

I look forward to seeing you in Toledo, Ohio this coming June.

Warmest Regards

Carlos A. C. Baptista
President
LETTER FROM
THE EDITOR

Dear Readers,

This morning, I typed “plastination” into PubMed. There were 195 records, including the subsets “plastination anatomy”, “sheet plastination” and “plastination technique”, with ten papers so far already published in 2016. Papers on plastination have been published in an astonishing one hundred and three different peer-reviewed journals, covering surgical anatomy, veterinary anatomy and anatomical education, as well as journals specialising in more arcane fields such as dysphagia, the voice, congenital anomalies, and experimental gerontology. Papers on plastination have been published in Polish, German, Russian, Japanese, Spanish, and Chinese, alongside the English majority. The most notable absentee from the list of 103 journals is, of course, The Journal of Plastination, which is not listed by the National Library of Medicine, and hence has no PubMed identifier. Last year I submitted an application to the Literature Selection Technical Review Committee (LSTRC) of the NLM, for the Journal of Plastination to be included in MEDLINE. Unfortunately the process is rather slow, so no decision has been reached at the time of writing. However, I am confident that we will be successful, which will be a notable way of marking the 30th anniversary of the publication of the first issue, in January 1987.

In this issue, you will find papers describing technical innovations in the field of plastination, including an ingenious approach by Chadhary et al. which describes a low-cost method of plastination using laboratory waste; a comparison of low-temperature and room-temperature techniques from Starchik and Henry; a modified polyester technique from China described by Sui and Henry; and an assessment of two different colouring techniques from Kang et al.

I have made the point before, that a journal can only be as good as the papers it publishes, and on behalf of the editorial board I would like to take this opportunity to thank all of you have submitted manuscripts for publication in the Journal of Plastination. We rely on you for the continuing success of your journal.

With best wishes,

Phil Adds, Editor, the Journal of Plastination
Hoffen P45: A Modified Polyester Plastination Technique for Both Brain and Body Slices

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.

| Day 0 | Prepare and freeze specimen in anatomical position. |
| Day 2 | Slice, rinse, clean and cool slices. |
| Day 3 or X | Immers in first cold (-25° C) acetone bath (100%). |
| Day 10 | Immers in second cold (-15° C) acetone bath (100%). |
| Day 17 | Immers 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-mixure (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
References


Product distribution:

Dalian Hoffen Bio-technique Co. Ltd, No. 36. Guangyuan Street, Lvshun Economic Developing Zone, Dalian, China, URL:http//www.hoffen.com.cn. Fax: (86)-411-3936-8715. E-mail: info@hoffen.com.cn.
TECHNICAL REPORT

Coloring Muscles and Vessels of Plastinated Limbs with Colored Silicone to Supplement Teaching

ABSTRACT:
Plastinated specimens, which have been painted or stained to highlight anatomical structures, can be helpful materials for medical students as well as others interested in studying anatomy. However, one problem with these specimens is that the paint often chips off due to being handled by many students and teachers. It may also wear off naturally if the specimen is kept for a long time. In this experiment, specimens were colored prior to the curing stage in an attempt to improve durability and better adherence of the color to the specimen. The results were then compared to techniques used by McCreary (2013). Colored specimens were challenged using methods which might be similar to routine handling during study. It was expected that the new coloring technique of applying the solution prior to the cross-linking stage instead of after the cross-linking stage would provide a stronger adhesion to the specimen. Although the color on the specimen applied prior to the cross-linking step demonstrated a more polished appearance compared to the color applied after the cross-linking stage, various durability tests confirmed no better efficacy or durability in adhesiveness of the silicone color to the specimen. Rather, some of the color came off when rubbed with fingers or latex-gloved hands.

KEY WORDS: S10 plastination; color; muscle, vessels

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Introduction
Coloring of plastinates can serve as a great learning tool for students who have difficulties distinguishing certain vascular structures and tissues. Various coloring techniques to highlight structures of the plastinated specimen have been developed by researchers, each with its own advantages (enhanced clarity of structures) and disadvantages (no method is student proof) (e.g. Marchese et al., 2008; McCreary et al., 2013; Raof et al., 2013). However, recent staining techniques used in our lab show long lasting durability (Concha et al., 2014). The goal of this research was to introduce the coloring technique at a different stage of the plastination process developed by von Hagens (1979a; 1979b, 1985) and implement various test methods to compare the durability of the coloration on a freshly-prepared lower limb specimen, and an upper limb prepared and described previously by McCreary et al. (2013) as well as the advantages and disadvantages of the new coloring procedure described here. In the upper limb specimen, color was applied after the cross-linking/curing stage of the plastination process (McCreary et al., 2013). In this modified protocol, however, color was applied to the muscles of the pelvis and lower limb (including the thigh, knee, and foot of the specimen) prior to cross-linking of the impregnation-mix (polymer-catalyst). In addition, the blue pigment, BiodurTM AC52 was used to color the veins of the leg specimen instead of the BiodurTM AC40 which was used in the previous research (McCreary et al., 2013) to color the upper extremity veins. Our expectation was that application of the color before curing/hardening the silicone polymer-mix in the specimen would result in a stronger adhesion of the paint to the specimen, and therefore result in better durability.

Materials and Methods

Specimen Preparation
The specimen for plastination was procured through the Lincoln Memorial University-DeBusk College of Osteopathic Medicine/Anatomical Donation Program. The left lower extremity of a human male was used. The fresh-frozen lower limb was harvested and immediately fixed by injecting 5% formalin solution into the tissue and submersion into a 5% formalin bath. The pelvis, femoral triangle, knee and foot were dissected. Dissection required about 100 hours. Due to the large size and thickness of the tissue, the specimen was stored in a 5% formalin bath for an extended period and maintained in this solution throughout the extended dissection in order to inhibit decay of the tissue during dissection. After
dissection was completed, the specimen was rinsed with tap water for 7 days to rid the specimen of formalin and all embalming chemicals. For plastination, the cold Biodur™ S10 plastination technique was used (deJong and Henry, 2007). In order to minimize tissue shrinkage, the specimen was dehydrated by submersion into cold -20°C acetone. Acetone baths were changed 4 times at weekly intervals. After 5 weeks of cold dehydration, the specimen and acetone bath were brought to room temperature for 3 weeks for lipid removal. Next, forced impregnation was carried out by placing the specimen into the silicone polymer/catalyst (S10/S3) mixture in the vacuum chamber at -15°C and gradually reducing the pressure. Impregnation time was five weeks.

Application of color to the specimen

Following forced impregnation, the coloring solution was applied to the surface of the muscles using a paint brush, to create a life-like, aesthetic appearance. Arteries and veins were colored to make the structures more distinguishable. The method of coloration described by McCreary (2013) was utilized for this experiment except that Biodur™ AC52 was used instead of Biodur™ AC40 to color the veins. The color solution for the muscles was made by mixing: 0.3 g of silicone (Akzo Nobel Clear All-Purpose Silicone Sealant, general residential caulking), 5 ml of MEK (methyl ethyl ketone), and 40 μL of Biodur™ AC51 (brown) dye paste. The solution mixture of 0.3 g of silicone, 5 ml of MEK, and 80 μL of Biodur™ AC50 (red) was mixed and applied to the arteries. The veins were colored with a solution containing 0.3 g of silicone, 5 ml of MEK, and 120 μL of Biodur™ AC52 (blue) dye paste.

Because of variation in muscle surface texture, color penetration into the surface of the muscle varied and repeated coats were required to achieve consistent coloration. In cross-sectioned muscles, the color solution was more easily absorbed, and fewer coats were required. In longitudinal muscles, however, several coats were necessary to gain color appreciation depending on size, thickness, and texture of the muscles. Similarly, the arteries and veins both required several coats to achieve the desired color.

Curing/Cross-linking

In the final step, the specimen was cured by using gas cure S6 at room temperature. Throughout the curing process, the specimen was manicured for polymer residue removal. Once the curing procedure was complete, the specimen was ready for results capture.

Durability and Testing

Color durability was investigated, taking into account the specimen’s expected handling for teaching and learning purposes. There are no known scales for rating the levels of abuse or handling of painted specimens. The tests were based on anticipated student and faculty handling mimicking a normal review period. Testing time was based on a normal specimen handling time by a student or professor during a class period.

Durability tests were carried out using the following four methods. The first involved using a latex-gloved hand and rubbing the surfaces of the colored structures of both proximal and distal portions of the specimens. In the second test, colored structures were rubbed with fingers.

For the third test and fourth tests, anatomy dissection instruments, specifically the blunt probe (third) and serrated forceps (fourth), were used to scratch both proximal and distal colored areas of the specimen. These methods engage or test the durability of the paint in real-life hands-on teaching scenarios.

Results

Application of color to the specimen

Different numbers of colored paint coats were necessary to achieve optimal color on the structures of each specimen. Two coats of color were applied to the cross-sectioned muscles of the specimen and 2 to 5 coats were applied to intact muscles. For the arteries, 5 coats of color solution were required while 4 to 6 coats were applied to the veins (Figs. 1-3).

Durability Testing

The first test (using a latex-gloved hand to rub the surfaces of the colored structures), resulted in a small amount of the brown paint peeling off when the proximal leg muscle was rubbed multiple times. The rest of the colored muscles, arteries and veins showed no peeling of the color, nor were stains observed on the gloved hands.
In the second test (rubbing the colored structures with fingers), as in the first test, a small amount of the brown color on the same muscle came off. Also, the blue color of the small veins on the foot started to peel off as well. The rest of the color on the muscle and vessels did not peel.

For the third and fourth tests (scraping with anatomy dissection instruments), the blunt probe (third) and serrated forceps (fourth), resulted in no peeling of the color on these regions.

Comparison with upper limb specimen

When tests 1 to 4 (as described above) were applied to the upper limb prepared by McCreary et al. (2013), no peeling of the paint resulted.

Discussion

This coloring technique demonstrated beautiful color appearance on the specimen (Fig. 4), however it did not meet our expectations for durability. The goal of this research was to develop better permeation and stability of the color to the specimen for long-term use in education. However, it was discovered that applying the color to the specimen prior to the cross-linking/curing stage resulted in no better durability when compared to the prior painting technique used by McCreary et al. (2013). For example, when the painted muscles and
vessels of McCreary et al.’s upper limb specimen (to which the original technique was applied) were rubbed with gloved hands, fingers, blunt probe and serrated forceps, no peeling of the paint resulted. Comparably, in this study, a small amount of the paint peeled off on the lower limb specimen.

Perhaps the different number of applied paint coats could have played a minor role in this unexpected result. For instance, while 2 to 5 coats to the muscles and 4-6 coats to the vessels of the leg specimen were applied in order to gain color appreciation, 2 coats of color on the muscles and 6 coats of the color solution were applied to vessels of the upper limb specimen (McCreary et al., 2013). The color tone was the same for both the lower and upper limb specimen except that the lower limb specimen appeared slightly more polished.

In the past, researchers from the University of Michigan Medical School have developed methods of coloration and plastination of specimens in order to produce stronger adhesion of the paint to the specimen (Marchese et al., 2008; Raoof et al., 2013). Their procedures involved applying the acrylic paint to the specimen prior to the curing process of plastination. As a result, these methods have been shown to improve the durability of the paint on specimens.

Although application of the paint to specimens prior to the curing stage demonstrated a greater durability of the paint in Marchese et al.'s (2008) experiment (Marchese et al., 2008; Raoof et al., 2013), it can be concluded from the research described here that the result could be influenced by a variety of factors. These factors include: use of different specimens containing different tissues with various textures, size, thickness, different number of applied color coats and different coloration material.

References


Comparison of Cold and Room Temperature Silicone Plastination Techniques Using Tissue Core Samples and a Variety of Plastinates

ABSTRACT:
A variety of organs, body regions and whole body specimens were plastinated using standard procedures for both cold and room temperature silicone plastination techniques. From these plastinates, advantages and shortcomings of both methods were evaluated. Criteria used for evaluation of plastinates included: duration of impregnation and curing, quality of plastinated specimens, need for extra equipment and its maintenance, as well as other cost considerations. To efficiently evaluate shrinkage and plastination duration, 3 cm pieces (core samples) of parenchymatous organs and 7 cm lengths from intestinal segments were collected, dehydrated and plastinated using standard procedures for both cold and room temperature silicone plastination techniques. Core sample volume was evaluated at the end of each stage of the process by fluid displacement. Shrinkage of samples was calculated after each stage of plastination. Evaluation of this information showed that the room temperature plastination technique takes about 35% less time for impregnation and curing, causes an average 8% less specimen shrinkage, produces life-like hair, fur or feathered specimens and it is more cost-efficient. The cold temperature plastination technique produces more flexible and elastic specimens and is preferable for whole body plastination.

KEY WORDS: cold technique; room temperature technique; shrinkage; silicone plastination

Introduction
The S10 plastination technique, using a cold impregnation reaction-mixture, was introduced by Gunther von Hagens in 1977 and now is the classic plastination technique which has been used worldwide (von Hagens, 1979a, 1979b, 1980, 1981, 1985; von Hagens et al., 1987; Bickley et al., 1981, 1987; Henry, 1987, 1995, 1998, 2007a; Weiglein and Henry, 1993; de Jong and Henry, 2007). The working material of this method is a silicone impregnation reaction-mixture, consisting of 99% polydimethylsiloxane with a relative molecular weight of 27200, plus 1% dibutyltindilaurate S3 as a catalyst (Chaynes and Mingotaud, 2004). This reaction-mixture becomes viscous when it is used or stored for several months at room temperature. To prolong the less viscous state needed for impregnation, impregnation is carried out in a deep-freezer; and when not in use, the polymer reaction-mixture is stored below -20°C.

In 1998, Daniel Corcoran, Dow Corning Corporation, proposed another sequence for combination of components for the silicone impregnation-mixture, and the room temperature plastination technique was developed (Glover et al., 1998; Henry et al., 2001; Latorre et al., 2001; Raoof, 2001; Glover, 2004; Henry, 2007b; Raoof et al., 2007). A non-reactive silicone of 92% low-molecular-weight polydimethylsiloxane and 5-8% of cross-linker CR-20 were used. This combination of silicone and cross-linker is stable at room temperature, therefore there is no need to impregnate specimens in a freezer. This is in contrast to the silicone-catalyst mixture, which is not stable at room temperature and needs to be used and stored in a deep freezer. Today about 80% of plastination laboratories in the world use the classical cold temperature technique; in spite of the ease of penetration of silicone and no need for additional equipment for the room temperature plastination technique, only 20% of all laboratories use this new plastination method (Starchik, 2014).

We assumed there were other quantitative and qualitative differences between cold and room temperature silicone plastination techniques besides polymer components and sequence of their combination. The purpose of this research was to compare...
methodologies and results of both standard methods and report the findings.

The following criteria were used to compare the two common silicone plastination techniques (cold vs room temperature) and two types of specimens (a. Normal plastinates [organs, regions of body and whole bodies] and b. Tissue core samples):

1. Time of impregnation and curing stages
2. Specimen shrinkage
3. External appearance, hardness, flexibility, elasticity, surface condition and final specimen performance characteristics after eight years of use
4. Technique process-dependent requirements and equipment maintenance
5. Polymer materials cost and consumption
6. Need for extra equipment and other technological costs

Specimens were collected and fixed in 10% formalin. To standardize experimental conditions two types of specimens were used:

a. Small cores (diameter 3 cm) from parenchymatous organs (liver, kidney, heart) and brain were extracted using a metal cylinder corer (Fig. 1). These cores of tissue were used to record shrinkage and plastination duration, and to ensure quantitative data would be collected and that samples could be measured. Also, 7 cm length segments of intestine were used as samples of hollow organs.

b. Also, parenchymatous and hollow organs, whole brains, parts of the body and whole body specimens, non-dissected fetuses (of similar size), fur-covered feet, fish and reptiles were plastinated. All the large specimens were used to compare qualitative parameters and check the other above-mentioned criteria during both cold and room temperature plastination techniques. Dehydration and degreasing were completed with acetone according to the standard method as described by de Jong and Henry (2007) for all the sets of experiments.

First set of experiments (cold temperature (CT) protocol)

Impregnation was carried out with silicone mixture consisting of 99% S10 (relative molecular weight of 23000 to 24000; content of hydroxyl groups 0.57 - 0.60 %; kinematic viscosity at 20°C 360 - 400 cSt), and 1% catalytic agent S3 (dibutyltindilaurate) at -20°C. Pressure in the vacuum chamber was lowered slowly from 200 to 3 mm Hg maintaining a moderate boil. After impregnation was completed and excess polymer-mix drained, the specimens were placed for curing into a gas curing chamber with cross-linker S6 (tetraethoxysilane) vaporized by an aquarium pump, for 10 - 12 hours (von Hagens, 1987).

Second set of experiments (room temperature (RT) protocol)

The impregnation mixture consisted of 95% low-molecular-weight silicone P-27 (relative molecular weight of 6400 to 6600; content of hydroxyl groups 0.7-0.8 %; kinematic viscosity at 20°C 50 - 60 cSt), with 5% content of a cross-linker agent P-27B (tetraethoxysilane). Impregnation was carried out at +20°C while lowering pressure in the vacuum chamber and maintaining a rapid boil. To prevent excess volatilization of the cross-linker (P-27B), pressure was not lowered below 30 mm Hg. Room temperature specimen curing was by spraying the surface with 30% Catalyst P-27A (dibutyltindilaurate).

The impregnation process was controlled by measuring pressure in the vacuum chamber with a pressure gauge and watching the bubbling intensity of the intermediate solvent on the silicone-mix surface. In addition, acetone vapors passing through the vacuum pump were cooled to -25°C and condensed in a cold reservoir. The quantity and density of the condensate were measured. In both experiments impregnation was judged complete after acetone collection had stopped.

Specimen shrinkage was measured by submerging the specimen cores and intestine segments in a graduated cylinder filled with the appropriate liquid (water, acetone or polymer) and recording the displaced liquid volume. The volume of the cylindrical cores and intestine segments was measured four times: after cutting, after dehydration and degreasing, after impregnation and after curing. Relative shrinkage was expressed as a percentage, and calculated according to the formula:

\[(\text{Initial volume} - \text{volume after plastination stage})/\text{initial volume} \times 100 = \% \text{ shrinkage}\]

Results

The duration of impregnation and curing for experimental tissue cores from both cold and room temperature experiments are shown in Table 1.
Comparison Room Temperature and Cold Silicone Plastination

<table>
<thead>
<tr>
<th></th>
<th>Cold Temperature</th>
<th>Room Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impregnation</td>
<td>54.5 ± 6.56</td>
<td>33.7 ± 4.31</td>
</tr>
<tr>
<td>Curing including:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Draining</td>
<td>38.7 ± 4.82</td>
<td>8.2 ± 2.48</td>
</tr>
<tr>
<td>Catalyzing</td>
<td>8.1 ± 2.79</td>
<td>24.7 ± 4.34</td>
</tr>
<tr>
<td>Sum</td>
<td>47.2 ± 5.24</td>
<td>32.4 ± 4.86</td>
</tr>
<tr>
<td>Total hours</td>
<td>101.7 ± 7.85</td>
<td>66.1 ± 6.89</td>
</tr>
</tbody>
</table>

Table 1. Average duration in hours of impregnation and curing stages for tissue cores (± SD; hours)

Cold temperature impregnation time of core samples averaged 54.5 ± 6.56 hours, whereas room temperature impregnation time was 1.62 times shorter (p < 0.05). Draining excess silicone after RT impregnation took 8.2 ± 2.48 hours only, which was 4.72 times faster than for CT (p < 0.05); but catalyzing (gas hardening/curing) in the CT was dramatically decreased in time, so the overall duration of the CT curing stage was 1.46 times shorter (p < 0.05) compared to the RT curing. However, the total duration of the two cold temperature plastination stages proved to be 1.54 times longer than that of room temperature plastination (p < 0.05).

Cold temperature impregnation of large plastinates was quite variable from 10 days to six weeks, whereas room temperature impregnation time was from 5 days to 4 weeks. Impregnation was judged complete when bubbles diminished and no acetone was collected in the cold reservoir from the cooled (-25°C) pump exhaust.

The silicone impregnation-mix for the RT technique, because of its low viscosity, drains very easily from impregnated hair, fur, and feathers, which is a large time-saver when using this method on hair, fur, or feathered specimens. In contrast, the polymer-mix for the CT technique drains much more slowly after removing the specimens from the impregnation bath, and stays in hair, feathers and fur. When this silicone started to cure it was difficult to remove, so animal and bird specimens with hair, fur or feather looked less attractive after the CT process.

The average shrinkage for different tissue samples in both plastination techniques is summarized in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Cold temperature shrinkage %</th>
<th>Room temperature shrinkage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>22.4 ± 3.97</td>
<td>15.5 ± 3.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>28.3 ± 5.68</td>
<td>19.6 ± 4.11</td>
</tr>
<tr>
<td>Heart</td>
<td>15.8 ± 4.52</td>
<td>12.7 ± 2.34</td>
</tr>
<tr>
<td>Brain</td>
<td>46.0 ± 8.19</td>
<td>28.5 ± 5.56</td>
</tr>
<tr>
<td>Intestine</td>
<td>17.1 ± 5.95</td>
<td>13.2 ± 3.27</td>
</tr>
<tr>
<td>Average</td>
<td>24.3 ± 2.42</td>
<td>16.2 ± 1.49</td>
</tr>
</tbody>
</table>

Table 2. Volume changes of various tissue cores from fixation to after curing in cold and room temperature plastination (± SD; %)

According to our data, at the end of curing, shrinkage was less in the experimental pieces plastinated via the room temperature protocol. The average difference in shrinkage calculated for all pieces plastinated by the RT technique (16.2 ± 1.49 %) was 1.5 times less, and statistically significantly different from that of the CT process (p < 0.05). Brain samples demonstrated maximal shrinkage (46.0 ± 8.19%) probably because they were defatted together with other tissue cores in room temperature acetone.

Sagittal head sections and non-dissected fetuses (same age and size), plastinated with both techniques, gave the opportunity to compare shrinkage visually. The cold plastinated head specimens had a larger subdural space when compared to room temperature plastinates (Fig. 1).

![Figure 1. Median sagittal view of brain stem in human head: A - cold temperature technique; B - room temperature technique.](image-url)
were harder and more fragile. The most elastic and flexible specimens were made using the CT protocol (first set of experiments) which were not exposed to the cross-linking agent vapors, but it took much longer for them to cure.

The room temperature technique silicone polymerized only on the outer surface of the specimen while it remained fluid inside after initial application of catalyst. This allowed small amounts of uncured silicone to ooze from the deeper layers, especially in whole-body specimens, for several weeks after curing. For that reason, repeated catalyst application was required to complete hardening, which lengthened the hardening stage for whole body specimens. This phenomenon was not always observed with smaller specimens (body parts or organs) and was not observed at all with the cold temperature technique. When the whole plastinated organs like liver and whole brain were cut after two years of curing, fluid (non-polymerized) silicone deep inside the plastinated specimens was observed with the RT protocol. All specimens from the CT protocol had only cured polymer inside, but the depth of silicone penetration for these specimens was visibly less than for specimens produced via the RT protocol.

Room temperature-impregnated fur and feather-covered specimens yielded life-like hair and feathers with minimal specimen manicuring, while cold-impregnated fur and feather specimens required many hours of manicuring to yield near life-like/unmatted specimens. Reptile and fish RT specimens yielded more natural-appearing specimens than specimens impregnated with the cold temperature products. Fungi/mushrooms seemed to have similar results when using either technique.

One of the properties of the cross-linking agent tetraethoxysilane, is hydrolyzation with water. As a result, the interaction between the cross-linking agent S6 and air moisture occasionally leads to white spots/precipitate on the surface of the cold temperature technique specimens (Fig. 2).

With the room temperature plastination technique, in specimens with more than 3% water content (which means inadequate dehydration), the cross-linking agent (P-27B) interacts with the water and forms small firm crystals on the specimen surface (Fig. 3).

We had to purchase an additional freezer for the vacuum chamber to impregnate specimens with the CT protocol and keep the impregnation silicone-mix in the freezer at
all times even when not impregnating. We noticed that when the CT silicone-mix had been stored at -25°C for more than 6 months it became more viscous and could no longer be used. In addition to that, we had to produce a special chamber equipped with a fan, an aquarium pump and desiccant for curing specimens with the CT protocol. There was no need for such equipment for curing according the room temperature protocol.

When silicone prices were compared in the Russian and USA markets, silicone compounds for the room temperature protocol were from 10 to 20% less than the products for cold temperature plastination.

Discussion

The same silicone chemicals are combined in different sequences for cold and room temperature plastination. Yet each combination requires a different methodology for impregnation and curing, as well as handling and storage of polymer, and each yields specimens with a difference in features and quality. Many of these findings correlate with previous study results (von Hagens et al., 1987; Henry et al., 2001; Smodlaka, 2005). Traditionally, silicone polymer used for the standard cold temperature technique (S10) contains silicone with a higher molecular weight, so it is more viscous. This CT silicone mixture penetrates into tissue depths relatively slowly, which, in turn, makes the impregnation stage longer. As a result, impregnation using the cold temperature protocol is 1.62 times longer than impregnation with the less viscous room temperature products.

Specimen shrinkage with the RT protocol was 1.5 times less than with CT, which is explained by less viscosity which results in higher penetration of the room temperature plastination-mixture with low-molecular-weight silanol (Henry, 2002b). It allows more polymer to impregnate the specimen, preserving its volume and shape. Higher penetration of low-molecular-weight silicone (room temperature) can be used for impregnating dense archaeological objects and longtime formalin-preserved specimens with good results (von Hagens et al., 1987).

One of the major advantages of the cold temperature plastination products is the ability to make more flexible and elastic specimens, because the cured silicone used with this technique has higher molecular weight and higher extension coefficient than the silicone used with the room temperature technique. Another advantage of the CT technique is the possibility to vary flexibility and elasticity of plastinated specimens during the curing stage by altering exposure time to S6. Specimens without exposure to hardening vapors of S6 are the most elastic and flexible. Plastinated specimens made with the room temperature techniques have harder and firmer surfaces. In the RT process, more flexible and elastic specimens can be produced by decreasing the percentage of the cross-linking agent (P-27B) in the silicone impregnation-mix. This means that we can control the flexibility of the final product. The multi-year observation of cold temperature plastinated specimens demonstrated a tendency towards surface deformation, especially in large skeletal muscle insertion.
sites (gluteal region, back and pectoral muscles). This phenomenon was observed mostly in gross whole-body specimens. It is likely that this happened because the cross-linking agent (S6) did not penetrate deep enough inside a large specimen and some of the silicone-catalyst mixture was left unhardened. Eventually, slow polymerization of the mixture results in stress within the tissue and slight long-term shrinkage of large specimen surfaces.

The silicone impregnation-mix for the CT protocol (first set of experiments) produces an endothermic reaction. This reaction, which lengthens polymer chains, is accelerated as temperature rises. This reaction is decelerated by lowering the temperature. Even when the CT impregnation-mix is stored in a freezer at -25°C, the reaction still proceeds slowly and the mix gradually becomes more viscous. When this partly-cured mixture is used for impregnation, it cannot penetrate as well as the more fluid room temperature mix. For good penetration of such viscous polymer-mix, impregnation time must be prolonged. For this reason, monthly checks of the cold temperature impregnation-mix is recommended with timely replacement of some of the viscous silicone-mixture to ensure penetration of polymer-mix. When the polymer is too thick, it will take much time and effort to remove the polymerized silicone-mixture from the vacuum chamber. Lining the chamber with a polyethylene film will help protect the inside of the chamber and facilitate removal of viscous polymer.

Another challenge with the room temperature technique is that the cross-linker (P-27B) vaporizes from the impregnation-mixture when the pressure nears zero. It may crystallize in the vacuum pump and impair its function. Therefore, frequent changing of the pump oil is recommended.

To conduct cold temperature plastination, an extra freezer is needed to house the vacuum chamber with the silicone reaction-mixture. In addition to that, a special chamber with an air/aquarium pump is helpful for vaporizing the S6 during the hardening process. These increase laboratory set-up expenditures, as well as increasing electricity usage and cost because of the extra freezer and the longer vacuum pump operating time during the impregnation stage. The cross-linking agent S6, which is used as a hardening component with the cold technique, can cause eye conjunctiva irritation. Therefore, it is advisable to allow recently cured specimens to air out.

Catalyst P-27A, used for room temperature curing, is an organo-tin compound with low toxicity. Respirators should be used when the specimen surface is sprayed with the catalyst, and airtight bags or wrapping specimens in foil should be used while RT specimens are curing (Henry, 2007b; Raoof et al., 2007). It is also necessary for the room being used for hardening to be equipped with an effective ventilation system, to ensure a proper exchange of room air with fresh air.

In summary, experiments reported here demonstrated that specimens plastinated with the cold temperature technique are more flexible and elastic, but this process takes longer and specimens tend to shrink more. This technique is preferable for plastination of hollow organs of the gastrointestinal tract, lungs and the whole body, especially when the goal to make specimens for exhibition. Room temperature plastination is more economical to set up the laboratory, and allows production of good quality specimens in a shorter period of time. Low-molecular-weight silicone, used initially for room temperature impregnation, improves penetrability and makes it possible to reduce specimen shrinkage and surface deformation. The RT protocol can be recommended for brain (for best brain specimens cold dehydration with no degreasing is recommended), parenchymatous organs, parts of the body, fetus, fur/hair/feather-covered specimens, and reptiles & fish. This technique is also preferable for plastination of formalin-fixed specimens, as well as for archaeological and fossil objects.

References


Non-Perishable Museum Specimens: Redefined Plastination Technique

Objective: The study was conducted to overcome specific limitations of formalin-preserved specimens at negligible cost.

Materials and Methods: The study was conducted on museum specimens collected from the Department of General Anatomy, Kothiwal Dental College. All the collected specimens were plastinated employing previously utilized laboratory consumables, such as disposable culture plates, and xylene, mixed together in a fixed proportion to form a homogeneous paste. After complete dehydration in alcohol, clearing was done in acetone followed by impregnation with reactive polymer under vacuum which was created with a modified suction apparatus. Curing of the specimen was done under direct sunlight.

Results: This technique has proved its advantages over formalin-preserved specimens as it has produced dry, life-like specimens. The only limitation of our technique was that it has showed marked shrinkage after curing.

Conclusion: We have devised a new and cost-effective method of plastination that involves no specialized equipment and prepares plastinated specimens that retain much of their natural features in a life-like manner.

KEY WORDS: formalin; heart; larynx; museum; specimen handling

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Introduction

Handling and managing gross specimens is an important part of the training in general and in maxillofacial anatomy and pathology. Unfortunately handling formalin-fixed specimens carries several disadvantages such as repulsive odor, loss of color vibrancy due to long-term fixation and difficulty in maintaining the 3D orientation of luminal and branching patterns of the specimens (Jain et al. 2014). The potential carcinogenicity of long-term exposure to formalin is also a considerable concern. Novel solutions have been explored over the years to overcome and eliminate these drawbacks.

A technique which carries inventive solutions for most of these difficulties is plastination. Dr. von Hagens in 1978 invented the technique of plastination for museum specimen preservation at the University of Heidelberg (Jain et al., 2014). Plastination is a word of Greek origin, taken from Plassein which has a literal meaning to shape or to form (Dudanakar et al., 2014). Due to its many advantages plastination has gained wide recognition all over the world, and the first paper describing this original method was published by Dr. von Hagens in 1979. Plastination is basically a combination of science, technological phenomenon and artistry along with cultural aspects of life and death. Plastinated specimens have several advantages over formalin-conserved ones in that the former are clean, dry, odorless, durable, non-toxic, non-infectious, do not exude damaging vapors, have finer esthetics, can act as educative tool in patient counselling, can be handled with bare hands and do not require any special storage conditions or care (Jain et al. 2014). According to the available literature, very few have tried this technique for oral specimens (Ravi & Bhat, 2011).

The technique of plastination, as described by von Hagens, and followed across the world, is quite effective and durable. However, it requires the use of specialized materials and equipment that make it unsuitably expensive. The challenge for us is to develop and adapt the technique in a way that is economically viable and suited for use on a routine basis. Here we describe a novel way to implement a plastination method that was developed keeping these requirements in mind. We
have tried to use locally-sourced, cheap and easily available materials, and, using only the routine lab armamentarium along with laboratory waste reagents, we have developed a practice for plastination that may be adopted easily in museums and labs across the country.

**Materials and Methods**

The anatomical specimens taken for plastination were those preserved in the anatomy museum of Kothiwal Dental College and Research Centre. These were being stored in 10% formaldehyde as study models including specimens of larynx, kidney, fetal stomach and fetal heart.

The materials to perform plastination in our laboratory were drawn from previously used xylene, disposable sterilized culture plates, acetone, alcohol, suction machine, eosin stain and hydrogen peroxide. The detailed list of materials is discussed in table 2.

Routine plastination methods were implemented utilizing the following procedures.

**Fixation**

Specimens utilized for plastination were prefixed in 10% formalin solution for several years for the purpose of teaching of anatomy. In the case of fresh specimens, fixation time depended on the size and nature of specimen. However, a standard 48-hour fixation time was followed for small specimens.

**Staining & Bleaching**

Stored specimens were discolored and brownish in appearance due to the long duration of fixation. A staining and bleaching step was introduced into the methodology to remove this pigmentation and achieve a more life-like appearance for the finished plastinated specimen.

The fixed specimens were first immersed in routinely used eosin-Y dye (10% in 80% ethanol) and 0.5% glacial acetic acid (1gm) in to it to sharpen the stain. Thereafter, the specimen was dipped twice into hydrogen peroxide (50%) for bleaching and removing the excess stain. This procedure improved color differentiation of the specimen.

**Processing**

Processing of specimens for plastination was performed under two steps that included dehydration and clearing. All the procedures were performed at room temperature (37°C). The specimens were immersed in 70% alcohol for 24 hours and 100% alcohol for next 48 hours with one change in alcohol (24 hours). Anhydrous copper sulfate was used as an indicator to ensure complete dehydration that was considered complete when colorless. For clearing, specimens were immersed in acetone (4-5 hours) depending on the size of the specimen.

**Formation of reactive polymer**

Reactive polymer for impregnation was formed using disposable culture plates and used filtered xylene. Autoclaved used culture plates (200gms) were dissolved into Xylene (1000ml) under adequate ventilation. Once the plates were fully dissolved, it formed a homogenous viscous fluid that was used as the polymer solution for impregnation.

**Impregnation**

Forced impregnation of polymer into the specimen was performed under vacuum (25-30mmHg). A clinical suction apparatus was modified to create the vacuum. The vacuum was created in a single jar of the suction apparatus by blocking its second jar, which generally helps to neutralize the excessive vacuum in the container. A safety valve incorporated in the design ensured that any pressure in excess of 30mmHg was released, thereby reducing the risk of any explosion. During the impregnation period, pressure in the vacuum chamber was consistently monitored using the meter indicator of the suction apparatus. Specimens were submerged in the polymer bath for 3-5 days depending on the size of the specimen. The end-point of impregnation was considered as the stage at which air bubbles were no longer seen escaping from the surface of the specimen. This indicated that the specimen was now saturated with the polymer solution.

**Curing**

Once specimen were completely impregnated with the polymer, they were taken out of the polymer bath, leaving the residual polymer in the jar, and allowed to stand for few hours at room temperature so that excess polymer drained from the surface of specimen. The
curing was performed under direct sunlight by placing the specimen in a transparent glass jar.

In previous trials with the polymer, we had found that it could be cured by exposure to direct sunlight, presumably by the UV present, and this method was used. The end point of curing was determined as the stage at which the specimen hardened and no residual xylene was detected in the specimen, typically after 2-3 days.

Results

Plastination produced odorless, durable, non-hazardous, easy-to-handle, formalin-free and life-like specimens that could be handled without personal protective equipment (Figs. 1-4). Eosin dye imparted a crisp and well-differentiated color to the different structures in the specimens. The structural integrity and anatomical details of the specimens were well preserved. On the other hand, a small amount of shrinkage was also observed in all the specimens.

Discussion

Plastinations are permanent, non-hazardous, durable, dry museum specimens, which are excellent tools for teaching the anatomy and morphological characteristics of various specimens. The use and interest in plastination has steadily increased since it was first developed. However, the degree to which it may impact the developing countries appears to depend on cost-effectiveness and practicability of execution as well as training of human resources. In the past, it cost approximately $15,152 to setup a laboratory for plastination (Torre et al., 2004).

The general belief is that production of plastinations including the cost of equipment is an expensive proposition and hence is not an economically viable option, particularly for small to medium scale labs and institutions. However, our method of plastination warrants a thorough rethink of this mindset. We have innovated a method using regularly-used laboratory equipment that includes a suction machine with suitable modifications, and laboratory wastes. With minimum expenditure in terms of materials and armamentarium, we have been able to create plastinations of the laryngeal complex, adult human kidney, fetal heart and fetal stomach. Our method is particularly useful and effective in the case of small to medium-sized specimens, particularly those routinely encountered in an oral pathology department. Our method of plastination has opened an era of great opportunities for oral pathologists to develop specimen libraries. Although this technique is not new to the field of oral pathology,
the reduced cost of polymer, insignificant cost of laboratory setup and impregnation unit gives exclusivity to our study.

However, our study and the method has limitations. For one, only specimens small enough to fit into the jar of the suction machine can be plastinated this way. It was also difficult to maintain adequate vacuum for extended periods in the modified suction machine. There was also the risk of explosion of the suction unit if the pressure inside exceeded beyond the safe level (30mmHg). Shrinkage of specimens noticed during impregnation and curing was also a drawback. In spite of these limitations, this technique was very advantageous in many ways including its cost-effectiveness and the fact that additional lab setup and impregnation unit were not required.

Utilizing long-term fixed specimens for plastination, resulted in good preservation of specimens for observation-based teaching. The structural integrity of the specimens remained sound and aesthetically agreeable. The preservation of anatomical specimens that retain much of their natural features has been a long-standing goal of pathologists in India. With this technique we can reduce the cost of plastination by 90%. The expenditure in our method was limited to the cost of acetone, eosin dye and hydrogen peroxide that is significantly less than the usual cost. We recommend further studies on larger number of specimens to explore other possible innovations in reactive polymer and impregnation units at lower temperature to reduce shrinkage.

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