Original Article

A Novel Method Of Manual Construction Of Small Format Paraffin Tissue Micro Arrays (PTMAs) In A Center With A Minuscule Budget

Ambereen A Imran

Background: To describe and assess a manual method of Paraffin Tissue Micro Array (PTMA) Construction using simple equipment available in ghe most elementary pathology set ups.
Methods: In this article is described a manual method of PTMA construction. It requires simple equipment available in the most elementary Pathology set ups. Paraffin blocks with preformed holes in a 3x3 grid pattern were created and filled with tissue cores obtained by bone marrow aspiration needles. A variety of tissue blocks was selected from the archives of Pathology Lab, PGMI Lahore, yielding a total of 108 cores.
Results: Encouraging results were obtained. Only two tissue cores were lost. All the others showed up well. There was no tissue folding, splitting or rolling up. Tissue cores were easily recognizable as to source owing to larger diameter.
Conclusions: The technique is easy to reproduce, economical, quick, and creates uniform blocks capable of yielding multiple sections. Though the number of cores per block is small, the larger core diameter offers advantages of easier tissue handling and more accurate diagnosis. It could form a modest foundation with a potential for improvement in future.
Keywords: PTMA, Tissue cores, arrayer, donor block and recipient block.

Introduction

The millennium turned amidst rapid and amazing developments in the fields of molecular biology, genetic engineering and virtual medicine; causing drastic shifts in our paradigm. These developments placed renewed demands on us to revisit our methodologies and adapt them to the changing needs. Paraffin Tissue Micro Array (PTMA) has emerged as a robust, high throughput technique which allows analysis of a large number of samples in a single slide.[1-3]

It basically involves taking only one (or more) tiny cores of representative tissue from a cohort of pre existing paraffin blocks --- called the donor blocks. These cores are then inserted into preformed holes in a single new block --- termed the recipient block; in a regular grid pattern. This new block now contains tiny but representative bits of tissue from upto a 1000 of original tissue blocks. This may then be cut on a routine microtome and sections obtained, which are ready to be submitted to one or a battery of staining procedures.[4] One or more "control cores" may be included in the block. The process by its inherent nature ensures that each and every core undergoes exactly the same treatment at every step so it is ideally suited for research purposes.[5] Here it must be emphasized that since the size of the cores is small they can never be used for diagnosis.[6]

Although the technique has been widely adopted in the Western world; as is borne out by the plethora of research papers on the subject its utilization in the underdeveloped countries is hampered by its high cost.[7] This has prompted innovative workers in these regions to develop techniques that could help create low density PTMAs.[5,8-10] Inspired by the expertise of the technical staff available in our lab I decided to try and develop a modest scale version of the technique whereby low density PTMAs could be manually constructed from readily available instruments with the hope that it might be improved upon in future.

Material and Methods

Special recipient tissue blocks were prepared in the following way. Paraffin wax was poured into Leuckhard's moulds and allowed to set halfway. At this stage 9 toothpicks (Care®, Al Fatah Departmental Store, Lahore) with a diameter of 2 Mm, were inserted vertically into each block, in a grid pattern of 3x3. The blocks were then cooled and when completely set the toothpicks were easily removed. This gave us a blank block with nine wells in a regular grid pattern. The holes ran the entire depth of the block.A group of 74 paraffin wax blocks was obtained from the archives of PGMI Histopathology

Laboratory. It included a wide spectrum of tissues types **(Table).** Bone marrow aspiration needles (16G) were used to punch out tissue cores. The needle tips were straightened by cutting off their beveled tips. Routine sections were cut from each block, stained with H & E, and areas of interest were identified. These slides were overlaid on the blocks and areas to be cored out were marked on the respective paraffin blocks. The blocks were brought to room temperature before being punctured by bone marrow needles to minimize the risk of block breakage. The needles were inserted vertical to the surface of the block. The cores thus obtained were about 3mm in length and 2mm in diameter. They were gently pressed out with the stylet and transferred to one of the holes in the blocks described above. Care was taken that the cores were flush with the surface. To blend together the wax from the tissue cores and that from the recipient block, the filled block was incubated at 60°C for 15 min as described.8,11 Blocks were trimmed taking care to maintain a 3mm border of wax around peripheral cores **(Fig-1)**.Twelve blocks were prepared containing a total of 108 tissue cores **(Table).** The blocks were chilled and 5 µm sections were obtained using a rotary microtome. Use of fresh disposable blades greatly improved the quality of the yield. Sections were stained with H & E and examined under the microscope. Features especially looked for were quality of sections, staining characters, recognisibility of tissue source, tissue loss, tissue folding, tissue splitting and tissue rolling up.

Results

Out of 108 tissue cores only 2 were lost. One of these was from a leiomyoma which was very hard and the core had been difficult to obtain. The other was from the central caseous area of a lymph node with extensive caseating granulomatous inflammation. All the other tissues including some quite friable carcinomas like Hepatocellular Carcinoma (HCC) and Transitional Cell Carcinoma (TCC) fared very well **(Fig 2).** Even tissues with caseating granulomas except the one mentioned previously stood up to the procedure remarkably well **(Fig 3).** Section quality and staining characters were excellent. No tissue folding, splitting or rolling up was seen in any core. Almost all cores could be recognized regarding the source owing to the large diameter **(Fig 2, 3).**

It was possible to obtain multiple sections from each block. The original blocks suffered minimal damage and were reusable even after yielding more than one cores.

Discussion

Traditional screening for new markers involves using a slide for each of several different patients.[12] With PTMAs multiple specimens can be simultaneously investigated with different techniques under identical laboratory conditions resulting in a dramatic time and cost saving. Another advantage is that this technique is less exhausting for the finite original donor material allowing increased number of tests to be performed on each case. The obvious advantages of this high throughput technique

Table: Details of types of tissue, numbers of blocks and cores taken.

Type of Tissue	No. Of Blocks	No. Of Cores
Nodular hyperplasia Prostate	24	41
Adenocarcinoma Prostate	6	9
Leiomyoma	10	14
Diagnostic D&C	6	8
Tuberculosis lymph node	6	10
Tuberculosis intestine	4	4
Mesentry	1	1
Transitional cell carcinoma	2	2
Renal cell carcinoma	4	6
Chronic pyelonephritis	4	4
Hepatocellular carinoma	3	5
Chronic active hepatitis	2	2
Squamous Cell Carcinoma	2	2
Total	74	108

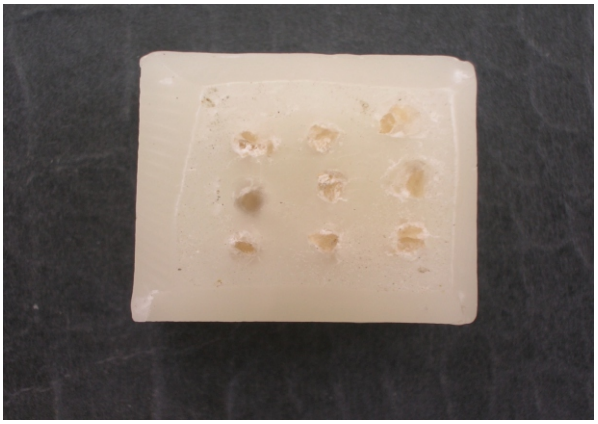

Figure-1: A finished block with 9 cores from

different donor blocks.

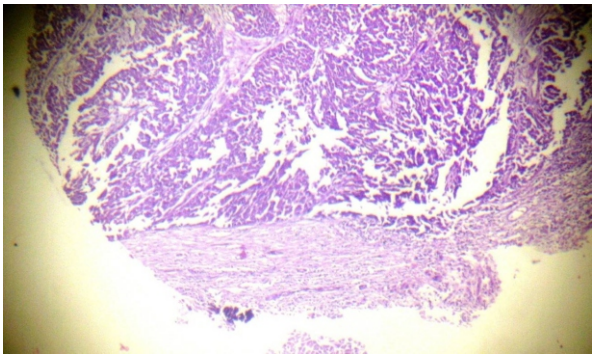

Figure-2: A core showing well recognizable tissue from a case of TCC (H & E, x200).

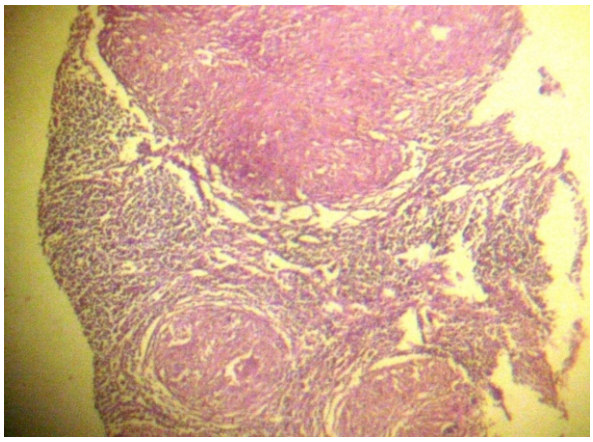

Figure-3: Well preserved core from a lymph node afflicted with caseating granulomatous inflammation (H & E, x 200).

have stimulated many workers to improvise and evolve new ways of PTMA construction.[1, 13] Encouraging results were obtained in the present study. The number of cores per block was small but other researchers have reported small format PTMAs too.[1, 5, 7-10] The major appeal is that no specialized equipment is required. The whole procedure is carried out using readily available materials, is neither very labor intensive nor time consuming.

Another advantage, albeit gained unwittingly, is the larger core diameter. This not only allows easier identification of cores, but also obviates the need for multiple cores described in other studies.[1, 14] The bigger diameter of cores was also the probable cause of avoiding problems of tissue loss, folding, splitting and rolling. These have been described as major technical nuisances.[2,11]

A variety of tissues was selected for the study and 98% of the tissues fared well. This included friable and crumbly tissues like HCC, TCC and lymph nodes with caseating granulomatous inflammation. Cores from all these sources revealed easily discernible morphology. Only two cores were lost during sectioning. One was from a leiomyoma which was very hard and difficult to core to start with. The other was from a purely caseous area of granuloma in a lymph node. It too was difficult to handle owing to excessive friability. It was possible to obtain multiple sections from each block. This was greatly facilitated if the disposable blades used were fresh and of good quality. Getting multiple sections is important because it could allow us to run a panel of immunostains on the whole cohort of cores; as is the standard practice in most research centers.[4, 12] The significance of good quality blades has been reported by other authors earlier.[8, 15] The donor blocks did not suffer any major damage, and though mildly mutilated were still reusable after yielding one or more cores. This assumes special importance in cases of needle biopsy specimens due to the finite nature of donor material; a situation we are likely to face more and more frequently in the coming years.[1,13]

Conclusion

By dint of its application in molecular and clinical research, PTMA technology has emerged as a robust tool which is both less labor intensive and more cost effective. Unfortunately, its own high costs hamper its access to low budget set ups. The technique described here requires readily available materials, and could serve as a modest foundation with a potential for further development.

Department of Pathology
Pakistan Postgraduate Medical Institute Lahore
theesculapio@hotmail.com
www.sims.edu.pk/esculapio.html.

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