Research Article

Economic tissue microarray using stamp and punch biopsy needle

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ABSTRACT

Introduction: Conventionally, paraffin blocks were utilized to diagnose the histological samples. Tissue microarrays make the analysis of these histological samples easy by compiling all the information on a single slide. A paraffin block with an array is prepared, and a needle transfers samples from the histological sample blocks into it. Different tissue samples from various tissue blocks are punched and re-embedded on a new paraffin block. **Aim:** The aim of this study is to fabricate an economic tissue microarray using stamp and punch biopsy needle. **Materials and Methods:** The stamp was fabricated with 2 mm cylindrical template arranged in three rows and three columns. The molten paraffin wax was poured into the leukardt mold. The stamp with the cylindrical template was placed over it when the wax was in the molten state itself after which the wax was allowed to set. After that, the stamp with a cylindrical template was retrieved. This creates numerous holes in the wax block. Punch biopsy needle 2 mm diameter with the plunger was used to punch the tissue from the previous paraffin-embedded tissues. The punched tissue from various paraffin blocks was transferred into the holes created by a stamp in the paraffin block. A hot plate is used to stabilize it after placing all the punches of tissue. The paraffin block with arrayed tissues was sliced using microtomy and transferred to the slide and viewed under microscope or can be used for molecular and immunohistochemistry procedures. **Results:** In this study, tissue microarray procedure was done using a stamp and punch biopsy needle which is economical and accurate. Nine tissues were transferred to slides, and both epithelium and connective tissue were seen clearly without any fold and tear.

KEY WORDS: Economic tissue microarray, Punch biopsy needle, Stamp

INTRODUCTION

Tissue microarray is a new innovation in the field of pathology.[1] A microarray contains many small representative tissue samples assembled on a single histologic slide and, therefore, allows a complete analysis of multiple specimens at the same time.[2]

Microarray, actually a "biopsy of biopsy," utilized to obtain punches from various tissue ranging from 10 to 1000. Biopsy of the malignant tissue is processed and embedded in paraffin wax and sliced into three microns thickness using a microtome. This section transferred to slide, stained, and viewed under microscope. Immunohistochemistry using a primary antibody and secondary antibody helps in better diagnosis. IHC of this array will reduce the antibody usage to manifolds.

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

Clinical TMAs include multitumor microarrays (samples from multiple histological tumor types), progression microarrays (samples of different stages of tumor progression within a given organ), prognosis microarray (samples from which clinical follow-up data are available), and cryomicroarrays (frozen samples that might be more suitable than formalin-fixed tissues for the detection of mRNA).[3]

The origin of tissue microarray was by Dr. Hector Battifora's "multi-tumour sausage blocks," in which a number of tissues, typically from different organs, were thrown together in the same block and tissue distribution of a particular antigen/protein was assessed.[4] There was another method with the alignment of the tissue specimens in a Cartesian coordinate system (checkerboard pattern) popularly known as "checkerboard tissue block" method.[4] Kononen used a cast of a small amount of melted paraffin to record the position of each punch specimen.[5] This led to the development of a TMA

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Received on: 17-01-2020; Revised on: 25-02-2020; Accepted on: 27-03-2020

precision microarray instrument with an X-Y Guide. This enabled real high throughput analysis with arraying of up to 1000 cores in the same block.[5]

Thus, tissue microarray is a method, in which numerous tissue samples from the previous paraffin blocked tissues are taken and re-embedded in a paraffin block. It can be sliced using microtome and transferred to slides.[4] The advantage of this method is that numerous tissues ranging from 10 to 1000 can be arrayed and studied at a single time.[2] IHC of this array will reduce the antibody usage to manifolds.

The commercially available kits are costlier and one kit costs around $820. Hence, we formulated a new method which is economic and elementary to fabricate.

MATERIALS AND METHODS

The stamp was fabricated with 2 mm cylindrical template arranged in three rows and three columns. The molten paraffin wax was poured into the leukardt mold. The stamp with the cylindrical template was placed over it when the wax was in the molten state itself, after which the wax was allowed to set. After that, the stamp with cylindrical template was retrieved. This creates numerous holes in the wax block. Punch biopsy needle 2 mm diameter with the plunger was used to punch the tissue from the previous paraffin-embedded tissues. The punched tissue from various paraffin blocks was transferred into the holes created by a stamp in the paraffin block. A hot plate is used to stabilize it after placing all the punches of tissue. The paraffin block with arrayed tissues was sliced using microtomy and transferred to the slide and viewed under microscope or can be used for molecular and immunohistochemistry procedures.

RESULTS

In this study, tissue microarray procedure was done using a stamp and punch biopsy needle which is economical and accurate. Nine tissues were transferred to slides and both epithelium and connective tissue were seen clearly without any fold and tear.

DISCUSSION

Tissue microarray, actually "biopsy of biopsy," utilized to obtain punches from various tissue ranging from 10 to 1000, re-embedded in paraffin template, and sliced. This one slide with 10–1000 tissues can be arrayed, stained, and examined under a microscope. IHC can be done for 1000 tissues at the same time using minimum antibodies.

Biopsy of the malignant tissue is processed and embedded in paraffin wax and sliced into three microns thickness using micro-frame. This section transferred to slide, stained, and viewed under microscope. Immunohistochemistry using a primary antibody and secondary antibody helps in better diagnosis.

There are two types of TMA technique, automated and manual.[6] In automated method, we can mark, edit, and save punch coordinates using an on-screen display and software tools, while visual selection can be performed during punching, using a magnifying glass or a stereomicroscope ASA guide. It is faster in the automated method with respect to punch and speed. The block capacity is 7 times more than the manual method. Video merge unit displays pre-marked slide images side-by-side to the donor block image in the automated method, while pathologist marks regions of interest to slides by hand before arraying in manual method. Punch sets of 0.6, 1.0, 1.5, and 2.0 mm are available. Like automated tissue microarrayers, manual tissue arrayers are also commercially available. Automated evaluation is also possible with a DNA microarray scanner.[7]

Automatic microarray and conventional microarray's punch and template costs from 30,000 to lakhs. To overcome this, we devised an instrument which costs only 500 so that it can be used by small laboratories.

Figures 1 and 2 explain the procedure of tissue microarray using a stamp and punch biopsy needle.

Stamp fabrication is simpler if we provide a design template to the manufacturers. The device can be easily obtained even by small laboratories. The device is economical when compared with conventional devices. The conventional silicone mold is Rs. 50,000, and we have fabricated it for Rs.500. Cost-effectiveness of this device will make this rare tissue microarray procedure a most common in histopathology. It further reduces antibody usage (which might cost Rs.30,000

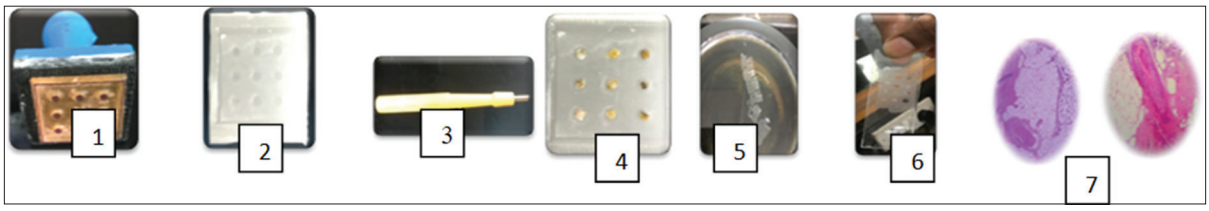

Figure 1: Procedure for tissue microarray

1. The stamp was fabricated with 2 mm cylindrical template arranged in 3 rows and 3 columns
2. The molten paraffin wax was poured into the leukardt mold. The stamp with the cylindrical template was placed over it when the wax was in the molten state itself after which the wax was allowed to set. This creates numerous holes in the wax block.
3. Punch biopsy needle of 2 mm diameter with the plunger was used to punch the tissue from previous paraffin embedded tissues.
4. The punched tissue from various paraffin blocks were transferred into the holes created by stamp in the paraffin block.
5. The paraffin block with arrayed tissues were sliced using microtome and transferred to the slide
6. Transferred to the slide

approximately for 20 slides) in immunohistochemistry procedures.

The newer invention in this device is that instead of mold, we used stamp. It can be designed easily and can be used multiple times. It is economic compared to conventional silicon mold which costs Rs.50,000. For punching the tissue, punch biopsy needles were utilized, which cost around Rs.200. Cost-effectiveness of this device will make the tissue microarray procedure a common tool for faster diagnosis. This can also be used for cryofixation during frozen sections. This device helps in fabricating the paraffin template accurately and tissue can be oriented properly.

CONCLUSION

An economic tissue microarray using a stamp and punch biopsy needle is fabricated.

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Source of support: Nil; Conflicts of interest: None Declared