Simple, Inexpensive, and Precise Paraffin Tissue Microarrays Constructed With a Conventional Microcompound Table and a Drill Grinder

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Abstract

In 1998, paraffin tissue microarrays (PTMAs) as paraffin blocks containing up to 1,000 cylindrical paraffin tissue core biopsy specimens (PTCBs) for high-throughput molecular profiling of tumor specimens were introduced. PTCBs can be constructed using a manual tissue puncher/arrayer (Beecher Instruments, Sun Prairie, WI; cost, at least $7,000). Furthermore, custom-built PTMAs such as the MaxArray are created by companies such as Zymed Laboratories (South San Francisco, CA; PTMA with 96 holes, about $900). In our search for a less expensive alternative, we constructed PTMAs with up to 558 PTCBs by using a drill grinder, a drill stand, and a microcompound table (Proxxon, Niersdorf, Germany; cost, <$300).

Materials and Methods

Paraffin Tissue Punches

To manufacture the paraffin tissue punches, conventional hypodermic needles (eg, Sterican 20 gauge, B. Braun, Melsungen, Germany; inner diameter, 0.6 mm), so-called percutaneous entry needles (eg, BARDSelect PEN70, C.R. Bard, Murray Hill, NJ; inner diameter, 1.0 mm), and aspiration needles (eg, B. Braun; inner diameter, 1.7 mm) were shortened and sharpened using a drill grinder fitted with a cutting disk (Micromot 50/E, Proxxon, Niersdorf, Germany; cost, $50). Moreover, skin biopsy punches of different diameters (2-6 mm)
were used as paraffin tissue punches (kai Europe, Solingen, Germany) Image 1. The existing channel in the plastic handle of these skin biopsy punches was focally enlarged by drilling to allow the insertion of the stylet. Other hypodermic needles (eg, the Microlance 3, Becton Dickinson, Drogheda, Ireland; outer diameter, 0.55 mm) or the trocar of a bone marrow biopsy needle (Manan Medical Products, Northbrook, IL; outer diameter, 1.7 mm) were shortened and used as stylets (Image 1). Before using the punches, metal grinding debris had to be removed, eg, by compressed air.

**Construction of Recipient Blocks to Become PTMAs**

The recipient paraffin blocks, which would become the PTMAs, were made of ordinary plastic cassettes and paraffin (Paraplast Plus, Sherwood Medical, St Louis, MO) poured in ordinary steel embedding molds. To avoid air bubbles being trapped under the plastic, the cassettes were warmed to 62°C before filling. After pouring, the paraffin blocks were cooled at room temperature to avoid cracks. To facilitate the separation of the paraffin blocks from the steel molds, the plastic cassette–paraffin-mold sandwiches were stored at 4°C in the refrigerator for a few minutes, if necessary. Before drilling, the recipient paraffin blocks were examined for air bubbles and paraffin cracks and sorted out if there were any. Holes of different diameters were drilled in the recipient paraffin blocks with the drill grinder (eg, diameter of 0.6 or 1.0 mm; distance between holes, 0.2-0.5 mm; depth, 4.8 mm). For this procedure, the paraffin block was mounted on a microcompound table (K70, Proxxon; cost, $85) in a drill stand (MB140/S, Proxxon; cost, $50). The coordinate structure of the PTMA was achieved by turning the handwheels of the stage with a minimum movement of 0.05 mm Image 2.

Drilling results were improved by cooling the drill by immersing the recipient paraffin blocks in a water bath made of polyvinyl chloride (cost, $50). The paraffin blocks fit exactly into the water bath and were fixed additionally with 4 plastic screws. The water bath was filled with distilled water at room temperature. Before the holes were filled with...
PTCBs, the PTMAs were allowed to sit so the water trapped in the holes could evaporate.

**Filling the Recipient Block**

The PTCBs were removed manually from donor paraffin-embedded tissue blocks [Image 3] and transferred to the holes of the PTMAs by using the self-made paraffin tissue punches. To establish the orientation within the PTMA, special types of tissues were deposited at certain positions (eg, melanoma in the right lower corner of the array). The PTCBs in the different holes of the PTMA were recorded in an MS Access-file (Microsoft, Redmond, WA).

**Validation of the New Technique**

To validate this new technique, several PTMAs (eg, PTMA 1: 558 holes; diameter of holes, 0.6 mm; distance between holes, 0.3 mm; Image 3) were constructed and filled with different types of tissue samples (eg, lung, melanoma, breast cancer, colorectal adenocarcinoma, myometrium).

**Cutting and Staining**

When all holes were filled, the PTMAs were carefully pressed down on a smooth surface (eg, a glass plate) to even the surface of the PTMA. Then the PTMAs were stored in an incubation chamber at 50°C for at least 15 minutes to make the paraffin specimens of the recipient block and the PTCBs sticky to produce a stronger bond. Following routine procedures, 2- to 5-µm-thick sections were cut from the PTMAs with a sliding microtome (Reichert and Jung, Heidelberg, Germany) and put on silanized slides. Alternatively, an adhesive-coated tape system (Instrumedics, Hackensack, NJ) was used. The slides were stained routinely with H&E, immunohistochemical stains, and for fluorescence in situ hybridization.

**Results**

In general, as shown in Image 3 and [Image 4], it was easy to construct PTMAs with different numbers of PTCBs, different diameters of the PTCBs, and different densities of the PTCBs, ie, different distances between the PTCBs. The self-manufactured paraffin tissue punches were well suited for manually punching the PTCBs from the donor blocks and transferring them into the holes of the recipient PTMA blocks. There was little damage to the donor blocks from the punches (Image 3). The heating of the steel molds and the plastic cassettes to 62°C before filling with paraffin usually was successful in preventing air bubbles from being trapped under the plastic cassette.

[Image 2] Drilling of the recipient paraffin block. The recipient block is fixed in a water bath (polyvinyl chloride) that is mounted on a horizontal x-y stage (microcompound table) in a drill stand (left and middle). The coordinate structure of the paraffin tissue microarray was achieved by turning the handwheels of the stage with a minimum movement of 0.05 mm. The drilled recipient block with 558 holes (diameter, 0.6 mm; distance, 0.3 mm) (right).
Drilling the holes in the PTMAs was easy. However, caution was required to prevent arithmetic positioning errors while turning the handwheels of the microcompound table. The water bath effectively cooled the drill. Furthermore, the drill chips floated on the surface of the water and could be removed easily for a good view of the paraffin block. However, it was not necessary to cool the drill if the holes were drilled at low rounds per minute, especially when drilling manually. In this case, the PTMA could be fixed on the microcompound table with a standard microtome clamp.

Most of the time, the manual transfer of the PTCBs to the PTMAs could be done easily. If a short PTCB was submerged in the PTMA, a second or even a third PTCB from the same donor block could be injected in the same hole. Care was needed to store the exact position of the deployed PTCB in the computer database.

Further processing of the PTMA with sectioning and staining could be performed according to routine methods. However, some problems occurred with rolling and folding of the PTCBs during sectioning with subsequent floating off the slide during the staining process. Surprisingly, consecutive sections of the same PTMA revealed different percentages of PTCBs being lost. The ratio of lost PTCBs ranged from 0% to 30% of all PTCBs in a PTMA. Furthermore, some sections split in the water bath after damage from the microtome blades, resulting in wastage of the precious PTMA sections. These problems of split sections and rolling up of the PTCBs during cutting, floating on the water bath, and staining were prevented successfully when the adhesive-coated tape system from Instrumedics was used.

Discussion

There is no doubt that the PTMA technique as described by Kononen et al. is a very effective technique in pathologic research (high-throughput molecular profiling) and routine work (on-slide control in immunohistochemical analysis or in situ hybridization) as shown by the growing number of articles about using this technique and commercially available PTMA products (eg, Zymed Laboratories). However, until now this technique has been restricted to institutions with the funds to buy a tissue puncher/arrayer (from Beecher Instruments, Sun Prairie, WI; cost, at least $7,000) or to let commercial companies do the expensive array construction (custom-built PTMA with 96 holes, about $900 [MaxArray System], Zymed Laboratories).
To lower the costs, an inexpensive and simple technique with which laboratories can construct PTMAs themselves was sought. In 2000, Gillett et al. described a self-made PTMA with 34 PTCBs with a core diameter of 3 mm (11 gauge). They also punched the holes of the PTMA with needles. The structure of the array was achieved by using the back of a standard R.A. Lamb processing cassette (Raymond A. Lamb, Durham, NC) as a template for punching. In 2003, Hidalgo et al. described their experience with a 36-hole PTMA created with bone marrow aspiration needles (14 and 16 gauge). The array structure was defined manually.

Like Gillett et al. and Hidalgo et al., we used conventional needles as paraffin tissue punches, as first described by Wan et al. in 1987. The construction of the paraffin tissue punches is not difficult. However, in contrast with the techniques used by Gillett et al. and Hidalgo et al., who constructed low-density PTMAs with PTCBs of larger diameters, we used a microcompound table to create the array matrix for high-density PTMAs with up to 558 PTCBs. Furthermore, the diameter of the cores was as small as 0.6 mm. Moreover, in contrast with Kononen et al., who punched the holes of the PTMA, and Lilischkis et al., who poured the PTMAs with metal spacers, the holes in the system described herein were drilled, making the system very flexible.

Ordinary hypodermic needles or biopsy punches are available for little money in a huge variety of diameters (0.6-6 mm) in every hospital. Drill bits can be purchased in any ordinary hardware store with diameters that match the inner diameter of the punches for a close fit between the paraffin samples of the PTMA and the PTCBs. The costs for these drill bits (<$5) are negligible compared with the costs of commercially distributed paraffin tissue punches. The drill bits can be
fixed in the chuck of the drill grinder. Because of the soft consistency of paraffin, drilling can be done quickly, and the drill bits do not become blunt. Just as with commercially available manual tissue arrayers, care must be taken when turning the handwheels of the microcompound table to avoid arithmetic positioning errors in making the holes. Even the manual transfer of the core tissue samples and the deposition into the holes of the recipient blocks worked well.

In contrast with the tissue puncher/arrayer with its depth stop, the manual transfer sometimes resulted in submerging the tissue samples in the PTMA holes. Fortunately, this submerging could be solved by deploying more than 1 PTCB per hole. The position of a tissue sample had to be recorded precisely in a data file. However, mistakes in filing may be recognized and corrected by depositing defined tissue samples at defined positions to provide a rapid overview of the structure of the PTMA. Such mistakes also are possible in arrays constructed with other methods. This is also true for the folding and rolling of the PTCBs at sectioning and for certain kinds of splitting of the section in the water bath.3,5,9

Up to 30% of the PTCBs were lost during the staining procedures, mainly owing to the detachment after folding during sectioning. In the water bath the coordinate structure also could be destroyed. Kononen et al3 suggested the use of an adhesive-coated tape system (Instrumedics) to overcome these disadvantages. However, this tape system might influence immunohistochemical and fluorescence in situ staining results.9 In the experience with the present method, a growing number of folded PTCBs was seen at sectioning with an increase in the thickness of the sections. Skacel et al9 solved this problem by briefly soaking the PTMAs in a low-concentration detergent solution before cooling on ice and cutting. Mengel et al5 found that the main reason for this folding was insufficient contact between the paraffin samples of the PTMAs and the PTCBs and solved this problem by fully melting the PTMAs before sectioning. By doing so, they reduced the percentage of lost PTCBs after staining to less than 1%.5 Hidalgo et al7 reduced the loss of PTCBs with a tight fit of the PTCBs in the holes of the PTMAs, the use of hot liquid paraffin on the surface of the PTMAs, and incubation of the PTMAs for 60°C for 15 minutes to blend the paraffin samples from the PTMAs and the PTCBs. The splitting of the sections during the water bath treatment was reduced considerably by prompt replacement of damaged, disposable microtome blades.

It was possible to construct high-density PTMAs with equipment costing less than $300. Arrays with various PTCB sizes, distances between holes, and different coordinate structures were manufactured. Most of the problems encountered also can occur with other PTMA construction techniques.

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Vogel and Bueltmann / INEXPENSIVE TISSUE MICROARRAYS

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References