Original Article

Plastination - A method for preservation of oral hard and soft tissue biopsy specimen v/s the conventional method of preservation with formalin

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Abstract

Context: Plastination is one of the most advanced method for preserving perishable biological specimen as well as tissue samples as for a longer period of time using polymers.

Aims: To evaluate the changes in dimension of hard and soft tissue specimens after plastination procedure and compare it with the conventional method of preservation in formalin.

Settings and Design: Institution-based retrospective study.

Methods and Material: The study included 20 formalin-fixed soft tissue and 10 formalin-fixed hard tissue specimens. All the specimens were plastinated which involved four basic steps of fixation, dehydration and defatting, impregnation with polymer and curing of polymer followed by finishing and storage. The specimens were analysed for shrinkage and dimensional changes and changes in colour and consistency between formalin-fixed specimen and plastinated soft tissue and hard tissue specimen.

Statistical Analysis Used: Descriptive statistics were used.

Results: After plastination, soft tissues showed average shrinkage of 3.49%with a range of 0.80–7.90% in comparison to the original size. In case of teeth and hard tissue specimen, there was no evidence of dimensional changes or shrinkage before and after plastination. Changes in colour and consistency of the soft tissue specimens were also noted before plastination and after plastination.

Conclusions: Although the plastinated specimens in the current study showed minimal shrinkage rate, they have proved to be an excellent alternative to formalin-fixed specimens as they are easy to handle and maintain, non-infectious, non-toxic, user-friendly.

Keywords: Cysts, hard tissue, plastination, soft tissue, teeth

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INTRODUCTION

Plastination is one of the most advanced methods for preserving perishable biological specimens as well as tissue samples for a longer period of time using polymers, which was introduced by Gounther Von Hagens, a German Professor in 1977.[1] This technique involves the removal of interstitial water and lipid from the tissues and their replacement by forced impregnation with curable polymers like silicone, epoxy or polyester resins with vast applications in medical fields of study. Although the technique rapidly expanded to many allied areas: human anatomy, biology, pathology, embryology and clinical medicine, as well as art. Its application in the field of Oral Pathology has received little attention even after three decades of its invention.[2,3]

The study of gross oral specimens is an integral part in learning oral pathology. However, the use of formalin for fixation makes their storage and handling difficult possibly because of concerns about carcinogenic potential of formaldehyde requiring the person dealing with the tissue to wear gloves to protect themselves from the fixative. Also, it makes the tissue wet and slippery. Hence, an innovative and alternative approach called 'plastination of oral hard and soft tissue biopsy specimen using locally available resin polymers' was studied which would produce life-like, dry and odourless specimens which are durable, non-hazardous and do not require any maintenance.[3] In addition, it can also be used for both light microscopy and ultrastructural studies following de-plastination, thus, also allowing to carry out a retrospective epidemiological study.

With this rationale, the objectives of the present study were to do long-term preservation of hard and soft biopsy tissues in a life-like state for teaching, to evaluate dimensional changes in both hard and soft tissue specimens after each step of plastination procedure along with the conventional preservative method in formalin, to provide a new three-dimensional perspective of oral biopsy tissue and its variations and to demonstrate that it is safer, more economical and also ensures the safety of the workers who are handling the specimens.

SUBJECTS AND METHODS

Oral specimen to be plastinated was taken from the archives of the Department of Oral and Maxillofacial Pathology and Oral Microbiology, A. B. Shetty Memorial Institute of Dental Sciences, Mangalore. Ethical Clearance for the study was obtained from the Central Ethics Committee, Nitte (Deemed to be University) (NU/CEC/DHR-07/2015 dated 07.01.2015).

Inclusion criteria

- Stored specimen both hard and soft tissue of the head and neck region (normal and pathologic)
- Cysts, tumours and bone lesions
- Old specimens stored in formalin for up to 10 years in a museum

Exclusion criteria

- Soft/hard tissues which are very small and in multiple bits.
- Tissues which are totally distorted.
- Fat tissues

The study included 30 formalin-fixed tissue specimens, which were broadly divided into two groups:

1. **Group I**: Comprised of 20 formalin-fixed soft tissue specimens like tumours of the jaw, cysts and periapical pathology.
2. **Group II**: Comprised of 10 formalin-fixed hard tissue specimens, which included normal teeth, tooth with developmental defects and anomalies, calculi, bone tissue of maxilla and mandible, etc.

Method of processing of teeth, soft and hard tissue specimen for plastination

The whole process of plastination involved four basic steps 1. Fixation, 2. Dehydration and defatting, 3. Impregnation with polymer and 4. Curing of polymer followed by finishing and storage.

Firstly, the specimens were irrigated with a mild detergent solution and were washed with dilute hydrogen peroxide to clean dirt, blood, etc.

Fixation

The first step was fixation of the specimen in 10 times the volume of 10% formalin for 48–72 hrs in case of fresh specimens. This procedure was skipped in case of old specimens fixed in 10% formalin for several years. The formalin was washed in running tap water for 12–24 hrs. Specimens that turned brownish in colour/discoloured due to the increased duration of fixation and storage, staining and bleaching were done to remove the pigmentation and to give a more life-like appearance for the specimen. Staining was done by first immersing in eosin-Y dye (10% in 80% ethanol) and 0.5% glacial acetic acid (1gm) to sharpen the stain and then for bleaching and excess stain removal, the specimen was dipped twice into 50% hydrogen peroxide.[2,4]

Dehydration and defatting

After fixation, the soft tissue specimens were taken for the dehydration and defatting process. The specimens

were transferred to a jar containing at least 10 times the volume of 60% alcohol. Thereafter, the specimens were transferred to 70%, 80%, 90% and absolute alcohol at intervals of a few days to few weeks, depending upon the size of the specimens.

For clearing, specimens were immersed in acetone. The volume of acetone used for dehydration was about 10 times the volume of the specimens.[5] The specimens were passed through three changes of acetone for 3 weeks or until the acetone did not turn yellow, which indicated total removal of fat. Some bigger specimens also required deep freezing with acetone at -25°C. Dehydration and clearing steps were not recommended for the plastination of teeth specimens, they were directly transferred to the reactive polymer.[2,4,6]

Next important step was plastination which involved preparation of embalming paste from polyethylene polymer and xylene. The mix is continually stirred with the glass rod under adequate ventilation. Once the polymer was fully dissolved, it formed a homogenous viscous fluid/ embalming paste [Figure 1a and 1b].

Impregnation

Direct impregnation - Smaller soft tissue and bone tissue, teeth were directly immersed in embalming paste and kept for two–four weeks. Specimens were stirred with glass rods to free any trapped air.

Forced impregnation - Forced impregnation of polymer into the specimen was done for larger tissues under vacuum (25–30mmHg). A specially designed vacuum chamber was made from a desiccator which was modified and a vacuum pump was attached to create the vacuum with a safety valve incorporated in the design to release pressure in excess of 30mmHg thereby reducing any risk of explosion. Specimens were submerged in the polymer bath in a specially prepared rectangular glass container and these containers were placed inside the desiccator under vacuum [Figure 1c and 1d]. The impregnation was done till the air bubbles stop escaping from the specimen surface, an indication that the specimen was saturated with the polymer.

Curing

Following the polymer impregnation, the specimens were removed from the polymer bath, and allowed to stand for a few hours at room temperature to drain the excess polymer from the surface of specimen. The specimens were then placed on an overhead projector (OHP) sheet at room temperature for curing under indirect sunlight until it becomes non-sticky and dry. The specimens were mounted on a base and oriented. Colouring of the specimen was done to highlight specific parts. Ultimately, this procedure resulted in non-toxic, dry, maintenance-free, durable, inexpensive, natural-looking specimens [Figure 2].

Figure 1: Images showing the steps of plastination. Polyethylene polymer mixed with xylene for preparation of embalming paste (a) Hard tissue specimen kept immersed in embalming paste (b) Soft tissue specimens inside a specially prepared rectangular glass container along with a modified desiccator and a vacuum pump for forced impregnation (c and d)

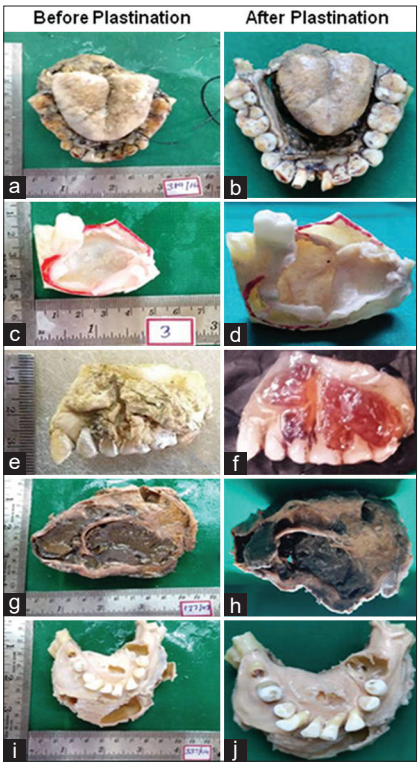

Figure 2: Soft and hard oral specimens before plastination and after plastination process. Mandible with tongue and hard tissues (a, b) Dentigerous cystic lining and cavity (c, d) Part of maxilla with hard and soft tissue (e, f) Solid multicystic ameloblastoma (g, h) Ameloblastoma in the lower anterior region (i, j)

Measurement of soft tissue and hard tissue was taken at two stages: a) after fixation with formalin and b) after plastination process to check for shrinkage.

Analysis of the plastinated specimen

The following parameters were analysed on the plastinated specimen:

1. Shrinkage and dimensional changes between formalin-fixed specimen and plastinated soft tissue and hard tissue specimen.
2. Changes in colour and consistency of formalin-fixed specimen and plastinated soft tissue and hard tissue specimen.

RESULTS

Plastination produced life-like specimens that were easy-to-handle without personal protective equipment and durable. The specimens were formalin-free and hence non-hazardous and odourless. After plastination, soft tissues showed average shrinkage of 3.49%with the range of 0.80–7.90% in comparison to the original size [Table 1]. In case of teeth and hard tissue specimens, there was no evidence of dimensional changes or shrinkage before and after plastination [Table 2]. Changes in colour and consistency of the soft tissue specimens were also noted before plastination and after plastination [Table 3]. Formalin-fixed soft tissue was appearing dull and light and was soft in consistency whereas plastinated specimen had a bright and glossy look and was firm and slightly flexible in consistency. Formalin-fixed hard tissue specimen appeared dull and was yellowish brown whereas plastinated specimen appeared to be white and glossy.

Table 1: Comparison of shrinkage between formalin-fixed specimen and plastinated soft tissue specimen

Volume of Formalin-fixed Specimen (cm^3)	Volume of Plastinated Specimen (cm^3)	Shrinkage (Percentage)
3	2.8	6.60%
7.5	7.3	2.60%
3.6	3.2	5.50%
5.6	5.5	3.60%
105.6	102	3.40%
7.5	7.3	2.60%
300	280	6.70%
255	235	7.90%
198	196	1%
245	240	2%
776.4	770	0.80%
190	194	2.10%
8.6	8.3	3.50%
3.5	3.4	2.80%
125	120	4%
375	370	1.30%
228.2	222	2.70%
87.3	84.1	3.60%
60.4	57.1	5.50%
185	182	1.60%

Table 2: Comparison of shrinkage between formalin-fixed specimen and plastinated hard tissue specimen

Volume of Formalin-fixed Specimen (Cm^3)	Volume of Plastinated Specimen (Cm^3)	Shrinkage (Percentage)
48	48	0
32.4	32.4	0
12	12	0
49	49	0
49	49	0
4.2	4.2	0
4.1	4.1	0
138.3	138.3	0
82.9	82.9	0
190	190	0

Table 3: Comparison of colour and consistency between formalin fixed specimen and plastinated soft and hard tissue specimen

	Soft Tissue		Hard Tissue	
	Formalin-fixed Specimen	Plastinated Specimen	Formalin-fixed Specimen	Plastinated Specimen
Colour	Lighter and dull	Darker and glossy	Yellowish brown & dull	White and glossy
Consistency	Soft	Firm and slightly flexible	Hard	Hard

DISCUSSION

Plastination has opened up new vistas in various fields of health science education including dentistry.[7–9] Over the years, the application of plastination techniques has been on the rise. However, challenges do exist in terms of its cost-effectiveness, training of human resources and practicability of execution. Generally, plastination is considered to be expensive and not an economically viable option, particularly for small to medium-scale labs and institutions owing to the cost of equipment.[10] However, in our method of plastination, we have used a regularly used laboratory equipment that includes a suction machine and dessicator with suitable modifications, and indigenous chemicals like acetone, xylene and a polymer and the whole procedure is done at room temperature.

Aarushi Jain *et al*.[11] in 2017 did a similar study on anatomy specimens by vacuum-assisted plastination using melamine. The authors calculated the post-plastination shrinkage, polymer cost and consumption, extra equipment cost and external appearance. The student's feedback post teaching with plastinates revealed that they were good for cross-sectional anatomy as the displacement of structures was minimal but had difficulty in visualising deeper structures. Although they were easy to handle, they were less flexible. The authors concluded that iplastination is cheap, executable and is excellent adjunct for teaching.[11]

Singh *et al*.,[2] in 2015, developed a cost-effective method of plastination for anatomy specimens where previously utilised laboratory consumables were employed and mixed with xylene to form a homogeneous paste followed by impregnation with reactive polymer under vacuum using a modified suction apparatus. However, marked shrinkage after curing was reported using this technique.

Megha Jain *et al*.[3] (2014) studied the efficacy of plastination technique in preservation of oral soft tissue specimens for museum purposes and in demonstration of root canal morphology of teeth. The authors evaluated the physical and dimensional changes, in both soft tissue specimens and teeth after each process. They reported that there was considerable shrinkage in plastinated soft tissue specimens but no significant changes were noted in tooth specimens. Hence, they concluded that plastination could be used for the study of root canal morphology.[3]

Vidya M *et al*.[4] (2009) in a study attempted plastination of oral specimens by using recycling of materials such as used plastic cups as an ecofriendly and cost-effective plastination technique. Aufdemorte TB *et al*.[12] (1985) reported that plastinated oral pathological soft tissue specimens were effective as a teaching adjunct and were preferred by both staff and students.

In our study, we attempted plastination of small to medium-sized specimens since they are routinely encountered in an oral pathology department, thus paving the way for establishing a low-cost library of portable real specimens and models which would be useful in teaching and research. However, we observed slight shrinkage of the specimens and change of colour which is a limitation of this technique.

CONCLUSION

Plastination is both art and science as it requires skill along with application of scientific knowledge. Although the plastinated specimens in the current study showed minimal shrinkage rate when compared to previous similar studies, they have proved to be an excellent alternative to formalin-fixed specimens as they are easy to handle and maintain, clean and dry, odourless, non-infectious, non-toxic, non-biohazardous, user friendly. In addition to teaching and research, they could serve as effective tools for patient education as the doctor can explain the anomaly or pathology to the patient using the plastinated specimens.

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Conflicts of interest

There are no conflicts of interest.

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