A New Method of Brain Plastination

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ABSTRACT

Introduction: Plastination is a unique technique for preservation of biological specimens used for teaching purposes. The protocol of flexible sheet plastination includes fixation, slicing, dehydration, force impregnation, casting, and curing (Hajian, Rabiei, Fatollahpour & Esfandiary, 2008). The procedure is done by using P87 flexible unsaturated polyester resin and provides heavy, gross, fragile, and bubbling plastinated sheets.

In this study, synthetic resin (P89) in the plastination laboratory at Isfahan Medical School is utilized with a new method for plastination of 3-mm human's brain slices without casting stage. Also, common plastination method with the use of P87 flexible resin was used and the products were evaluated and compared in the laboratory with new method products. This method is also compared with specimens made by P35 resin sandwich method.

Methods: This study was carried out on 3 human brains. Initially, according to the conventional methods, the brains were fixed in 10% formaldehyde, cut sagitally, coronally, and horizontally into 3-mm thickness slices by meat slicer, and then dehydrated in cold acetone (-25°C) and immersed in P89 unsaturated polyester resin at 25°C. Finally, the specimens were taken out from vacuum chamber and exposed to room temperature. When both surfaces of specimens became dry, they were taken to P89 polyester resin pail again. We repeated this stage 10 times.

Results: P35 specimens had high tensile strength compared to P89 specimens. Also P89 specimens had high bending capability compared to P35 specimens made by sandwich method. Likewise, P89 specimens were lighter compared to P35 specimens. In the naked survey of specimens, P35 specimens with white spot in the tissue indicate discoloration for plastination of brain specimens without casting stage.

Conclusion: Our study showed that P89 technique is a cheap, quick, and less expensive method for producing sheet plastinated specimens which are suitable in teaching neuroanatomy.

Key Words:
Plastination, Brain, Polyester resin

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1. Introduction

Plastination is a new method that stops anatomical specimens decay. This method preserves biological specimens by replacing the tissue water and lipid with curable polymers. Plastination was invented in 1946 by Romaniak who used unpolymerized resin. Later, it was modified by Von Hagense in 1987 who applied polymerizing resins for replacement of intermediary solvent [1-5].

The standard protocol of flexible sheet plastination includes fixation, slicing, dehydration, force impregnation, casting, and curing (Hajian, Rabiei, Fatollahpour & Esfandiary, 2008). The procedure is done by using P87 unsaturated polyester resin to provide heavy, gross, fragile, and bubbling plastinated sheets [6, 7].

Through this project, we omitted the casting stage and saved polyester resin. Then, we compared fragility, weight, and bubbling of the new sheets with those of sheets provided by the standard method. In this study, synthetic resin (P89) in the plastination laboratory at Isfahan medical school was utilized with a new method for plastination of 4-mm human's brain slices without casting stage. In the same way, common plastination method with the use of P87 flexible resin which were previously made and evaluated in the laboratory was compared with new specimens for better evaluation of new method. This method was compared with the specimens made by P35 resin sandwich method too.

2. Materials and Methods

This study was carried out on 3 human brains of middle-aged men who had donated their bodies to medical school. Two fresh brains were immersed in the fixative materials with 10% neutral buffer formalin, while suspended by the basilar artery over a period of 12 weeks [8-10]. Along this time, we changed the fixative fluid with fresh formalin every week to keep fixed the concentration of the fixative fluid.

Next, the brains were sliced by knife into several parts for better fixation, after 10 days of fixation period. The third brain has been previously fixed in 10% neutral buffer formalin, 2 years ago [11]. After fixation, brains were submerged in tap water for 2 hours to remove fixative materials. The brains were then prepared for slicing [11]. They were carefully cut sagitally, coronally, and horizontally into 3-mm thickness slices by meat slicer (Barnet and Burland, 2005). The slices were put in stainless steel grid [9-10].

The stainless steel grids containing brain slices were first placed into cold acetone bath (-25°C, not less than 99% purity) for one week (Barnett, 1997). In other words, tissue fluid was replaced with acetone as an intermediate solvent. During the first week, the concentration of acetone was diluted to almost 92%, measured by acetonometer. Then, the stainless steel grids with specimens were put into a new 99% acetone bath, every week for 1 month. The last bath of acetone showed no less than 99% pure acetones. This stage was necessary to avoid white spots in finished slices [9-10].

Next stage was forced impregnation. The specimens on the stainless steel grids were put in vacuum chamber, while immersed in P89 unsaturated polyester resin at 37°C for 7 days. The pressure of vacuum chamber decreased gradually from 760 mmHg to 5 mmHg in 7 days. The slices remained in 5 mmHg atmosphere for 2 days. In this stage, acetone evaporated and was replaced with P89 polyester resin. Impregnation was checked by watching the bubble formation on the surface of the polymer. When forced impregnation finished, no more bubble formation was observed [12-15].

The next step would be curing. The specimens were taken out from vacuum chamber and exposed to room temperature. When both surfaces of specimens became dry, they were taken to P89 polyester resin pail again. We repeated this stage 10 times. Finally, when slices were almost dried, we put them between 2 glass plates of 20×20 cm which were banded together with one attached clamp on each side. Through this technique in this stage, air bubbles and shrinkage in specimens were removed with the glass pressure. Curing was carried out at 37°C and completed for 4 days. After this time, the glass plates and clamps were removed and flexible and light plastinated sheets of human brain were provided [12-15].

P87 Method

This method was done by our new P87 resin and according to the conventional (P35) plastination method, which includes fixation, cutting dehydration, impregnation and casting is done.

P35 Sandwich Method

In this method, the brain's specimens were plastinated with P35 resin without casting by sandwich method to be compared with our new P89 method. At first, specimens

were fixed by formalin solution 10% and cut with the 4-mm thickness and then dehydrated in the cold acetone. In the next step, samples are placed in resin P35 in the vacuum chamber at -25°C. Then, 1% (w/w) of MEKP was added to resin bathroom and specimens was impregnated. The specimens were placed in the polyester sheet and the adequate amount of resin that include initiator and accelerator is poured over specimens and the second sheet is put over it. After bubbling, sheets that include specimen were placed between 2 glasses with suitable size in order to be cured. Finally, specimens were come out from sheet and were polished.

Mechanical Tests and Weight Test

To compare our new plastination method (P89) with that of the conventional methods (P35 and P87) for plastinated specimens, we used tensile test (to show strength of specimens), bending test (to show flexibility of specimens), and weight test (to show difference between P89 with that of the conventional methods P35 and P87 weight). Three groups (n=6) of plastinated specimens consisted of group 1: brain plastinated with P89 resin, group 2: brain plastinated with P87 resin, and group 3: brain plastinated with P35 resin. For weight test, 18 slices (n=6 for each group) were weighed after plastination. For mechanical tests, 18 slices cutting in the same block in size 5×1×1cm were used (n=6 for each group). In tensile test, for comparison of samples strength, we determined maximum force to break the specimen in constant speed (V=5mm/min) by tensometer. In bending test, for comparison of samples flexibility, we determined maximum deflection (mm) of the specimen in constant force of 31.00 N by instron [7, 13, 15]. The results are shown in Tables 1 and 2 and Figures 1 and 2.

Statistical Analysis

Mann-Whitney test was used to compare the result of tensile test, bending test, and weight test among the 3 groups (P89), (P87), (P35) of plastinated specimens. Differences with $P<0.05$ were interpreted as significant.

3. Results

The weight of plastinated sheets with P89 was about one tenth of the weight of plastinated sheets with P87. The mean weight of flexible sheets by P89 method was 12.50 g, while in standard method it was 107.17 g (Figure 1, and Table 1).

The difference of mean weight between the 2 methods of plastination was significant ($P\leq0.05$). Some degree of gross fragility was observed in standard method, while it was not seen in P89 flexible sheets. The mean flexibility of P89 was 74.17 N, while in standard method it was 73.17 N (Figure 2 and Table 2), which showed no significant difference ($P>0.05$).

Detailed anatomical structure of the brain such as brush appearance of corona radiate was seen in specimens provided by both methods (Figures 4-6). Also, the color of specimens in both methods was similar. The mean thickness of P89 flexible slices was almost 3 mm, while in standard method, it was about 5 mm because of casting stage (Figures 4-6). P89 plastinated slices showed excellent differentiation between white and gray matter of the

Table 1. Mean and standard deviation of bending test (F=31.00 N), tensile test (V=5mm/min), and weight test for 3 groups (n=6) of plastinated specimens.

Deflection (mm)		Force (kN)	Weight (g)
P8989	2.7168±0.3	73±2.28	1.18±0.09
P87	6.1049±0.24	114.8±16.4	3±0.17
P3535	2.2125±0.13	131.17±21.2	2.2±0.13

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Tables 2. Mann-Whitney test results (P-value) for comparison of 3 groups of plastinated specimens with regard to bending test, tensile test, and weight test.

	P89 & P87	P89 & P35
Weight (P-value)	0.001	0.001
Force (P-value)	0.001	0.001
Deflection (P-value)	0.001	0.004

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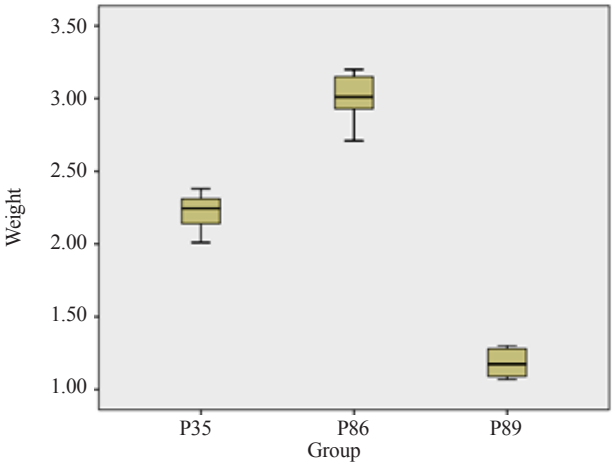

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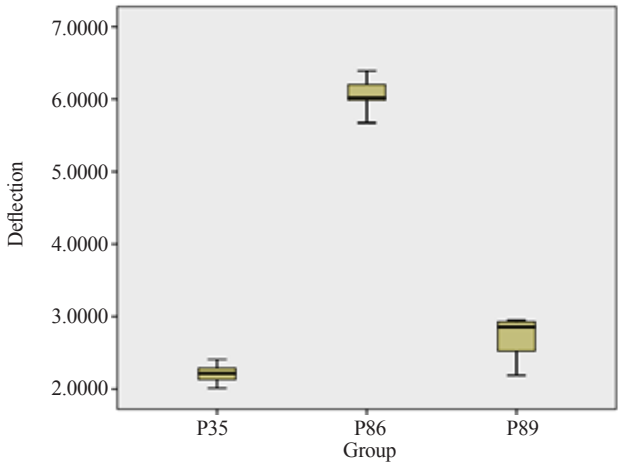
Figures 1. Comparison of the weight of plastinated brain sections produced by P35, P87 and P89 techniques.



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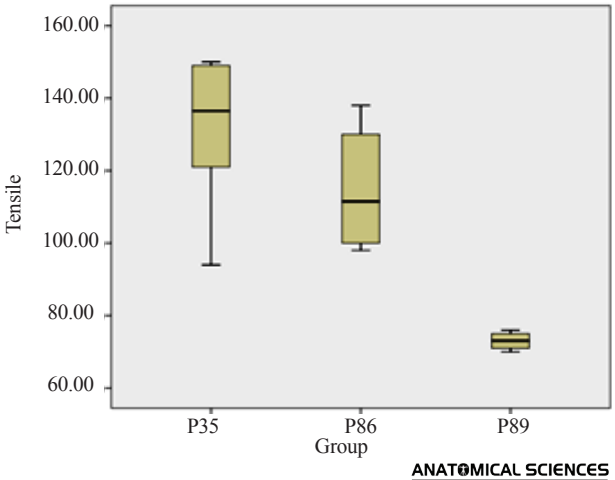
Figures 2. Comparison of the deflection of plastinated brain sections produced by P35, P87 and P89 techniques.



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Figures 3. Comparison of the tensile of plastinated brain sections produced by P35, P87 and P89.

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Figure 4. Plastinated brain section produced by P87 flexible polyester resin through the standard method, Department of Anatomical Science, Isfahan, Iran.

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Figure 5. Plastinated brain section produced by P89 flexible polyester resin with omitted casting stage, Department of Anatomical Science, Isfahan, Iran.

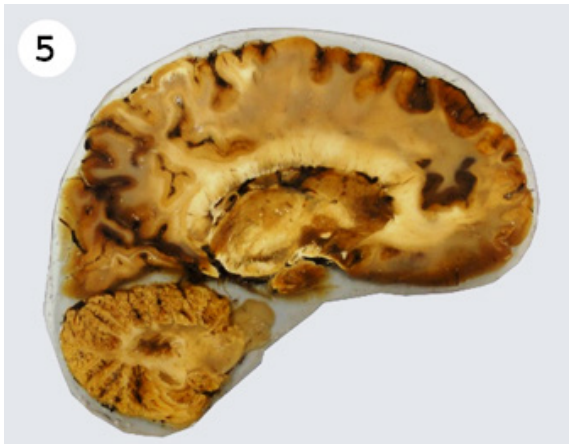

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Figure 6. Plastinated brain section produced by P35 resin through the standard sandwich method.

brain fixed for 2 years, while the fresh brains which were fixed for 12 weeks showed less differentiation.

4. Discussion

In 1987, Von Hagens et al. plastinated brain sections with 1.0-2.0 cm thickness by S-10 technique for preparation of half and whole brains plastinated specimens, which were used for neuroanatomy courses [5]. In 1992, Sheet plastination of the brain introduced by Weber and Henry produced more detailed, durable, and easier to handle specimens with 4 mm thickness by P35 technique [8]. P40 plastinated brain slices had high contrast between white and grey matter that were resulted from fresh brain removed from post-mortem cadavers in thin sections of 4, 6, or 8 mm [10]. In 1996, Weiglein compared S-10 and P35 techniques and produced very thin (1 mm or less) brain slice with band saw [16]. Barnet prepared coronal and horizontal brain slices using P40 technique, and compared them with those of P35 techniques [9]. In 2005, Barnet et al. used P35 technique for brain plastination taken from embalmed cadavers [9]. In previous techniques, casting stage was carried out after vacuum impregnation.

Our plastinated specimens were odorless and suitable for handling. Their examination could be done without the burden of gloves. They were clean, non-fragile, non-toxic, odorless, not sticky, light, and dry to touch. All of them maintained their original shapes and natural looks and were easy to carry in briefcase. In our study, casting stage was omitted and therefore, our ultimate product was light and easy to be used for teaching purposes [17, 18]. The comparison of the results of Mann-Whitney test (Table 2) for deflection, force, and weight indicated that there was significant difference among 3 groups (P35, P87, P89) of plastinated brain. With regard to obtained results from tensile test, bending test and weight test, it is observed that P35 specimens had high tensile strength compared to P89 specimens. P89 specimens had high bending capability compared to P35 by with sandwich method and in the same way, P89 specimens were lighter compared to P35 method. With the apparent survey of specimens, P35 with white spot in the tissue indicates discoloration for plastination of brain specimens without casting stage (Figures 1-3).

Our new technique has many advantages. First, the weights of plastinated specimens without casting were one tenth of casting plastinated sheets. Second, thicknesses of P89 flexible slices were much less than P87 flexible slices. Third, in our plastinated specimens we did not see any gross fragility and bubbling in comparison to the casting method specimens. Fourth, this method is more cost-benefit compared to casting method. Fifth, lack of casting problems such as spending long time and expenses. The only disadvantage of this method is the less firmness of P89 flexible sheets compared to casting technique [17, 18].

In conclusion, our study showed that P89 technique is a cheap, quick, and less expensive method for producing plastinated sheets, which are suitable for teaching purposes.

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Conflict of Interest

The authors of this study declared no conflict of interests.

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