APMIS 118: 739–743


DOI 10.1111/j.1600-0463.2010.02652.x

# A simple and economical method for the manual construction of frozen tissue arrays

SHU-CHUAN TSAO,[1] CHUN-CHIEH WU,[1] CHIEN-HUI WEN,[1] YA-CHUN HUANG[2,3] and CHEE-YIN CHAI[1,2,3]

[1]Department of Pathology, Kaohsiung Medical University Hospital, Kaohsiung; [2]Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung; and [3]Department of Pathology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Tsao S-C, Wu C-C, Wen C-H, Huang Y-C, Chai C-Y. A simple and economical method for the manual construction of frozen tissue arrays. APMIS 2010; 118: 739–43.

Tissue microarray has been developed to enable multiple cores of tissue in one or more new paraffin blocks. Currently, almost all tissue microarrays are made by coring cylindrical tissues from formalin-fixed and paraffin-embedded tissues. The disadvantages of formalin-fixed and paraffin-embedded tissues include the poor preservation of antigenicity of certain proteins and mRNA degradation induced by the fixation and embedding process. However, frozen tissue array construction presents technical difficulties, and tissue array devices are expensive, particularly for small- and medium-sized laboratories. We describe a simple manual method for producing well-aligned tissue arrays by a capsule freeze method that allows us to successfully perform hematoxylin–eosin and immunohistochemical stain. All 120 tissue samples were collected and constructed into blocks by this capsule freeze method. The capsules were not affected during the sectioning process, and the capsule material always disappeared during the aqueous steps of the stain processing. The frozen tissue arrays were smoothly sectioned without the use of a tape transfer system and immunohistochemical study was performed with satisfactory results. This alternative method can be applied in any laboratory, and is both simple and economical.

Key words: Frozen tissue; tissue array; capsule.

Chee-Yin Chai, Department of Pathology, Kaohsiung Medical University Hospital, No. 100, Tzyou 1st Road, Kaohsiung 807, Taiwan, ROC. e-mail: cychai@kmu.edu.tw

Tissue microarray (TMA) has been developed to enable multiple cores of tissue in one or more new paraffin blocks (1). It is increasingly being used for high throughput analysis of the diagnostic, predictive, or prognostic value of candidate biomarkers for large numbers of samples (2, 3). Currently, almost all TMAs are made by coring cylindrical tissues from paraffin-embedded, formalin-fixed tissue donor blocks and transferring cores into a paraffin-recipient block (4, 5). One difficulty with paraffin-embedded tissue relates to antigenic changes in proteins and mRNA degradation induced by the fixation and embedding process (6, 7). Recently, an optimum cutting temperature (OCT)-embedding method was developed to prepare TMAs from unfixed fresh tissues (8). It provides an excellent way to store and analyze tumor samples and may prove useful for identifying novel molecular targets for diagnosis, prognosis, and therapy of cancer, but it requires the use of an expensive TMA instrument. However, due to a limited budget, a small laboratory is not always able to afford an expensive tissue array device. Here, we report on our modified method for the manual construction of frozen tissue arrays by the capsule freeze method that allows us to successfully perform hematoxylin–eosin (HE) and immunohistochemical (IHC)

Received 12 January 2010. Accepted 1 June 2010

staining. Furthermore, we envisage that this simple and economical technique could be applied in many pathology laboratories.

## MATERIALS AND METHODS

### Tissue collection

All 120 tissue cores from different human tissues (including thyroid, liver, breast, uterine, lung) were tested for preparing frozen tissue array and then were subdivided into three blocks.

### Frozen tissue array construction

Unfixed fresh tissue was embedded in cryomatrix embedding medium (Shandon, Pittsburgh, PA, USA) and frozen. HE staining was performed and the slides were examined using light microscopy. The representative area of each frozen tissue block was marked using a color pen. A tissue punch extractor (Pin-Extractor; Ho Hua Electricity, Taichung, Taiwan) was used to obtain two cylinders 3 mm in diameter from the selected area of each frozen tissue block at −5 to −10 °C inside the cryostat microtome. We then modified a previously reported method (9). Each size '01' gelatin-based capsule (Da-Ming Capsule Co., Ltd., Taichung, Taiwan) was filled with two to three drops of cryomatrix embedding medium immediately before placement of the specimen. Using a pair of forceps, two tissue cylinders were carefully placed within a capsule followed by one to two drops of cryomatrix embedding medium. The capsule was then immediately frozen at −80 °C for 1–2 min (Fig. 1A). After freezing, a part of each capsule was cut using a scalpel for unit tissue appearance. Frozen TMA was prepared according to a previously published method (10). The stainless steel container contained a thin layer of cryomatrix embedding medium to hold the capsules (Fig. 1B) in the cryostat chamber at −25 °C; one recipient box could accommodate 20 capsules (40 frozen tissue cylinders). After all capsules were aligned in the stainless steel container, the cryomatrix embedding medium was gently poured into the container until it was full. The container was then frozen at −80 °C for 10–15 min and the frozen block was removed from the container. Five-micrometer thin frozen sections were cut from the array

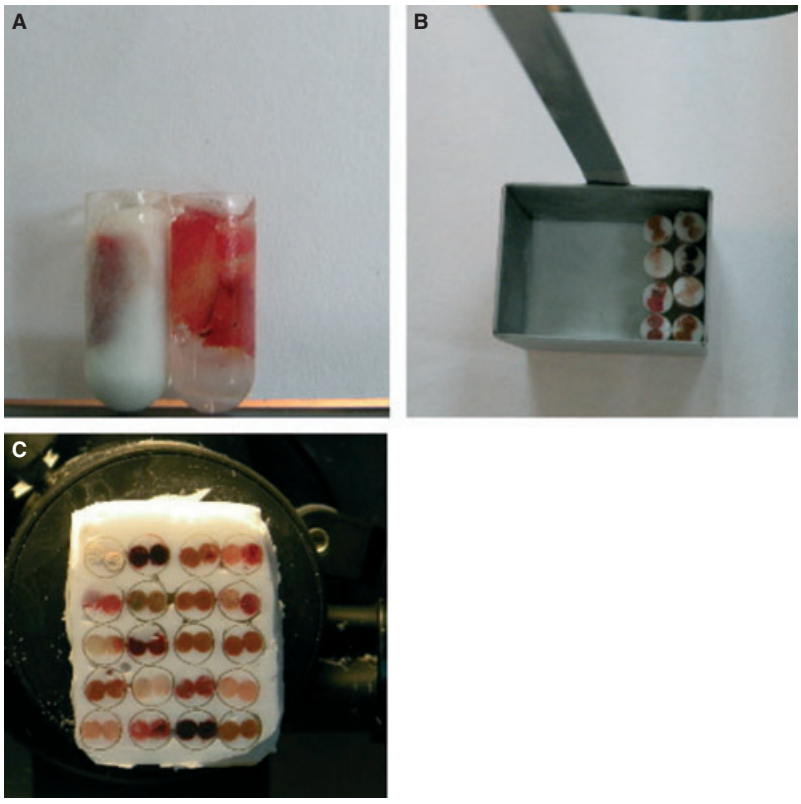


**Fig. 1**. (A) Two tissue cylinders were carefully placed within a capsule. (B) The stainless steel container contained a thin layer of cryomatrix embedding medium to hold the capsules. (C) The finished frozen tissue array block.

block using a cryostat microtome. The remaining frozen tissue array was stored at −80 °C and HE or IHC staining was performed.

### Immunohistochemistry staining

The slides were fixed in 100% cold acetone for 5 min. Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide for 5 min. The sections were first incubated with a cytokeratin monoclonal antibody for 30 min. Immunohistochemistry was performed using standard streptavidin–biotin–peroxidase complex (SABC) method, with 20-min incubation for both steps (second antibody and SABC). Between each stage of the procedure, careful rinses were performed with several changes of phosphate-buffered saline. Finally, diaminobenzidine was used as a chromogen and arrays were visualized and photographed using standard light microscopy.

## RESULTS

As shown in Fig. 1C, the frozen tissue array block contained 40 unfixed frozen tissues. We constructed the frozen tissue array, so the capsules were not affected during the sectioning process, and the capsule material always disappeared during the aqueous steps of the stain processing. HE and IHC staining were performed successfully (Fig. 2). The frozen tissue arrays revealed a smooth section, and one sample was lost during the HE staining process. However, the number of tissue losses did not exceed 1–2 per slide during immunostaining. Figure 2B shows the HE stain of liver tissue, and Fig. 2C demonstrates the result of IHC staining of cytokeratin. Tissue morphology and architecture are rather well preserved in the sections. In the IHC cytokeratin stain, the hepatocytes show positivity in their cytoplasm; however, the blood vessels reveal negativity in staining. The staining result is consistent with the paraffin section.

## DISCUSSION

Of late, we have tried different methods to build satisfactory frozen tissue arrays, and we have found the method described herein to be most successful. The critical point is encapsulation and a kind of freezing embedding medium. We found the frozen tissue cylinders without capsules were lighter than the frozen tissue cylinders with capsules, and so when the embedding medium was poured into the container, all frozen tissue cylinders easily floated and touched each other. To improve this situation, we used the capsules to support the tissue cylinders and

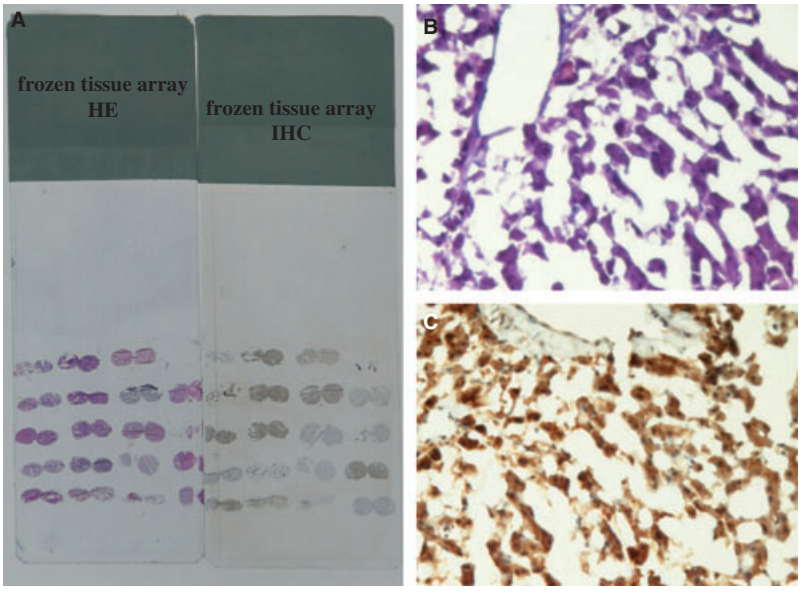


**Fig. 2**. (A) Hematoxylin–eosin (HE)- and immunohistochemical (IHC)-stained slides of a frozen tissue array. (B) HE staining in the frozen tissue array section (200×). (C) IHC staining for cytokeratin (200×).

avoid them collapsing because of the additional freezing embedding medium in the container. In another method, we tried to use two different freezing embedding media for preparation of the frozen tissue array block, and we found that the Shandon cryomatrix embedding medium was softer and easier to section than the Jung tissue freezing medium (Leica, Germany) (not shown).

The preparation of frozen tissue arrays is similar to that of paraffin arrays except that to avoid ice crystal artifact, all coring and preparation must be performed at subzero temperatures. First, we punctured frozen tissue at −5 to −10 °C inside the cryostat microtome because the temperature range is easier for manual coring without compromising morphology and RNA/protein quality (11). Second, when frozen tissue was added into capsules containing cryomatrix embedding medium, the tissue and capsule would quickly become softer and more difficult to handle, therefore tissue cores should be placed into capsules for not too long a period and must be immediately frozen.

Tissue microarrays have been widely used for a number of years in human medicine, although a lack of consensus still exists on how many core biopsies should be representative of whole tissue section. Most authors are in concordance with increasing the number of cores collected from each sample and/or to increasing the size of the single core biopsies to ensure representation of the whole tissue (12–17). In this study, placing two frozen tissue cores into the capsule also allowed us to increase the number of tissue cores; the number and size of which sufficed to produce recognizable histologic features. In constructing a frozen tissue array, a ''known'' and labeled tissue can be arranged at the top-right or top-left corner of the block for the purpose of recognition and proper orientation of the frozen tissue array block.

Some methods designed for frozen tissue array have been reported by Scicchitano et al. (8) and Zhou et al. (11). In these methods, the OCT compound was used as the array recipient block matrix to prepare high quality TMAs. However, the former required the use of an expensive tissue array device and a tape transfer system. The latter did not need an expensive tissue array device but they needed a special instrument – a steel stamp. In comparison with the OCT-base technique, even though the frozen tissue array that we describe contains small numbers of tissues compared with other frozen tissue arrays (which can contain up to 96 samples), it is still a good alternative when budgetary constraints are important, a tissue array device is not available, or only a limited number of samples need to be tested.

In summary, the simple and economical method for the construction of frozen tissue array by the capsule freeze method can be applied in most pathology laboratories, thus obtaining an easily aligned frozen tissue array without the need for specialized equipment or extra cost.

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