

A Novel *Cold Boat (Cold Plate)* Method to Overcome the Irregular Solidification of Paraffin Wax during the Time of Preparation of Blocks for Microtomy

Santhoshkumar R*, Santhosh R, Shibu B, Hareesh kumar H and Gayathri SS

Post Graduate Department and Research Center of Botany, Mahatma Gandhi College, Thiruvananthapuram, Kerala, India

Abstract

Melted paraffin wax is widely used for the preparation block for anatomical sectioning. But very often the process encounters with irregular solidification. So the need for a modified procedure for block preparation in microtomy with an aim to control the irregular solidification of wax in a simple way is discussed here. For this purpose temperature controlling system was found to be successful. The temperature control system was developed based on a simple way, for this purpose a paper boat was prepared and a layer of paraffin wax was coated to the paper-boat. This help to control the entering of water to the paper-boat. Then the boat fixed in the tray with cold water. After this, the molted wax poured to the boat and it help to form the basal layer of the wax, following this, the material fixed in the basal layer and again added molted wax to the sample to create the upper layer. The cooling system helped to the solidification starts from the basal portion to the upper level at a controlled state.

Keywords: Paraffin wax; Microtomy; Anatomical sectioning

Introduction

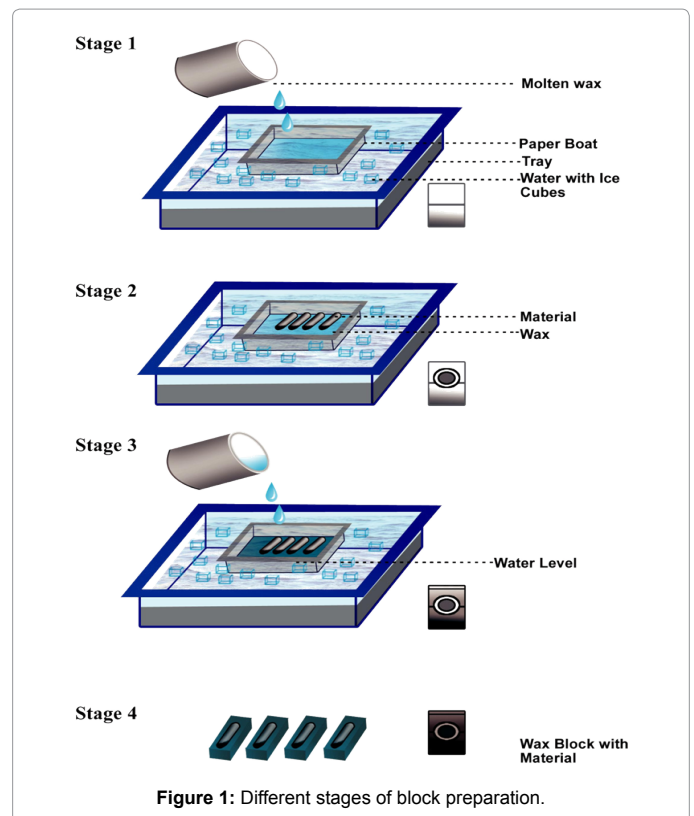
Paraffin wax embedding is one of the oldest and widely used methods in the micro-technique studies. This method is very useful for different cellular pathological as well as modern histological studies [1,2]. Some published articles were available in connection with paraffin block preparation [3,4]. The steps involved in this process includes fixation of samples, infiltration, embedding, sectioning, mounting and labeling. Most of these steps are time consuming especially, the paraffin wax embedding step where melting temperature of paraffin wax is very crucial (approximate 58°C is recommended). Since it becomes difficult to maintain the recommended temperature simultaneously, irregular solidification of wax results. Hence the present study deals with a simple technique to overcome the irregular solidification of wax in the paper boat. Besides this, the time schedule of process of fixing and the formation of blocks were found to have reduced when compared to the conventional method.

Materials and Methods

Paraffin was melted with the help of an electric oven. After the process of infiltration, the samples were used for embedding with paraffin wax. Before embedding, a little bit of changes were incorporated to the conventional technique. The appropriate paper boats were prepared for the experiment based on the standard protocol. Then these boats were coated with a layer of paraffin wax to avoid the entry of water to the boat. The boats were then fixed in a tray with water. The melted paraffin wax (melting temperature was not noted) was poured onto the boat up to an appropriate level and then the experimental material was transferred to the upper layer of wax during which the wax starts to harden slowly from the base region of the paper boat. In the next step, the remaining wax was used to create the top covering layer of experimental material (Figure 1).

Results

By following this modified technique the paraffin wax hardening was found to start slowly from the lower portion to the upper portion of the paper boat due to the low, and controlled temperature in the tray. After some time the block was detached from the paper boat and kept in cold water for some time to hasten the hardening process. The block was then used for sectioning.



*Corresponding author: Santhoshkumar R, Post Graduate Department and Research Center of Botany, Mahatma Gandhi College, Thiruvananthapuram, Kerala, India, Tel: 9495765921; E-mail: santhoshkumar30576@gmail.com

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In the above result shows that a definite uniform pattern was formed for the formation of the paraffin block, from base to upper part of the paper block. This help to control the irregular solidification of wax in the paper-boat. The coating of paper boat helps to overcome the entering of water from the tray to the paper boat. Otherwise the boat may contact with water and damage it. A small brush was used to paste the wax to the boat. The outer as well as inner part of the boat was coated with paraffin wax.

Discussion

In conventional method the temperature control is more important otherwise the hardening of wax was in irregular way. The uneven section was the result of the irregular hardening of paraffin wax. The present study is a very simple and an easy method for the preparation of blocks for anatomical studies. Although the conventional method is the most widely used and accepted one the proposed study was found to have much more efficiency over the conventional method. So the boat prepared with paper is preferred. Hence we are anticipating having a metallic boat with automatic cooling system in near future to improvise research in microtomy.

Conclusion

The preparation of high-quality sections for histological studies requires skill and experience. This article has been useful to newcomers to microtomy and paraffin section preparation. Various steps are involved dealing with the preparation of blocks and microtome-sectioning. Most of the steps are time consuming but comparatively easy for handling even an inexperienced persons in this field. However, the block preparation with paraffin wax is some time difficult to inexperienced peoples. The paraffin wax method was widely used for the preparation of blocks for anatomical sectioning. For this method the particular temperature is needed for wax melting otherwise the

irregular solidification of wax in the paper boat may form. After result of the irregular solidification, the sectioning may be very difficult with a rotary microtome. In normal condition 58°C was recommended for melting the paraffin wax, however, the maintenance of temperature somewhat difficult due to the laboratory condition. If paraffin wax molted at high temperature it will solidify irregularly. This may cause the formation of paraffin block with irregular shape and very difficult to take sections with microtome. The result of this the formation of uneven sections is formed.

The modified temperature controlled block preparation method may useful for the anatomical sectioning especially for the inexperienced beginners in this field. The low temperature at the bottom may help to start the hardening of paraffin from the bottom to the upper portion of the paper boat. This will help to the formation of block with suitable size and shape. This block may help to produce clear section for microtomy studies. In addition to this there is no botheration about the melting temperature of paraffin wax.

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