SHORT REPORT

Depositing archived paraffin tissue core biopsy specimens in paraffin tissue microarrays using a paraffin tissue punch modified with a countersink

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Paraffin tissue microarrays (PTMAs) introduced by Kononen *et al* in 1998 have become a widely used technique in routine pathology and even more so in research. Kononen used a tissue puncher/arrayer (Beecher Instruments, Sun Prairie, WI, USA) to take paraffin tissue core biopsy specimens (PTCBs) of 0.6–2 mm in diameter from routine paraffin tissue blocks and transfer them to another paraffin block with up to 1000 holes. As pointed out by Mengel *et al*, however, it is not possible to use the Kononen/Beecher system to construct PTMAs out of archived PTCBs. To overcome this drawback in the extremely popular Beecher system, the paraffin tissue punch was modified by incorporating a conical 4 mm deep countersink. This countersink was milled with a conical precision cutter that can be bought in an ordinary hardware store (cost <US\$5). The countersink facilitates the insertion of an archived PTCB into the paraffin tissue punch and enables the construction of PTMAs with previously archived PTCBs using the widely distributed Beecher system. Moreover, this paraffin tissue punch can be used for other systems to create PTMAs, such as the low-budget systems designed by Vogel.

Paraffin tissue microarrays (PTMAs) introduced by Kononen *et al*¹ are a powerful tool for research and in routine pathology. By using a tissue puncher/arrayer (Beecher Instruments, Sun Prairie, Wisconsin, USA), Kononen constructed PTMAs with up to 1000 paraffin tissue core biopsy specimens (PTCBs). Punches made of thin steel tubes are used to take PTCBs of different diameters (0.6–2 mm) from so-called donor blocks—that is, for example, routine paraffin tissue blocks. These PTCBs are immediately transferred to holes in a recipient block. In 1987, Wan *et al*² suggested that tissues embedded in paraffin wax (eg, tumour tissues) could be stored as PTCBs in vials for later use in tissue microarrays.² However, until now, it has not been possible to use these stored PTCBs to construct PTMAs with the Beecher tissue microarrayer system or with the low-budget systems devised by Vogel.³ We overcame this disadvantage by modifying the paraffin tissue punch of the Beecher system with a countersink.

MATERIALS AND METHODS

A commercially available tissue punch (Beecher Instruments; inner diameter about 1 mm) was modified by milling a 4 mm deep countersink with a cone-shaped precision cutter (diameter 2.3 mm; Proxxon, Niersdorf, Germany; cost <US\$5) in the upper opening (fig 1A–D). The equipment for the milling was purchased in an ordinary hardware store and comprised a drill grinder (Micromot 50/E, Proxxon, Niersdorf, Germany; cost US\$50), a drill stand (MB140/S, Proxxon; cost US\$50) and a machine vice (MS4, Proxxon; cost US\$20). Optionally, the opening of the countersink can be enlarged for the first millimetre with a larger cone-shaped precision cutter (eg,

3–4 mm in diameter). The metal shavings were removed from the paraffin punch with adhesive tape and pressurised air. PTCBs of different paraffinised tissues were punched out manually and stored in microtubes (fig 1E). Tweezers were used to transfer the stored PTCBs into the countersink hole in the punch (fig 1F). The stylet of the corresponding paraffin tissue punch was used to push the PTCBs into the punch (fig 1G) and finally press them into the hole of the recipient paraffin block. The holes of the PTMAs were constructed by punching (Beecher system), pouring with spacers (Mengel system) or drilling with a conventional drill grinder (Micromot 50/E, Proxxon; cost US\$50)³ or a computer numerical control drilling machine (Maho MH400E, Deckel-Maho-Gildemeister, Bielefeld, Germany; cost US\$10/PTMA).³ Before the PTCBs were inserted into the countersink, the orientation of the PTCBs was checked to ensure that the tissue-bearing end of the PTCBs was on the surface of the PTMAs. After the holes were filled, the PTMAs were cut and the sections stained according to routine procedures.

RESULTS

The countersink was easy to construct (fig 1A–D). As fig 1H shows, we were able to construct PTMAs with up to 322 archived PTCBs. Less than 2% of the PTCBs broke when they were handled with the tweezers or the stylet. No difference in the utility of the paraffin punch was seen whether the holes were prepared by punching, pouring or drilling. The time needed to fill a PTMA with stored PTCBs was similar to that needed with freshly punched and inserted PTCBs, despite the added step of picking up the PTCBs with the tweezers.

DISCUSSION

Introduced by Battifora in 1986⁴ and perfected by Kononen *et al* in 1998,¹ PTMAs represent a powerful tool in routine pathology and research. PTMAs can be constructed as high-density PTMAs with up to 1000 PTCBs /for high-throughput molecular profiling or as low-density PTMAs with up to 100 PTCBs for use as controls in immunohistochemistry.⁵ PTMAs are generally made by punching PTCBs from routine paraffin tissue blocks and immediately transferring these PTCBs into the holes of the PTMAs. However, as described by Wan *et al* in 1987,¹ PTCBs may be archived in vials for later use in PTMAs² or, as outlined by Mengel *et al* in 2003,⁶ for DNA and RNA analyses. Until now, use of these stored PTCBs for PTMAs was restricted to the multitissue “straws” system of Wan *et al*² and the Max-Array system, invented by Mengel *et al*⁶ and now probably used by Zymed (Zymed Laboratories, South San Francisco, California, USA). By modifying the tissue punches with a countersink, we have made it possible to use archived PTCBs with the popular, globally distributed Beecher system. Moreover, these punches can also be used for other systems to

Abbreviations: PTCB, paraffin tissue core biopsy specimen; PTMA, paraffin tissue microarray

Figure 1 (A–E) Construction of a paraffin tissue microarray (PTMA) using archived paraffin tissue core biopsy specimens (PTCBs). (A) Drill stand with a cone-shaped precision cutter (diameter 2.3 mm) clamped to a drill grinder. The paraffin tissue punch (Beecher Instruments; inner diameter about 1 mm) is fixed in a machine vice (overview). (B) Similar to (A) (higher magnification). (C) The paraffin tissue punch with the already milled conical countersink. (D) Cone-shaped precision cutter. (E) PTCBs stored in microtubes. (F) Transfer of a PTCB to the countersink with tweezers. (G) PTCB pushed out with the stylet. Paraffin tissue punch not clamped to the arrayer. (H) PTMA with 322 formerly archived PTCBs. (I) PTCBs with tissue portions of different lengths (lower row). Short paraffin tissue rods composed of different PTCBs cut to equal length (upper row).

construct PTMAs. It does not matter whether the holes in the PTMAs are punched (Beecher system), poured (Mengel system) or drilled with a conventional drill grinder or a computer numerical control drilling machine (low-budget Vogel systems for PTMA construction). The loss of PTCBs by mechanical destruction from the tweezers or the stylet is minimal (<2% of the PTCBs). Working with archived PTCBs can save time and boost efficiency, because the tissue of interest in a paraffin block has to be identified only once and not every time a PTMA is constructed. Furthermore, rapid access to defined tumour material is ensured for further examinations such as DNA and RNA analyses. Working with archived PTCBs makes the different lengths of the PTCBs more obvious because the PTCBs are clearly visible and not hidden by the metal of the punch (fig 1I). The height of the tissue within the donor block, the height of the donor block itself and, thus, the length of the PTCBs can vary tremendously, especially when routine paraffin tissue blocks are used as donor blocks. Using PTCBs of different lengths in PTMAs means that some PTCBs are missing in later sections, reducing the efficacy of the PTMA technique. Before the PTCBs are put into the punch, it is possible to cut the cores to an equal length using a

cutting board.³ Parts of the PTCBs which stand out are cut away and can be used for non-morphological investigations such as polymerase chain reaction. Thus, PTMAs can be constructed with PTCBs of equal length, making full use of the PTMA technique from the first to almost the last section. Moreover, stored PTCBs may be useful for consulting pathologists. It seems possible to punch one or more PTCBs out of a paraffin block sent in by the referring pathologist without destroying the paraffin block itself. In this way, the specialist can rapidly and easily collect a large number of interesting cases in his or her particular field of pathology which can later be used for PTMAs as needed. Furthermore, from a legal point of view, consulting pathologists may also benefit from the PTCBs stored in their archives.

In summary, paraffin tissue punches modified with a countersink allow the use of archived PTCBs with all their advantages to construct PTMAs, especially with the extremely popular Beecher system.

Competing interests: The University of Tuebingen is applying for an international patent.

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Take-home messages

- By modifying paraffin tissue punches with a countersink, archived paraffin tissue core biopsy specimens (PTCBs) can be used to construct paraffin tissue microarrays (PTMAs) with the popular, globally distributed Beecher system.
- Working with archived PTCBs has several advantages:
 - Efficient construction of PTMAs by identification of the tissue of interest in a donor paraffin block only once.
 - Rapid access to defined tumour material for further examinations such as DNA and RNA analyses.
 - Construction of PTMAs using PTCBs of equal length.
 - Collection of PTCBs of interesting cases by consulting pathologists for further use in PTMAs.

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