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Tissue micro array; establishing a cost-effective tool for cancer biomarker research in Sri Lanka

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ABSTRACT

Tissue micro array is a cost effective tool for cancer biomarker discovery and for the validation and external quality control in immunohistochemistry. It has not been utilized in Sri Lanka before, although widely used in cancer research centres world over. Scarcity of cancer biomarker research in Sri Lanka is partly due to the unaffordable cost of laboratory consumables including antibodies. TMA is produced using tissue cores from multiple tissue blocks. It reduces the cost and improves the consistency in immunostaining and adds validity to the assessment. In this brief report, we describe the technique of producing TMA and technical issues faced and how we could overcome them.

Key words: *TMA, cancer biomarkers*

The concept of embedding tissue of different samples into one tissue block goes back to 1986 when the 'sausage' tissue blocks were developed for immunohistochemical assessment (1). The advantage of sausage block was that all of the tissue samples are treated equally during immunostaining and most sources of variation are eliminated which facilitates comparative studies. It was recommended for large scale inter-laboratory quality control processes. This concept was further developed and Tissue Micro Array (TMA) was designed to its current format by Kononen *et al* in 1998 (2). Now it is an invaluable research tool in cancer biomarker discovery.

TMAs are paraffin wax blocks (recipient blocks) constructed with tissue cores extracted from multiple tissue wax blocks (donor blocks). TMAs are sectioned and histology slides are prepared and can be stained with any routine histological stains and immunohistochemistry. It is a high-throughput technology useful in histology based laboratory tests and can be used in florescent in situ hybridization as well (3). TMA can also be used to assess molecular parameters (DNA, RNA) by molecular techniques. While TMA is made, a template per block is prepared indicating the reference number to map the clinical details of the patient with the biomarker score. Once made, TMAs can be used for subsequent assessment of multiple markers. Therefore, TMAs can be used as tissue libraries for future research. TMA cuts down on the cost for antibodies and reagents by many folds as a small core of representative tissue is carefully selected instead of a routine tissue sample. The selected size of the core can be 0.6 to 2 mm. Therefore, a TMA block can be built with hundreds of tissue cores minimizing the variation that can occur during staining procedures improving the validity and increasing the cost-effectiveness. In this brief report, we intend to describe our experience in how this technique can be established in a routine histopathology laboratory.

TMA blocks can be constructed manually or by using precision instruments. Automated forms of tissue micro arrayers are also available but less cost effective for a country like ours. In our histopathology laboratory we used a TMA Builder (Thermo Fisher™) and manually constructed TMAs for a research project on immunohistochemical biomarker assay for a cohort of breast cancer patients who's clinic-pathological and survival details were available for mapping and subsequent analysis.

Making a TMA block

The TMA Builder consists of a mould and a punch extractor. The base of the mould has 24 pins which makes 24 pits in the recipient block. The mould-top has an inset for C-ring and two lifting screws (Figure 1).

Paraffin wax pellets were melted at 60 °C in an oven to bring to liquid state. The base of the TMA mould was placed on a flat surface and a plastic C-ring was fitted into the inset in the mould-top. Molten wax was poured slowly to fill the C-ring which was then left to solidify. Once wax was solid enough, the C-ring filled with wax was removed from the metal mould by screwing down the two lifting-screws. The prepared wax block now has 24 pits to receive 24 tissue cores.

The donor tissue blocks were first examined for its physical suitability. The haematoxylin and eosin (H&E) stained slides of each case were reviewed. The best representative tumour region with minimum fixation artifacts was selected and marked for tissue extraction. The slide was superimposed on the corresponding donor block to identify the area in the tissue to be punched.

From each of these donor blocks, a core of 2 mm diameter tissue was extracted using the punch extractor of the TMA Builder™ (Thermo Fisher). The cores were transposed/injected into the pits in the recipient TMA wax mould prepared previously.

A core of brain tissue from a wax block was transposed into the 24th pit in the mould as a guide to identify the rows and the columns of the TMA. A template for each TMA block was prepared to link the biomarker score to clinico-pathological data of each case. We made 53 such TMA blocks containing breast cancer tissue of 1200 patients. Since the diameter of each tissue core was 2 mm which covers a sufficient surface area, tissue cores were not taken in duplicate (4).

TMA block was labeled in accordance with the template and was kept in the oven with the wax surface with tissue cores facing down on a flat metal surface. Temperature was set to 58 °C and left for 15 minutes to anneal the block and to bring the tissue cores to the cutting surface. Sections were cut at 4µ on a traditional microtome. Slides were kept overnight in the incubator at 60 °C before staining was done. Sections were assessed by light microscopy. TMAs also can be digitally scanned and displayed on a high resolution monitor (4). Scoring of the biomarkers on TMA was done blinded to the clinico-pathological data reducing the potential for bias. We were able to link this data to survival outcome and were able to prove the prognostic significance of immunohistochemical assessment of biomarkers in breast cancer which included new biomarkers (5). There are no other published reports on TMA technique being used for cancer research in Sri Lanka.

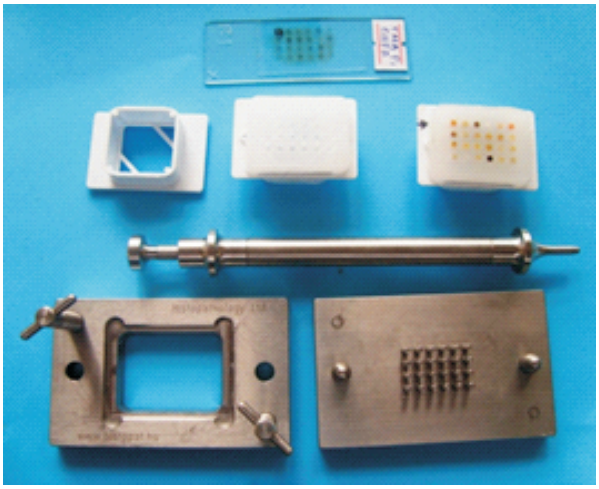

Figure 1: This shows (items from top to bottom and from right to left) 1. A TMA slide stained for EGFR antigen; black circle indicating the guide core, 2.C-ring, 3. A recipient TMA paraffin block with 24 pits built on a C-ring, 4. A TMA block containing 24 tissue cores, 5. Punch extractor, 6. Mould-top of the TMA Builder with inset for C-ring and two lifting-screws in place, 7. Base of the TMA Builder with 24 pins.

Problems identified / troubleshooting

1. Locating the correct area to extract in a core biopsy donor block was difficult. The shape of the core of tissue which appeared on the block and matching it with the corresponding slide was used as a guide.
2. Since it is important to leave some diagnostic material in the block as archive, many core biopsies had to be excluded from our study. This was a limiting factor in preparing TMAs from core biopsies.
3. The depth to which the punch should cut into the donor block has to be first determined by trial as the extracted cores should be of the same length to fit into the pits in the recipient blocks.
4. Some tissue blocks were already sectioned extensively for the diagnostic process leaving only a thin piece of tissue and wax. The cores obtained from such blocks were very short compared to the depth of the TMA pit. The problem of shorter cores not reaching the cutting surface of the recipient block was resolved by keeping the TMA blocks in an oven as described in the annealing process. However, tissue loss was observed as such cores wore off after a few sections were obtained.
5. When the tissue in the recipient block contains fat around the tumour, correct superimposing of the slide to mark the correct site for core extraction was difficult. Inked resection margins, if available, were of help in such situations.
6. Breaking off of the outermost column of the tissue cores was frequently observed when the TMAs were sectioned. This occurred when the TMA blocks were fixed to the microtome through the plastic wings of the C-ring. The problem of breaking of blocks was overcome by fixing the TMA block through the frame of the C-ring.
7. Applying ice cubes on the surface of the TMA block just prior to sectioning, further reduced breakage of blocks.
8. Overnight incubation of tissue sections at 60 ^{o}C in the hot air oven, prior to immuno-histochemical staining, minimized the loss of tissue cores. This does not replace the necessity for a good section adhesive or a charged slide. Loss of tissue cores was minimal with H&E staining. When the guide core of the tissue section was lost during immunohistochemical staining, the H&E stained slide was very useful in identifying the location of the guide core. Therefore, it is advised to have the first section to be stained with H&E.
9. Folding of tissue sections was encountered at times. This occurred when the sections were too thick. Good microtomy skills were of utmost importance in obtaining sections of correct thickness without folds or cracks.

We believe that the information given in this brief report will be of value for Sri Lankan Histopathologists who wish to research into tumour biology and validate biomarkers using the plethora of cancer specimens they report and to find new knowledge on biomarkers at an affordable cost. It will be also useful in establishing external quality control system for immunohistochemistry laboratories in the country, which is a long-felt need.

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Compliance with ethical standards

This research project was granted approval from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Conflicts of interest

Author SNG received a monthly stipend as research assistant from the funding authority. The other authors declare that they have no conflicts of interest. The funding agency had no involvement in the study other than providing sufficient funds to conduct the research.

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