

Original Research Article

An easy and economical method of construction of tissue microarrayDr Amit Kumar Adhya¹, Dr Nidhi Shukla²¹Associate professor, ²Post graduate student, Department of pathology, KIMS, Bhubaneswar, Odisha, India***Corresponding author**

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Abstract: Tissue microarray is very useful in research. However the automated equipment is very costly and cannot be afforded by many laboratories. We describe a very simple and cheap manual method of preparation of tissue microarray using readily available materials. Materials and methods: tissue cores were punched out from the donor blocks using a 16 g bone marrow trephine biopsy needle. The cores were aligned in a matrix of 6x5 on a transparent adhesive. L moulds were placed around the matrix and molten paraffin wax was poured to form a paraffin block. Results: The block was then cut with a microtome and sections were used for immunohistochemistry and H & E. Excellent results were obtained from the method. Discussion: We have described a very simple and cheap method of construction of tissue microarray by using readily available materials, which does not require any special training or expertise. The method can be followed in any histopathology laboratory and its uses are many in the field of research, standardisation of antibodies and in situ hybridization.

Keywords: tissue microarray, trephine biopsy needle, immunohistochemistry

INTRODUCTION:

Tissue micro-array (TMA) was developed by Kononen *et al.*; [1] in 1998. The basic premise of a tissue array is relocating tissue from many archival histological paraffin blocks, on to one recipient block, making it possible to study many different tissues quickly and efficiently thus saving time, effort and reagents. Tissue microarray (TMA) is becoming a useful tool for research and quality control in immunohistochemistry (IHC) and molecular techniques such as gene amplification surveys in tumor and *in situ* hybridization methods [2, 3]. TMA has been reported to be very useful for immunohistochemical antibody testing as well [4].

Array construction is done with the help of automated tissue arrayers, which are expensive and thus not suitable for laboratories in the developing world. Recently, different manual methods have been described for the development of inexpensive yet efficient microarrays [5]. Most of the techniques described, use a preconstructed paraffin donor block, into which holes are punched for inserting the tissue cores [6]. This is fraught with many difficulties, such as block breakage during punching, nonalignment of holes and mismatched length of the tissue cores and recipient holes. With the desire to construct a cost effective TMA

while overcoming the above-mentioned difficulties, we tried an easy method for the same without using prefabricated recipient blocks. This method, which is a modification of the one initially described by Ni Chen *et al.*; [7] requires no special instruments and has proved to be quite effective and reproducible. The tissue arrays thus constructed can be smoothly sectioned using a standard microtome and performed for a panel of immunohistochemical study with satisfactory results.

MATERIAL AND METHODS:

For preparation of tissue microarray we used paraffin embedded tissue blocks of our department which were received between 2014-2105. Thirty cases of colon cancer were selected. The representative H and E slides were seen under the light microscope and the most suitable areas were marked with a marker pen. The slides were matched with their corresponding paraffin blocks and the corresponding areas were marked on the tissue surface of the paraffin blocks. A 16 g bone marrow biopsy needle was used to punch out the tissue cores from the marked area. The tissue core was then extracted from the needle by pushing it out by the blunt stellate. Thus 30 cores were obtained from 30 blocks. The cores were then put in an array of 6x5 on an adhesive tape. To ensure proper alignment of the tissue,

transparent tapes were used, which were put of paper marked with dots designating the location of each of the tissue cores. The tissue cores were put on the adhesive tape in an erect position taking care that each of them adheres to the tape firmly. Then L moulds were put around the cores and molten paraffin was poured gently

taking care that the tissue alignment was not disturbed. Thus new paraffin blocks were constructed having a tissue microarray of 6x5. The block was then cut with a microtome in the usual manner to obtain slides. The slides could then be used for H and E staining or Immunohistochemistry in the usual manner (Figure 1).

Steps of construction of Tissue microarray



Fig 1: steps of construction of tissue microarray

RESULTS:

Tissue microarrays were constructed using the above method. Construction of one tissue microarray of 6x5 dots took approximately 1 hr. The donor blocks used did not show any significant damage or loss of tissue. Satisfactory alignment of the tissue cores could be achieved. A total of 50 slides could be cut from the blocks, before the microarray was exhausted. The slides thus obtained were subjected to hematoxyline and eosin stain to assess the quality and adequacy of the material obtained. The slides showed satisfactory staining

quality and adequate amount of tumor tissue. Sections were also taken on poly L lysine coated slides and subjected to immunohistochemistry. There was some apprehension that the tissue could float away from the slides during antigen retrieval step which involved subjecting the slides to microwave. However none of the slides showed tissue loss during antigen retrieval. Immunohistochemistry using a two-step method was done with primary antibodies to AMACR, Ki 67 and p53 for the purpose of a research work on colon cancer tissue. Immunohistochemistry could be satisfactorily

performed and the results were used for completing thesis work of postgraduate students. The use of microarray resulted in marked reduction of the efforts and price of doing immunohistochemistry because without microarray the procedure would have to be done individually for each of the 30 cases. Moreover as all the 30 tissue were subjected to same treatment, the immunohistochemistry results were also uniform and comparable.

DISCUSSION:

The technique described here is inexpensive and reliable alternative to automated instruments for constructing tissue micro-arrays. The major advantages of this method over automated methods is that it is very cheap, does not require expensive instruments, can be easily mastered by the histotechnicians, done with readily available instruments and can be done in any laboratory small or big. It has immense role in research where immunohistochemistry is being done. As it incorporates many tissue from different patients, so that one parameter can be studied in one go, thereby significantly reducing the cost of reagents and the time and effort required to study the same parameter in different cases individually. Moreover it ensures the same quality of immunohistochemical results for all the samples under study, which improves the quality of the results. Control tissue can also be incorporated into the microarray along with other cases which will serve as internal control, thereby increasing the quality of the results obtained by immunohistochemistry.

The advantage of our method over other manual methods described previously [5, 6] is that we have used a bone marrow biopsy needle which provides a tissue core of larger diameter, hence multiple tissue cores are not required from the same donor block. Use of finer bore needle requires multiple tissue cores from the donor block which many times leads to damage of the donor block and also is more laborious. The microarray block was prepared using fresh molten wax and we did not use preformed recipient block, thus overcoming the greatest disadvantages of using a prefabricated paraffin block namely breaking and suboptimal blending of tissue with wax. Our method is very similar to the method described by Pathak GS [8]. They have used a double adhesive tape for the same where as we have used a transparent adhesive tape which is easier to mark and maintain alignment of tissue cores.

Adequacy of the tissue obtained by TMA has also been critically discussed. In our experience the tissue obtained from the sections cut from the TMA blocks yield adequate tissue for interpretation. Recently construction of TMA using tissue from slides where blocks were not available has also been described. [9]

The major limitation of this method is that we have been successful in making a microarray of 6x5 only whereas the automated methods can prepare a microarray block or upto 200 dots. Again the time taken to prepare a microarray block is much less (10 minutes for a 9x9 microarray) and alignment of tissue is much better with the automated methods. Time taken to prepare the microarray block by our method is around 1-2 hrs. There could be misalignment of tissue cores making the interpretation difficult. However these are minor problems as compared to the immense benefit our method provides with regards to the cost effectiveness. These problems could be easily overcome with practice.

CONCLUSIONS:

We have described a very simple and cheap method of construction of tissue microarray by using readily available materials, which does not require any special training or expertise. The method can be followed in any histopathology laboratory and its uses are many in the field of research, standardisation of antibodies and in situ hybridization.

Acknowledgement: none

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