Notes

8

EMBEDDING

8.1 INTRODUCTION

Embedding is the process in which the tissues or the specimens are enclosed in a mass of the embedding medium using a mould. Since the tissue blocks are very thin in thickness they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin, synthetic resins, gelatine, etc.

OBJECTIVES

After reading this lesson, you will be able to:

- describe embedding
- explain embedding media
- describe types of moulds
- explain various devices for tissue embedding.

8.2 EMBEDDING

The choice of embedding media depends upon

- Type of microscope
- Type of microtome
- Type of tissue eg. hard tissue like bone or soft tissue like liver biopsy

Paraffin wax with a higher melting point (56 to 62^{o}C) is used for embedding. The molten wax is filtered inside the oven through a course filter paper into another container. This will protect the knife edge.

8.3 OTHER TYPES OF EMBEDDING MEDIA

- **Carbowax**: It is a water soluble wax. Therefore tissues are directly transferred to water soluble wax after fixation and washing.
- **Methacrylate**: It is easily miscible with alcohol and gives a clear and hard block when polymerised Polymerization takes place in the presence of a catalyst. Any trace of water causes uneven polymerization and formation of bubbles in the block around the tissue.
- **Epoxy Resin (Araldite)**: Epoxy polymers of araldite is used in higher resolution work and to see greater details. Epoxy resins are used for electron microscopy. Epoxy polymers of araldite differ from methcrylate in that they are crosslinked causing the cured solid block of araldite to be insoluble in any solvent. Longer filtration is required because the viscosity of resin is greater than methacrylate.

  For electron microscopy araldite is obtained as casting resinCY212, ahardner DDSA and an amine accelerator, DMP (ditrimethylamino methyl phenol) Blocks are suitably cured before sectioning for 48 to 60 hours at 60^{o}C.
- **Agar embedding**: It is mainly used in double embedding. Multiple fragments and friable tissue may be impregnated in one block when sectioning on the cryostat. Another use of agar embedding is for FNAC specimens.
- **Celloidin media**: Celloidin is a purified form of nitrocellulose. It is used for cutting hard tissues.
- **Gelatin**: Its melting point is less than the melting point of agar. Gelatin may be used when frozen sections are required on friable and necrotic tissues.

8.4 TYPES OF MOULDS

A variety of moulds are used for embedding. These may be LEUCKHARD embedding moulds (L mould) paper blocks, plastic moulds. Most of the laboratories use L moulds. L moulds are made up of metal, easy to procure, reusable and may be adjusted to make different size of blocks. One limb of the"L" is longer than the other. The two "Ls" are jointed to form a sides of the rectangular box that act as a cast to make the mould.

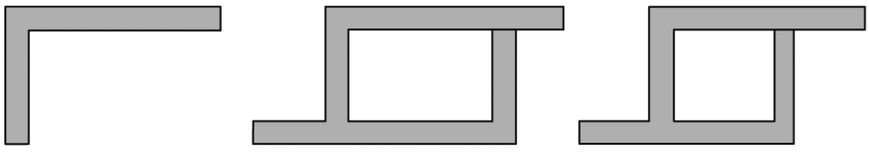


Fig. 8.1: L moulds

Notes

Plastic moulds: Most of the laboratories use plastic embedding rings now. These are relatively inexpensive, convenient and support the block during sectioning and are designed to fit it on the microtome. This eliminates the step of mounting or attaching the block on a holder (metal or wooden holder).

1. **Tissue-Tek System1 or Mark1 system**: In this system plastic embedding rings with stainless steel moulds allow rapid embedding and cutting of tissues. In this system the blocks are stored with the plastic rings; the angle does not change for further requirement of sections.

   The disadvantage of this method is that the space required for storing is more.

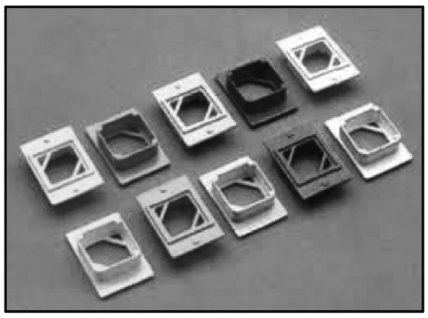


Fig. 8.2: Plastic Embedding Rings

Fig. 8.3: Stainless Steel Moulds

2. **Tissue-Tek system 2or Mark 2 system:** The Mark 2 system has provided a cassette to hold tissue during processing and has a stainless steel lid on the plastic cassette. The cassette has a rough surface on one side of it with a slope where the accession number or the marking is done using a permanent marker.

Fig. 8.4: Plastic Embedding Cassettes

Advantages

- Since the cassette is processed with the tissues and afterwards used for embedding, the writing has to be done once.

- Cassettes are thin so less wax is required.
- The space required for filing the blocks is less.

Disadvantages

- A special clamp has to be used in the microtome for this technique.
- The cassettes are shallow hence thin sections should be taken for processing.

8.5 PARAFFIN WAX ADDITIVES

Various substances can be added to paraffin wax in order to modify its consistency and melting point to improve the efficiency during microscopy.

Additives increase the hardness of blocks. This helps in cutting thinner sections at higher temperature. Stickiness of the medium is increased so better ribbons can be obtained. However if larger quantities of additives are added, undesirable side effects may be seen.

Commonly used additives

- **Ceresin** – It is hard white substance derived from mineral ozokerite. Its melting point is between 61 to70^{o} C. The addition of 0.3-0.5% is sufficient to reduce the crystalline structure of paraffin wax.
- **Bees' wax** - It is yellow substance with melting point of 64^{o} C. This also reduces the crystalline structure of the paraffin wax and improves the ribbon quality.
- **Bayberry wax** - It is a vegetable wax and present in the peal of bayberry. It is extracted from the peal of the fruit. Its melting point is 45° C.

Devices for tissue embedding

Devices designed specifically for tissue embedding are available for laboratories in need of such equipment. These machines vary in size and design depending on the number of samples they are designed to process. Some are designed for specific embedding media, including proprietary compounds intended for specific kinds of histopathology applications. Tissue embedding equipment tends to be expensive. Manufacturers have sales representatives who can provide information and advice when a lab is selecting new or replacement equipment.

Tissue embedding machine

All the blocking steps can be performed with the help of tissue embedding machine. The embedding machine contains the following parts -

- Mould warmer, cassette bath, working surface warmer with a nozzle for pouring the wax, forceps well and cold plate.
- The cold plate is of high efficiency refrigeration system having temperature control ranging from different freezing points to 4 or 5 degree C. It can occupy about 50-60 blocks.
- Large 3-5 litre capacity paraffin reservoir with adjustable temperature of 45-75 degree C.
- Optional vacuum lids, which allows for vacuum infiltration of tissues.
- It has a forceps warmer convenient drain for excess wax.
- The embedding machines are available with many other features.

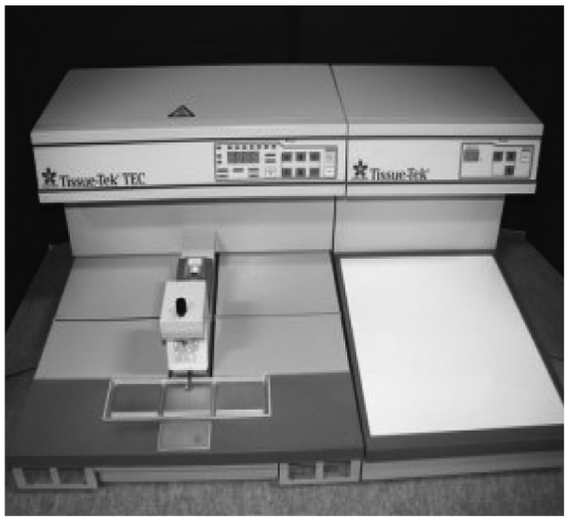


Fig. 8.5

Method of Embedding

1. Open the tissue cassette, check requisition form entry to ensure the correct number of tissue pieces is present.
2. Select the mould; there should be sufficient room for the tissue with allowance for at least a 2 mm surrounding margin of wax.

   Leuckhart mould method-This is the traditional embedding method. The "L moulds are adjusted according to the shape and size of the tissue. Glycerine may be applied to the L pieces and also to the metal or glass plate on which the moulds are placed for embedding. Simple glossed wall or floor tiles may also be used in place of glass plate.
3. Fill the mould with paraffin wax.

4. Using warm forceps select the tissue, taking care that it does not cool in the air; at the same time.
5. Place the tissue in the mould according to the side to be sectioned. This side should be facing down against the mould. A small amount of pressure may be used in order to have more even embedding.
6. Chill the mould on the cold plate, orienting the tissue and firming it into the wax with warmed forceps. This ensures that the correct orientation is maintained and the tissue surface to be sectioned is kept flat.
7. Insert the identifying label or place the labelled embedding ring or cassette base onto the mould
8. Add more paraffin into the mould to fill the cassette and mould.
9. Cool the block on the cold plate.
10. Remove the block from the mould.
11. Cross check block, label and requisition form.

Orientation of different tissue - During embedding the orientation of tissue is important. Correct orientation of tissue in a mould is the most important step in embedding. Incorrect placement of tissues may result in diagnostically important tissue elements being missed or damaged during microtomy.

During embedding it is important to orient the tissue in a way that will provide the best information to the pathologist. At the time of grossing, mark with India ink may be put on the side of the tissue opposite that to be cut. The embedding should be done according to the type of tissue. The requisition form should always be read during embedding for proper orientation.

INTEXT QUESTIONS 8.1

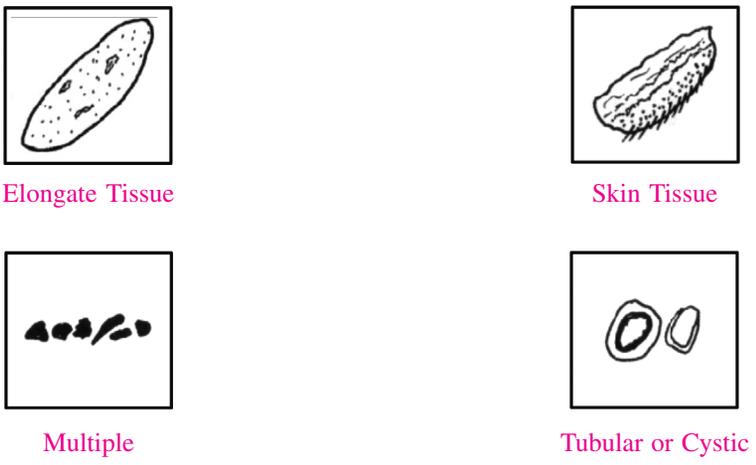
1. ..... is the process by which tissues or specimens are enclosed in a mass of embedding medium
2. The supporting medium is called ..... medium
3. ..... is used for embedding
4. ..... is used for electro microscopy
5. Agar embedding is used for ..... & for .....
6. ..... is used for clotting hard tissues
7. ..... increase the hardness of blocks
8. ..... in a mould is most important step in embedding

Notes

Some general considerations are as follows:

- Elongate tissues are placed diagonally across the block.
- Tubular and walled specimens such as vas deferens, cysts and gastrointestinal tissues are embedded so as to provide transverse sections showing all tissue layers.
- Tissues with an epithelial surface such as skin, are embedded to provide sections in a plane at right angles to the surface (hairy or keratinized epithelia are oriented to face the knife diagonally).
- Multiple tissue pieces are aligned across the long axis of the mould, and not placed at random.

Incorrect placement of tissues may result in diagnostically important tissue elements being missed or damaged during microtomy. In circumstances where precise orientation is essential, tissue should be marked or agar double embedded. Usually tissues are embedded with the surface to be cut facing down in the mould.



Elongate Tissue | Skin Tissue | Multiple | Tubular or Cystic

Fig. 8.6

WHAT HAVE YOU LEARNT

- Embedding is the process in which tissues or specimens are enclosed in a mass of the embedding medium using a mould
- Embedding medium are supporting medium into which the tissue block are embedded

- Various embedding substances such as paraffin wax, celloidin, synthetic resins, gelatine are used depending the type of microscope, type of microtome, type of tissue.
- Epoxy resin is used for electron microscopy
- Agar embedding is used in double embedding and FNAC specimens
- Celloidin media is used for cutting hard tissues
- Gelatin is used when frozen sections are required on friable tissues
- A variety of moulds are used for embedding. There may be L moulds or plastic moulds
- Various substances can be added to paraffin wax in order to modify its consistency and melting point to improve efficiency during microtomy
- Additives increase the hardness of block
- Correct orientation of tissue in a mould is the most important steps in embedding

TERMINAL QUESTIONS

1. Define embedding
2. Explain the types of embedding media
3. Explain the types of moulds

ANSWERS TO INTEXT QUESTIONS

8.1

1. Embedding
2. Embedding
3. Paraffin wax
4. Epoxy resins
5. Double embedding & FNAC
6. Celloidin media
7. Additives
8. Correct orientation of tissue