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REVIEW ARTICLE

TISSUE MICROARRAYS- A REVIEW

Pardeep Goyal[1], Nadaf Imtiyaz[2], Indu Goyal[3]

[1]Senior Lecturer, [2]Reader, [3]Tutor, Department of Oral and Maxillofacial Pathology, Adesh Institute of Dental Sciences & research, Bathinda.

ABSTRACT:

This review discusses about the various aspects of the Tissue microarray (TMA) technology and its applications in modern molecular and clinical research. Tissue microarrays (TMAs) allow a rapid, cost-effective, high through-put analysis of thousands of molecular markers at nucleic acids or protein level (1). This is a recent innovation in the field of pathology. A microarray contains many small representative tissue samples from hundreds of different cases assembled on a single histological slide, and therefore allows high throughput analysis of multiple specimens at the same time. Various types of tumors, at various stages of disease progression can also be studied at a single platform, in identical conditions in less labor-intensive and cost-effective ways. Tissue microarrays are paraffin blocks produced by extracting cylindrical tissue cores from different paraffin donor blocks and re-embedding these into a single recipient (microarray) block at defined array coordinates. Using this technique, up to 1000 or more tissue samples can be arrayed into a single paraffin block. It can permit simultaneous analysis of molecular targets at the DNA, mRNA, and protein levels under identical, standardized conditions on a single glass slide, and also provide maximal preservation and use of limited and irreplaceable archival tissue samples(2). This versatile technique, in which data analysis is automated, facilitates retrospective and prospective human tissue studies. It is a practical and effective tool for high-throughput molecular analysis of tissues that is helping to identify new diagnostic and prognostic markers and targets in human cancers, and has a range of potential applications in basic research, prognostic oncology and drug discovery. This article summarizes the technical aspects of tissue microarray construction and sectioning, advantages, application, and limitations.

Keywords: Paraffin Blocks, Tissue microarray, Histology, Immunohistochemistry.

Corresponding Author: Dr. Pardeep Kumar, H No.13243-B, Street-11, Namdev Marg, Bathinda -151001, Punjab, India , Email: drpardeepgoyalmds@gmail.com

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INTRODUCTION

TMA: a recipient paraffin block in which up to 1000 separate tissue cores are assembled in array fashion to allow multiplex histological analysis. Where hundreds of tissue cores are arranged in a single glass slide for analysis like IHC, ISH, FISH. Such a method evaluates numerous samples of tissue in a short period of time.

HISTORY

Tissue microarrays have been developed as a method to evaluate numerous samples of tissue in a short period. Battifora (1986) first introduced the concept of putting together multiple pieces of tissue in a single block called a sausage block. Kononen et al (1998) used this mechanism for examining several histological sections at one time by arraying them in a paraffin block.

Today's tissue microarrays use multiple tissues in a single paraffin block using a precise size and shape to prepare the recipient block. Histological techniques have an important role in the development of molecular biology (3).

ADVANTAGES OF THE TECHNIQUE

- ✓ This technology enables researchers and pathologists to study and evaluate diseases at an early stage. A hundred or more tissue samples are placed in one block. Many sections can be cut from the array block depending on the experience of the technologist and size of the cores. Hundreds of tissue cores are placed on a single slide for the pathologist to review.

- ✓ The technique is used for a wide range of staining procedures: immunohistochemistry, in situ hybridization, fluorescent in situ hybridization, special stain control samples, and quality control sections for H and E stains. Only a small amount of reagent is used to analyze one slide, so it is cost effective in reagents used for immunohistochemistry and in situ hybridization techniques (4).
- ✓ TMAs (tissue microarrays) have been widely used in immunohistochemistry for several years for quality control and quality assurance. They demonstrate the antibody thresholds on a single slide, which is used to optimize where the high and low signal intensities are seen.
- ✓ Experimental uniformity
- ✓ Allows amplification n conservation of *scarce tissue samples*
- ✓ Saving the assay volume
- ✓ Reduces the number of slides examined
- ✓ High-throughput
- ✓ Analysis can be automated and data can be computerized

TISSUE MICROARRAYS CAN BE DIVIDED INTO FOUR GROUPS

1. Prevalence TMAs – are assembled from tumor samples of one or several types without attached clinical and pathological information. These TMAs are used to determine the prevalence of a given alteration in the areas of interest in a tumor.
2. Progression TMAs – contain samples of different stages of one tumor type. They are used to discover associations between tumor genotype and phenotype. For example, an ideal breast cancer progression TMA would contain samples of normal breast from patients with and without breast cancer history, several different non-neoplastic breast diseases, ductal and lobular carcinoma in situ, invasive cancers of all stages, grades, and histological subtypes as well as metastases and recurrences after initially successful treatment(5).
3. Prognosis TMAs – contain samples from tumors available with clinical follow-up data. They represent a fast and reliable platform for the evaluation of clinical importance of new detected disease-related genes. Validation studies using prognosis TMAs readily reproduced all established associations between molecular findings and clinical outcome. Significant examples of these associations are found between estrogen or progesterone expression of HER-2 alterations and survival in breast cancer patients, between vimentin expression and prognosis in kidney cancer, and between Ki-67 labeling index and prognosis in urinary bladder cancer, soft tissue sarcoma, and Hurthle cell carcinoma.
4. *Experimental TMAs* – are constructed from tissue like cell lines. Cell line TMAs are optimally suited for screening purposes, e.g. tumor samples from TMA archives are also used in experimental TMAs.

DESIGNING THE GRID

- Depending upon the purpose of the array, the design varies and the pathologist and technologist must determine the guidelines.
- Once the grid sheet is completed and has been reviewed and signed off, the array process can be started. The purpose for constructing the array is to assemble a simple series of 50 or more patients into one or several blocks.
- It is important to plan in advance how many samples will be arrayed and create a map or grid sheet which will be easy to follow.
- A large number of samples (high density) can be arrayed in a 37 × 25 × 5 mm block.
- If working with a smaller number of samples (low density), use a 24 × 24 × 5 mm block.
- Normal tissue controls and control cell lines are placed in columns between the tumors and normal tissues asymmetrically at one end of the block; be careful not to lose the orientation of the block.
- It is helpful to place a notch at the end of your cassette block to help with this. Archived blocks are used as a source of control tissue without destructive sampling. Making the tissue arrays is a project that involves many steps. Selecting the slides, collecting the blocks, and designing the grid consume the time, rather than the array process (6).
- Setting up this process can take several weeks to a month before the array process actually will begin. Standardize the construction by making it easy to follow.

NEEDLE SIZES

- ✓ 0.6, 1.0, 1.5 or 2.0 mm needles can be used.
- ✓ 1.0 or 1.5 mm needles are recommended for general use.
- ✓ 0.6 needles can be used if you are coring 200 or more blocks.

- ✓ The use of 2.0 mm needles is not recommended by this author since damage can occur to the donor blocks.

DATABASE FOR TISSUE MICROARRAY ANALYSIS

- The first step in construction of a TMA is the selection of cases from a database and creation of a template or spreadsheet that identifies the position of each case and controls in the TMA block.
- During viewing and photographing of the slide, refer to core cases by position. Each case in the database is identified by the unique positions of each core on the template.
- To acquire the image, each core in the TMA is identified based on the row and column position on the slide. One image per core is taken and saved as a compressed file, then named as the position identifier. Each image acquisition takes less than 1 minute, including field selection, manual focusing, and identification.
- It is sensible to save a set of images for each stain in a folder with a name that you can identify.
- The next step is linking the images with the database, and the last is data entry. Several images from different stains can be viewed on the screen, scored, and the data entered manually into additional cells, adjacent to the image cell in the spreadsheet. This method allows examination of multiple cores and multiple stains of the same case with ease, allowing flexibility that is impossible at the microscope as it requires changing slides, stains, light sources, and identifying the correct core.
- Investigators can check scoring, and images can be printed and shared over the network. The collections of these images are used as an educational tool.

PREPARATION OF THE DONOR BLOCK

- ✓ A pathologist reviews the file slides and blocks to determine which blocks will be arrayed.
- ✓ The area of interest to be sampled is marked by circling with a pilot pen or permanent fine-point marker. Some pathologists prefer to mark the blocks rather than the slides.
- ✓ Once the slides are reviewed and marked, the block is matched to the glass slide. Circle the area of interest on the block to match the marked slide. It is important that the block is marked in the same area of interest as the marked slide.
- ✓ Donor blocks must be at least 1mm thick to be suitable for constructing the arrays. An area site marked that is not 1mm thick requires two cores from the same site and they are stacked on top of each other.
- ✓ When marking the slides and blocks we use the following colors as indicators:
    - Red – cancer
    - Green – normal
    - Black – pre-invasive
- ✓ It is important to keep the blocks and slides together; a filing system of archival blocks is used for controls and a system of case studies for arraying. A sectioned H and E slide is filed behind the block.

ARRAYERS: There are several different arrayers on the market today.

1. The automated arrayer is easy to use and includes a specimen tracking software system. The instrument marks, edits, and saves punch coordinates using an onscreen display and software tools. It pre-marks the punch areas. The video merge unit displays pre-marked slide images side by side to the donor block image. Some 120 to 180 cores can be punched per hour. The automated arrayer is ideal for a laboratory with a high volume of TMAs.
2. Using a manual arrayer you have to rely on your map or grid sheet.

- ❖ Visual selection while punching depends on the technologist, who uses a hand-held magnifying glass or magnifying lamp attached to the counter or a base to hold the magnifying lamp in place.
- ❖ The pathologist marks all areas of interest on the slide and the technologist makes the movements.
- ❖ Cores punched per hour depend on the experience of the worker. The average number of cores punched per hour using the manual arrayer is 30 to 70.
- ❖ To prepare microarray blocks for special stain controls, or QC controls for Hand E staining, an inexpensive pen extractor would be suitable.
- ❖ No matter which arrayer you decide to use you need to have a quiet environment where you will not b distracted and to become familiar with the equipment by constructing a practice array block.
- ❖ The number of specimens per array depends on the size of the punches and the desired array density.

- ❖ Using a 0.6 mm needle you can construct tissue arrays with 400 or more cores per block. Using a 1.0 mm needle, you can construct about 200 cores per block.
- ❖ Using a 2.0 mm punch allows you to construct 50 to 100 cores per block. We prefer using the 1.0 mm needle, allowing us to get a desirable core and leaving little distortion to the donor block.

PREPARATION OF THE RECIPIENT AND DONOR ARRAY BLOCK

- ✓ A blank paraffin block is prepared and used as the recipient for the tissue samples. It is best to use soft paraffin and to make sure there are no holes in the paraffin block caused by air bubbles.
- ✓ Typical core spacing and number of cores using various needle sizes.
- ✓ To ensure the alignment of the punches, first move the recipient punch into position and make a mark in the paraffin.
- ✓ Then do the same for the donor punch. Move the needles to the position of the first punch with the X or Y micrometer adjustment controls.
- ✓ The position of the punches over the block can be made by gently pushing down on them until a mark is made in the paraffin. Continue to make adjustments with the micrometer knobs until the desired position is attained, then zero the micrometers.
- ✓ The empty recipient block is placed in the holder and the attachment screws are tightened to keep the block from slipping.
- ✓ Place the recipient block notched edge to the left of the block holder. Making a hole in the first position begins the array process. The smaller needle is used to create the hole.
- ✓ First the depth stop is adjusted and its nut is tightened to stop the needle at the correct depth.
- ✓ The needle is pushed downwards by hand; the depth stop limits this motion and the handle in the needle is used to rotate the needle. The downward pushing pressure is relieved and springs will pull the needle upwards.
- ✓ The stylet is used to empty the needle. Do not remove the stylets from the needles during the array process.
- ✓ The donor block bridge is placed over the recipient block and the turret is moved to switch the larger needle into a vertical sampling position. The donor block is moved under the sampling needle.
- ✓ The larger needle is used to retrieve the sample. The H&E slide is moved out of the way and the needle is pushed downwards to retrieve the sample.
- ✓ The depth stop does not block the needle motion from the donor block.
- ✓ Care must be taken to prevent the needle from entering too deep. Depending on the tissue type and the purpose of the study it is best to have three punches of the same site.
- ✓ This way the tissue sample is well represented in evaluation of prognostic markers. Use of a four block indexer allows four replicate blocks to be made at the same time.
- ✓ Cut down the length of the donor core if it is too tall to fit in the recipient punch; this can be done by ejecting the core with the stylus and placing it on a clean flat surface.
- ✓ Use a clean razor blade to cut the core to the desired length; the core is then placed into the recipient block using a pair of forceps.

SMOOTHING AND SECTIONING

The array block must be smooth and level before sectioning. There are two methods in use in our laboratory.

❖ Method 1

Place a clean microscope slide on top of the array block and warm in a 35-37°C oven checking at intervals of 3 minutes. Do not leave the block in the oven or the paraffin will melt causing loss of tissue orientation. Move the slide in a gentle circular motion and press. You will notice circles on the slide. Place the slide and block in the refrigerator or freezer. Once cooled, the slide will separate from the array block.

❖ Method 2

Heat a clean microscope slide to around 70-80°C and touch it to the array block surface. The surface of the block will begin to melt. Move the slide in a circular motion and place the slide and block in the refrigerator or freezer.

MICROTOMY

- ✓ Set the temperature in your water bath at 37°C. Gently face off the array block on a dedicated microtome. Cut sections at 4-5 microns.
- ✓ Always use positively charged slides for the microarray sections. A hundred or more sections, depending on the skill of the technologist, can be cut from the array block. Place the sections on the slide in the same orientation.

- ✓ Stain one slide for Hand E. The unstained slides are placed in a box and stored at -20°C. Sections can be cut a day or two before they are stained.
- ✓ To avoid contamination place in a slide box and store. Sectioned TMA blocks should be dipped in paraffin to avoid loss antigens.
- ✓ Excessive soaking or freezing can cause the tissue to swell and keep the array block from ribboning well.
- ✓ The ability to study archival tissue specimens is important.
- ✓ To collect samples, cores can be placed into Eppendorf tubes and labeled.

TROUBLESHOOTING AND TIPS

- Core does not come out of the punch easily – punch tip is bent or distorted. Change the punch.
- Tissue core was pushed too deep – remove the sample with the small punch and place a new sample in the same position.
- Insufficient spacing of cores – can cause minor cracks or stress on the core when sectioning.
- Thinning of TMA cores in block – this is a result of repeated sectioning of the same block where cores are uneven in block.
- Loss of tissue on water bath – due to folds, wrinkles, and mishandling of ribbon.
- Re-facing block – when sections are cut to accommodate slides for stains requested. If the block is filed and then pulled for cutting of extra slides, the block is repositioned and re-faced. This is why it is important to use a dedicated microtome.
- Re-facing angle – shortens the life of the tissue micro-array block which is called thinning. Make sure the cassette is completely flat on top of the mold.
- One of the major drawbacks of TMA technology is the high cost of TMA facilities. The high cost of the array machines limits its use in general practice in many countries. The tissue microarray machines from Beecher instruments, San Prairie, Wisconsin, USA costs anywhere from 12,000 USD to 42,000 USD (7). Even the manual ones cost around 1200 USD, which are not be affordable to many laboratories in developing countries. Efforts have been made by many researchers to devise more cost effective TMA construction techniques.[7-12]

APPLICATIONS:

1. Immunohistochemistry
2. Staining of: H & E, ISH
3. In situ PCR
4. RNA or DNA expression analysis
5. TUNEL assay for apoptosis
6. Morphological and Clinical characterization of many patient tissue samples

FROZEN TISSUE MICROARRAY TECHNOLOGY FOR ANALYSIS OF RNA, DNA, AND PROTEINS:

- ✓ One difficulty with paraffin embedded tissue relates to antigenic changes in proteins and mRNA degradation induced by the fixation and embedding process.
- ✓ Consequently, there is a need to identify additional methods that allow for the optimal preservation of DNA, RNA, and proteins in tissue microarrays.
- ✓ Researchers have developed a new method for preparing tissue microarrays using non-fixed, fresh frozen tissue. Sections prepared from these tissue microarrays provide excellent target material for the study of DNA, RNA and proteins as each section can be fixed in a manner specific to the corresponding technique used.

ADVANTAGES: Allows fixation sensitive reagents to work effectively. Procedures requiring fixation can be conducted in samples fixed in an identical manner, providing greater uniformity than with paraffin embedded tumor microarrays. Each tissue array slide can be individually fixed in a manner specific to the corresponding technique used, allowing optimal fixation for DNA, RNA and protein targets.

CONCLUSION:

The major limitations in molecular clinical analysis of tissues include the cumbersome nature of procedures, limited availability of diagnostic reagents and limited patient sample size. The technique of tissue microarray was developed to address these issues.

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