Advances in Tissue Engineering & Regenerative Medicine

Short Communication

Etched analytical note on various histological staining protocols

Abstract

In-vitro Microarray method is based on the concept of nucleic acid hybridization which involves comparative analysis of DNA or RNA molecules of identical sequences in the test samples. It also involves various steps of histopathology in a highly sensitive technique, three-dimensional micro-physiological in-vitro cultures, called organoids and spheroids. It is more promising approach for more quick and efficient finding for patient clinical diagnosis relevance with more reliable therapeutic productivity in various case studies. Even so, at present days, more potent and effective characterization methods for three-dimensional structures are controlled in small groups of evaluation of simplistic live/dead techniques. Various protocols proposed for sectioning and staining in-vitro micro tissues which are considered by clinical scientists for examining and visualization of various molecular and biochemical structures like DNA, RNA and protein targets. So that, their respective various morphological examination and immunochemistry staining for in-vitro tissue cultures were performed to test more than hundred numbers of samples at one time or in one go. The cellular or tissue microarray that are explore for high throughput screening and analysis for large number of biological molecules to get more reliable and predictable observation of tissue samples. Microarray involves the evaluation of many similar or same kind of sample having same purpose in a single go rather than performing them individually following their respective immuno histochemistry, immuno fluorescence in situ hybridization.

Keywords: tissue microarray, tissue spheroids, micro physiological system

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Introduction

With the advances of molecular genetics and gene biology approach, molecular medicine and diagnostic tools are invented to find various molecular sample preparations of lethal and fatal pathogenesis and diseases for interpreting various tumour or cancer models. Tissue microarray method permits parallel evaluation of molecular profiling of various clinical samples. This method is helpful for immune chemists and histopathologists for quick, cost effective and efficient analysis of large-scale testing and interpretations of spheroids and organoids using immunohistochemistry based methods, fluorescent studies of in situ hybridization and ribonucleic acid in situ hybridization. Various proposed 3D cell spheroid and melanoma models can be considered to find out various therapeutic effects of drugs for studying various clinical aspects of tissue development, regeneration and oncogenesis.[1–3] The importance of these models is enhanced by high throughput 3D spheroid culture technologies allowing pathologists and clinical researchers to culture spheroids and organoids under different experimental variables. Microarray technique is considered more efficient approach for carrying out various proposed pathohistological sectioning/embedding/staining that are feasible for vast three-dimensional cell spheroid sample sets. Different types of Hematoxylin and eosin (H&E) stained cell lines including other staining protocols can be well very helpful to interpret histology of tissues. Hence, this various staining protocols including microarray method are used to optimize tissue/spheroids models/ stem cell models of many fatal pathogenesis like cancers, benign-tumour and tumour for tissue based engineering, safe examination with effective evaluations in tumours and cancer research.[4–7]

Materials and methods

Preparation of hematoxylin and eosin (H&E) paraffin block

Paraffin blocks is prepared by isolating cylindrical tissue core called biopsies from paraffin donor blocks and performed the re-embedding of them into a single recipient called microarray block at defined array coordinates as per experimental design. Firstly, donor blocks which invariably stored paraffin blocks are fetched and section cutting was done to prepare standard microscopic slides which are examined with hematoxylin and eosin staining (H&E).[3,4] A tissue microarray set up is used to prepare tissue core from the donor block and this core is further placed in an empty paraffin block called recipient block. Paraffin infiltrated agarose microarrays preparation were mounted in microtome and prepared core is placed at accurate recorded and assigned coordinate, typically on a Microsoft Excel.[4,5] All hepatic tissue specimens have been prepared in 7.5-20 µm sized sections and stained with Hematoxylin and eosin. Tissue microarrays are consisted of tiny tissue cores up to size of 0.6 mm in diameter, exact from specified regions of interest in prepared paraffin embedded tissues. These prepared tissue cores are customized by using hollow needles of fixed diameters by inserting in to paraffin blocks. These tissue cores are further placed into set slots in recipient paraffin block in a precisely pattern and area of interest is delineated accordingly. Sections of tissue array block are incised by using microtome, mounted on a microscope slide, and analysed.[6,7]

IHC (Immuno Histochemistry) procedure

Immuno histochemistry technique is used to check the antigen-antibody reaction in the tissue sample used to observe its anatomical, physiological, immunological interpretations. To avoid any fungal contamination for long preservation of collected tissue samples, add thymol and filter. The sections were placed on slide and keep it on the hot plate and deparafinize. Keep the slide in 3% hydrogen peroxide for at least 25 minutes. The sample was retrieved for the antibody in microwave for six minutes followed with washing by distilled water and TBS buffer. Protein block was added for 10 to 15 minutes and wipe it followed by pouring the primary antibody and keep it overnight. Next day, it was washed with TBS and added secondary antibody for proper binding of primary antibody. Further it was washed with TBS and added tertiary antibody 10 to

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20 minutes followed by adding DAB solution (DAB buffer +DAB brown coloured chromogen) which allowed to get better intensity of visualization of antigen-antibody complex in observed tissue when mounted with DPX.

Preparation of oil red staining

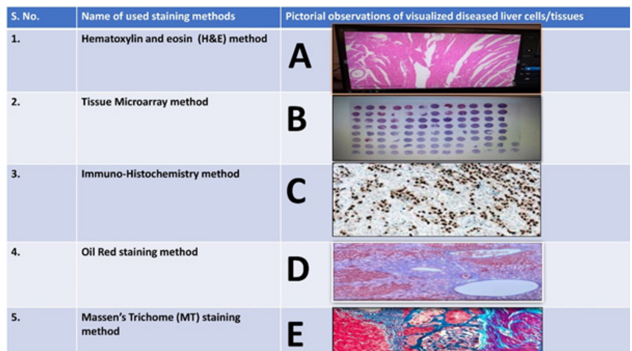
This stain is used to observe neutral fats in liver tissue in various pathological studies. Staining procedure is needed cryopreservation storage (80 degree Celsius) followed by melting for 25 minutes. Tissue was placed on the chuck plate and performs embedding using cryomatrix in the cryotome instrument for getting their sectioning of about seven micro meter sized slices. The trimmed slide was kept on the hot plate for fixation followed for staining to be placed in oil red stain jar for 1 to 2 hrs.

Preparation for massen's trichome staining (MT Staining)

MT staining is used to detect collagen, cytoplasm and nuclei. Trichome stain is consisted of combination of three different types of stain. Collected sample was deparaffine and dipped in Bouin's fluid solution for more than 1 hr in water bath at 25 degree Celsius. Prepared slide was rinsed under running tap water for 5 to 10 minutes. It was further counter stain with haematoxylin followed by washing in distilled water. Stain with Beibrick scarlet stain was used to stain the slides keep the slides dipped for 10 to 15 minutes followed by washing in distilled water. Phosphotungsten stains were dropped on slides and keep it untouched for 6 to 8 minutes. Wiped out the Phosphotungsten and Anline blue was poured and again kept the slide untouched for 8 to 15 minutes. It was followed by washing with glacial acetic acid and alcohol.[8]

Results and discussions

The visualization of the slide which is stained with Hematoxylin and eosin (H&E) (Figure 1A) was showed the following results where pink coloured areas are visualized and light pinkish blue marks are spotted when subjected to light microscopic examinations. The Haematoxylin stains the cell nuclei were observed in blue colour because it is a basic dye and cationic in nature that stains the acidic structures. The Eosin stains the cytoplasm in the pink colour and stained cell components such as muscular fibres, intracellular and extracellular fibres. The analysis is done on the computer named as Invitrogen "EVOS" FL Auto 2 Imaging System.[7,8] Studying the spheroid microarray, 10 cell-traces as spheroids were cultured and embedded them in mixed cell line microarray. Hematoxylin and eosin staining was used to study spheroid morphology. Even so, micro sized sample of each tissue core was compared with single tissue section in the range of 0.6–3.0 mm in diameter being as representative of donor array block. Block of cancerous tissue is mostly to have some area of normal tissue as well and random core sampling was planned to highlighted precise area of normal liver tissue. Because of this, more than two cores are chosen from a donor array block and transferred into the Tissue Microarray recipient block for examining the areas of targeted critical lesions microscopically (Figure 1B).[6,7] Some cell line traces longer form spheroid were prepared in loose aggregates as a substitute although the fulfilment charge for transfer of spheroid of loose aggregates got minimized. At the same time as a few cell traces are observed together with the pancreatic cancers that derived formed compact round spheres in the tissue microarray block (Figure 1B).[8,9] When results was analysed with Immuno-histochemistry (IHC) protocol in the microscope, brown coloured was observed in the slides where antigen –antibody reaction was occurred (Figure 1C). When interpretation was done with analysis of oil red staining methods, visualized that fat cells and adipose cells were appeared red and nuclei appeared in blue colour (Figure 1D). While studying the results of Massen's Trichome staining (MT) staining, it was found that fatty tissues having fibrosis or any muscle fibre and cytoplasm was appeared red and collagen resting area was appeared blue; nuclei stained black and background is stained remain red (Figure 1E). Spheroid or tissue cores have been showed necrotic and well visualized with intense effect for their best histological examination or interpretations when observed in the Hematoxylin and eosin (H&E) staining (A) as compared with other methods (Figure 1) whose results are well akin with previous cited observations.[10–12]



S. No.	Name of used staining methods	Pictorial observations of visualized diseased liver cells/tissues
1.	Hematoxylin and eosin (H&E) method	A
2.	Tissue Microarray method	B
3.	Immuno-Histochemistry method	C
4.	Oil Red staining method	D
5.	Massen's Trichome (MT) staining method	E

Figure 1 Pictorial comparative visualized observations of hepatic tissue segments by using different staining methods/procedures 1 Hematoxylin and eosin (H&E) method, 2 Tissue microarray, 3 Immuno-Histochemistry method, 4 Oil red staining method, 5 Massen's Trichome (MT) staining method

Conclusion

In-vitro tissue microarray is considered more powerful cost effective, quick and reliable diagnostic tool for clinical interpretations of many fatal diseases or cancers. Hence, this technique have also set benchmarks in diagnostic and therapeutic studies and also studying the biomarker for various proposed experimentation based on drug expression. Tissue microarray methodology is found to be main backbone in the routine pathology staining examinations to predict various tissues morphology including abnormal pathology or any response of cancer therapy given to patients. It also has wide range of implicit and incise explorations in prognostic oncology and drug discovery. But better visualized results were reported for H&E staining (hematoxylin and eosin staining) and this protocol was remain first choice for histopathologists for more decades worldwide and considered this protocol as the Gold Standard in cancer/tumour diagnosis even when used for tissue microarray method. Precise and potent morphological examination of cell nucleus and cytoplasm was done with ease by using hematoxylin and eosin staining (H&E staining) and volume bright-field imaging can be subjected for enhancing the efficacy of the present method. Hence, diagnostic criterion that are based on H&E staining might be proposed to study tumours spatially and the stain can reveal specific chemical components of cells for providing more precise subcellular structural information of lesions of examined biopsy samples. All these features are lacked in other stains and techniques, so that H&E staining is preferred most easy and reliable staining method for histological examinations among other most popular histological staining protocols including tissue microarray.[13,14]

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Conflicts of interest

The author declares no conflicts of interest.

References

1. Ivanov DP, Grabowska AM. *In Vitro* Tissue Microarrays for Quick and Efficient Spheroid Characterization. *SLAS Discov*. 2018;23(2):211–217.
2. Packeisen J, Korsching E, Herbst H, et al. Demystified...tissue microarray technology. *Mol Pathol*. 2003;56(4):198–204.
3. Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM. Histological Stains: A Literature Review and Case Study. *Glob J Health Sci*. 2015;8(3):72–79.
4. Underwood J, Lowe D. Recent Advances in Histopathology 20. London: *Royal Society of Medicine.* Chap 6, Tissue arrays: construction and applications. 2003.
5. Sauter G, Mirlacher M. Tissue microarrays for predictive molecular pathology. *J Clin Pathol.* 2002;55(8):575–576.
6. Horvath L, Henshall S. The application of tissue microarrays to cancer research. *Pathology.* 2001;33(2):125–129.
7. Rubin MA, Dunn R, Strawderman M, et al. Tissue microarray sampling strategy for prostate cancer biomarker analysis. *Am J Surg Pathol*. 2002;26(3):312–319.
8. Deeb S, Badii M, El-Begawey M, et al. Tissue microarrays and their use for preparation of reference slides for educational purposes in histology and histopathology. *African Journal of Health Sciences*. 2008,13(3),66–69.
9. Feller JK, Yang S, Mahalingam M. Immunohistochemistry with a mutation-specific monoclonal antibody as a screening tool for the BRAFV600E mutational status in primary cutaneous malignant melanoma. *Mod Pathol.* 2013;26(3):414–420.
10. Simon R, Sauter G. Tissue microarrays for miniaturized high-throughput molecular profiling of tumours. *Exp Hematol*. 2002;30(12):1365–1372.
11. Biunno I, Paiola E, De Blasio P. The Application of the Tissue Microarray (TMA) Technology to Analyze Cerebral Organoids. *J Histochem Cytochem*. 2021;69(7):451–460.
12. Gabriel J, Brennan D, Elisseeff JH, et al. Microarray Embedding/Sectioning for Parallel Analysis of 3D Cell Spheroids. *Scientific Reports.* 2019;9(1):16287.
13. Hoos A, Urist MJ, Stojadinovic A, et al. Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. *Am J Pathol*. 2001;158(4):1245–1251.
14. Moraes GS, Wink MR, Klamt F, et al. Simplified low-cost methodology to establish, histologically process and analyze three-dimensional cancer cell spheroid arrays. *Eur J Cell Biol*. 2020;99(5):151095.

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