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Wax embedding technique- innovative and cost effective method of preservation of human cadaveric specimens

Ezhilarasan S[1,*], Muthuvel Vijayan K[2], Jeyanthi M[3]

[1]Professor, [2]Assistant Professor, [3]Associate Professor, Dept. of Anatomy, Govt. Theni Medical College, Tamil Nadu

***Corresponding Author:**
Ezhilarasan S
Professor, Dept. of Anatomy, Govt. Theni Medical College, Tamil Nadu
Email: sathuruvelarasan@gmail.com

Abstract

Human cadaveric organs obtained from cadavers during routine dissection can be kept as dry specimens for teaching and mounting in museum by this new wax embedding technique. This is an innovative technique developed by us using simple chemicals like acetone, xylene and paraffin wax. The procedures for this technique are very simple, cost effective and no prior training is needed. We developed this technique mainly as an alternative to plastination, which needs high end plastination equipment's making the total cost higher.

Keywords: Fixation, Dehydration, Clearing and impregnation

Introduction

In medical curriculum, dissection of human cadaver is very important to learn Anatomy. Usually organs are preserved in 10% formalin solution. Normally, most of the dissectors and students get irritated eyes and sneezing due to formalin fumes and vapors. This wax impregnation method is innovative and no previous study has been done with relation to this.

Aim of the study

To prepare dry specimens of human cadaveric organs (organs obtained during routine dissection) using paraffin wax. Normally plastination is the only technique for preparing dry specimens. Since the technique of plastination is difficult and costly, we prepared dry specimens of human cadaveric organs using this wax embedding technique. In this study our aim is to discuss our experience and results of this wax embedding technique.

Materials and Method

Human specimens collected from cadavers during routine dissection done in the Department of Anatomy, Government Theni Medical College, Theni were used for this technique. The technique is exactly similar to the tissue processing done during histological slide preparation. Wax embedding technique includes the following steps:

Fixation: This step must be done as soon as possible as most cells contain lysosome which will carry out cell autolysis and release digestive enzymes to break down cell components after the cells have died (Bancroft & Gamble, 2002). The fresh organ or animal specimens must be fixed with 10% formalin for a period of 12 hours.

In our present study since the organs were collected from the cadavers, which are already embalmed and stored in formalin, fixation was not needed.

Washing in running water: Organs collected from the cadavers were washed in running water for a period of 24 hours to remove the excess formalin, to soften the tissue, rendering easy wax penetration.

Dehydration: Dehydration is carried out to remove all water in tissue substrate so that water droplets will not trap and affect the specimen from being processed (Gormley, 2012). Dehydration can be done either with Alcohol (Ethyl or Isopropyl alcohol) or with Acetone. Dehydration will be quick with Acetone.

In our study we used 99% acetone for dehydration. For proper dehydration, the volume of acetone must be 10 times the volume of the organ or tissue (in the ratio of 10:1). Depending up on the temperature and the texture of the organs, the dehydration period varies from 1 to 18days. At least, three changes in acetone are essential for complete dehydration. For tissues like skeletal muscle, heart, and dural folds at room temperature the total dehydration time was only 24 hours (8 hours for each change). The same tissues when kept at -10°C, the total dehydration period was 3 days (1 day for each change) and at -25°C the total dehydration period was 6 days (2days for each change).

For parenchymal organs like kidney, liver even at room temperature the total dehydration time was about 6 days (2days for one change). At -10°C the total period was 9 days (3 days for each change). At -25°C the period of dehydration was 12 days (4 days for each change).

For organs like brain at room temperature the total period for dehydration was 9days (3 days for each change). At -10°C the total period was15 days (5 days

for each change). At -25°C the period of dehydration was 18 days (6days for each change).

This obviously shows that dehydration was much slower at lower temperatures. But dehydration in low temperatures causes minimal shrinkage of tissues. Total duration for dehydration also varies with the texture of the tissue. Dehydration was quicker with soft tissues like muscle and slower with hard parenchymal organs like liver and brain. In our present study, most of the specimens were dehydrated at -10°C in deep freezer.

Table 1: Showing the duration of dehydration

Organs	Skeletal Muscle	Dural folds	Heart	Liver	Kidney	Brain
Room Temp 28.8°C	18 hours	18 hours	24 hours	6days	6 days	9 days
-10°C	3 days	3 days	3 days	9 days	9 days	15 days
-25°C	6 days	6 days	6 days	12 days	12 days	18 days

Clearing (Use of an intermediary solvent)

Wax is insoluble in either ethanol or acetone. Clearing agent that was miscible in both acetone and wax are required. As xylene has least effect on tissue substrate (Bancroft & Steven, 1990), it was used in this study .However, xylene can harden the tissue, and thus the immersion time cannot be prolonged (Bancroft &Steven 1990).

Clearing must be done in stainless steel containers. Again the volume of xylene must be 10 times the volume of the organ or tissue (in the ratio of 10:1). Approximately for a specimen of human kidney three litres of xylene must be used as an intermediatery solvent. Usually three changes (for a single change one litre must be used) are needed and the total duration again varies with the type of tissue and temperature. Total duration was about12 hours to 4 days.

Note: Temperature of xylene can be increased by keeping in an incubator or hot air oven. During this process, heated xylene can fire. So, it is advisable to use xylene at room temperature rather than playing by increasing the temperature of xylene and getting fired.

In our present study, clearing of all organs were done at room temperature.

Table 2: Showing the duration of clearing

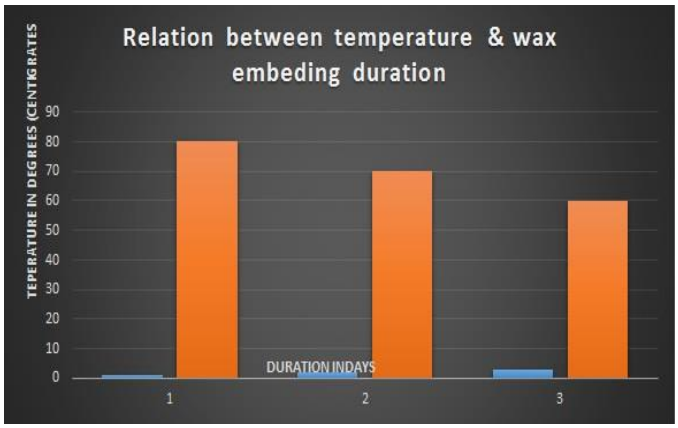
Organs	Skeletal Muscle	Dural folds	Heart	Liver	Kidney	Brain
Room Temp 28.8°C	12	12hours	12 hours	3 days	3 days	4 days
50°C	8 hours	8 hours	8 hours	2 days	2 days	3 days
55°C	4 hours	6 hours	6 hours	1 day	1 day	2 days

Wax embedding: This is the final stage. After the clearing process, tissues were then infiltrated with wax. In this process, paraffin wax which had a melting point of about 54°C to 58°C was used (Gormley, 2012). The organ or specimen immersed in xylene was transformed to a bath containing melted paraffin wax (melted at 60 to 80 degrees). The paraffin bath was kept in an incubator. Temperature of the wax was kept 2°C to 3°C above the melting point of wax (60°C) so that the wax will remain as liquid form throughout tissue infiltration process(Bancroft & Gamble, 2002). Again the duration for wax embedding, depending on the size and nature of the organ (solid organs like liver or kidney needs longer duration) varies from 12hours to 3 days. However the duration can be reduced by increasing the temperature of the wax bath to 80°C.

Note: Very high temperature can cause charring of the tissues and firing. Optimal temperature for clearing in our study was 70°C.

Table 3: Showing the duration of wax embedding

Organs	Skeletal Muscle	Dural folds	Heart	Liver	Kidney	Brain
60°C	24hours	24hours	24 hours	2days	2days	3 days
70°C	12 hours	12hours	12 hours	1 day	1 day	2 days
80°C	6 hours	6 hours	6 hours	12 hours	12 hours	1 day



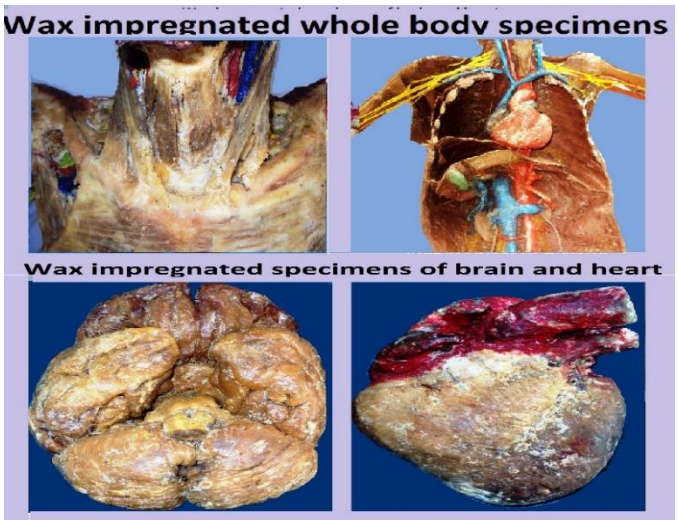


Plate 1: Specimen showing Root of Neck and Pectoral region, Painted Specimen showing internal organs, brain & heart

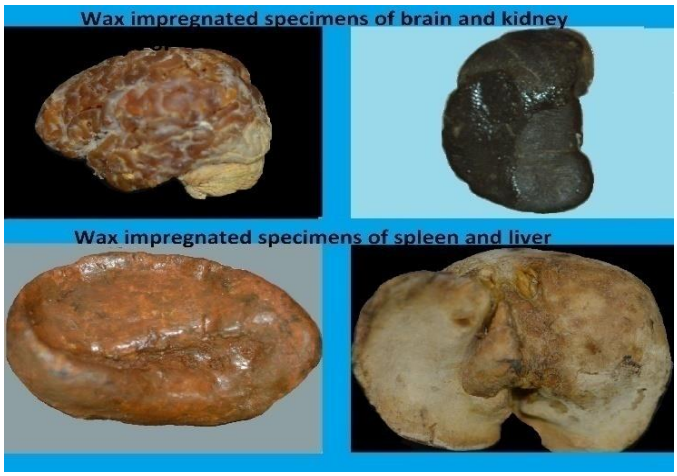


Plate 2: Specimens of brain, kidney, spleen and liver

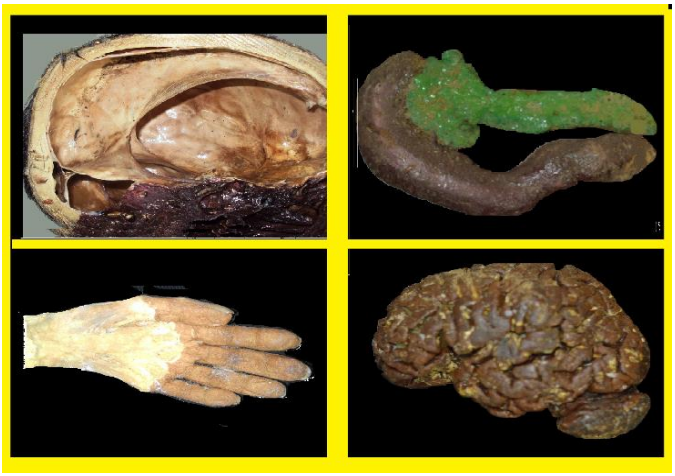


Plate 3: Specimens of dural folds, duodenum with pancreas, hand and brain

Hardening

The specimen taken from the wax is allowed to cool in running water and then allowed to dry. Paint can be applied over the specimen based on the need.

Table 4: Showing the general protocol for the technique

Step 1	Washing in running water
Step 2a	Immersed in first cold (-25c) acetone bath (90%) acetone. 1:10 specimen: acetone ratio.
Step 2b	Immersed in second cold 100% acetone bath.
Step 2c	Immersed in third cold 100% acetone bath.
Step 3	Immersed in a container containing xylene.
Step 4	Immersed in a wax bath (melted at 60C to70C) kept in an incubator.
Step 5	Specimen allowed to cool.

Results and Discussion

It is very easy to handle the specimen. After 1 to 2years (if needed), we can give a wax coat to the specimens. Durability of the specimens has been evaluated and the specimens were found to retain their original size and shape for more than five years.

Conclusion

It is a cheap and cost effective method of preparing dry specimens. It can be used for any sort of biological and medical specimens. It is a boon to the students and teaching academicians. It help them to be free from formalin fumes. It is much cheaper than plastination but the only disadvantage is the considerable shrinkage of specimen size (10% shrinkage). But the texture and external shape of the specimen or organ was not altered.

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