An introduction of an easy-operating and economical technique for tissue microarray preparation

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ABSTRACT

Aim Tissue microarray (TMA) is a powerful and effective tool for in situ tissue analysis. However, manual TMA construction methods showed varied qualities. This study aimed to raise a standardised TMA preparation technique that can be easily operated and is economical.

Methods A sampling needle was used to punch the tissue rods from the donor block and holes in the recipient block. To indicate the dots' positions and ensure vertical punching, a novel auxiliary device made using commercial three-dimensional printing technology was attached. The TMA block was made up of tissue rods and a recipient block.

Results A 77-rod (7×11) TMA block was constructed. The rows and columns were fixed in straight lines. There was no specimen loss during the process of embedding.

Conclusions An alternative method for the construction of TMA blocks that met the basic requirement of many laboratories and can be effortlessly performed was presented.

INTRODUCTION

With the progression of histopathological techniques, more and more methods are used for conducting research, such as immunohistochemistry (IHC), fluorescence in situ hybridisation (FISH), mRNA in situ hybridisation (RNA-ISH) and so on. However, most of these are highly expensive. Analysis of more samples in a single experimental procedure is of wide interest.

In early 1986, Battifora[1] made a preliminary exploration regarding the analysis of multiple samples in a single approach. He published the technique named multitumour (sausage) tissue block that contained about 100 different tissues on one slide. The target tissues were made into paraffin blocks, excised by knife and then wrapped in the small intestine of small mammals. In the following years, several methods have been proposed, but the results are far from satisfactory. Until 1998, Kononen *et al*[2] had developed a novel high-throughput technique, which is known as the tissue microarray (TMA). They have raised the sample size to 1000 with the help of highly precise punching instruments. Cylindrical tissue cores were punched from the donor block and then fixed in the recipient block, which subsequently becomes a primary method to construct TMA blocks.

Nowadays, the instruments for TMA are developed as semiautomated or even fully automated. Efficiency of these has been greatly improved. It has become more convenient to obtain a TMA block. However, a professional instrument is costly in many laboratories. Most of the researchers are still under urgent need for an appropriate and economical TMA technique.

The publications were reviewed and searched with the term 'tissue microarray' on PubMed. Several other manual methods have also been described[3–10] (table 1), and a series of TMA preparation methods were reviewed by Vogel.[11]

In most of the manual methods in table 1,[4–7] the TMA spots are of varied shapes and askew arrangement, since no device is used to assist punching to guarantee accuracy. Some others are neat and ordered,[9 10] as they rely on self-made equipment to punch tissue rods vertically. However, the equipment requires complex processing steps and is certainly expensive. Although cheaper than the commercial ones, they are still hard to obtain for a general pathological laboratory. None of these meet the need of convenience, accuracy and economy at the same time. Hence, our research might provide a compromise and cost-effective alternative for TMA preparation.

MATERIALS AND METHODS

Samples

One gastric cancer and four colorectal cancer specimens were collected in our hospital for the study. All tissues used were the residuals after diagnosis. Twenty tissue paraffin blocks were used as donor blocks.

The auxiliary device for punching

There were two versions of the auxiliary devices. The version 1.0 (v1.0) device was designed to be a combination of 6×11 cylinders on a base (figure 1Aa, v1.0). The diameter of the cylinder was 2 mm, and distance between each cylinder was 1 mm. The cylinder array was used to point the positions of the TMA spots.

The version 2.0 (v2.0) auxiliary device was a cuboid with 7×11 holes (figure 1Aa, v2.0). The diameter of each hole was 2 mm, and the spacing of both rows and columns was 0.8 mm. The v2.0 auxiliary device had the two basic functions of indicating the location of each spot in the TMA and ensuring the sampling needle punches vertically.

The two auxiliary devices were made of SOMOS Imagine 8000 resin (DSM, Holland) using three-dimensional (3D) printing equipment (Uniontech G400, China). The modelling of the two auxiliary devices was achieved by 3D Studio MAX 2012, and the files can be available upon contacting the author. The commercial 3D printing technique was available on http://www.wenext.cn/.

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Table 1 Publications on the tissue microarray (TMA) technique in PubMed

Year	Author	Recipient block	Sampling tool	Capacity of TMA blocks	Disadvantages
2016	Srinath[3]	Preformed, constructed by silicon moulds	Dermal punch biopsy needle	3 mm, 8 (2×4)	Low capacity
2016	Frosina[4]	Preformed, carrier-based block	Dermatological circular punch devices	0.5 mm, 91 (7×13)	No positioning reference system or auxiliary device
2013	Shi[5]	Preformed, checkerboard-paraffin block	Self-made blade-shaped knife	2 mm, 70 (7×10)	Shapes and sizes of specimens varied
2013	Kim[6]	Preformed, agarose-paraffin block	A modified 16-gauge needle	2 mm, 80 (8×10) 1 mm, 220 (11×20) 2 mm, 108 (9×12) 1 mm, 192 (12×16)	Manual operation: no positioning reference system or auxiliary device Premade ready-to-use recipient blocks: use an electric hand punch and X-Y table (requires special devices)
2011	Shebl[7]	Preformed, paraffin block	Mechanical Pencil tip	1 mm, 36–72 (6×6–12)	No positioning reference system or auxiliary device.
2010	Vogel[8]	Preformed, paraffin block	Skin biopsy punch and a used bone marrow trephine biopsy set	1.4 mm, 187 (17×11)	Shapes and sizes of specimens varies.
2006	Pires[9]	No use	Customised needles	1 mm, 325 (25×13)	Use a hand-press grommet inserting machine (require special devices).
2004	Dan[10]	Preformed, paraffin block	A set of holing, sampling needles and related components	1.3 mm, 112 (8×14)	Use a set of holing, sampling needles and related components (require special devices).

Extraction of tissue rods

As an important part of the TMA block, the tissue rods were excised from the donor block (figure 2F) and were generally cylindrical. A stainless-steel tube with an inner diameter of 2 mm acted as a sampling needle (figure 2E), which was frequently used as agar puncher in agar gel electrophoresis (Shanghai Yubai Industrial, China). It had a blade at one end for cutting tissue, ensuring a smooth surface of the tissue rod. After punching, the tissue rods were pushed out from the other end by a straightening paper clip. In addition, the sampling needle was also used to punch holes in the recipient block.

Construction of recipient block by v1.0 auxiliary device

Liquid paraffin was poured into a mould (figure 2A) with a size of 3.5cm×2.4cm. After the frozen platform of the Tissue Embedding Console System (Tissue-Tek TEC-5, Sakura, Japan) was cooled down, a blank paraffin block (figure 2B) was then prepared. The block was preheated to soft the surface, and the v1.0 auxiliary device was put on it to leave markers of the array. The recipient block was constructed via punching holes according to the markers on the surface by the sampling needle.

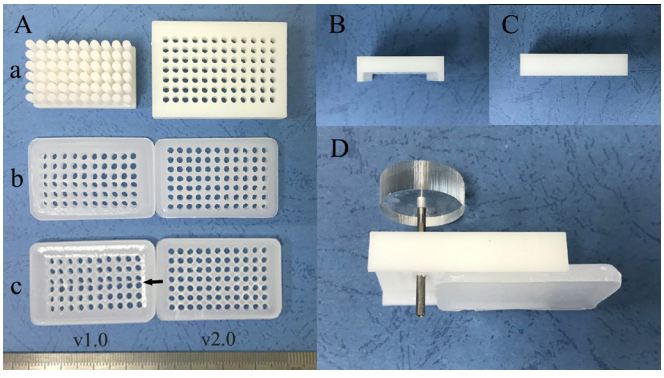

Figure 1 Auxiliary device. A: two versions of the three-dimensional (3D) printing model. (A) (a) Resin models. (b) Surface side of recipient block. (c) Bottom side of recipient block. The holes were irregularly placed on v1.0 block (black arrow). (B) Sagittal view of the v2.0 model. (C) Coronal view of the v2.0 model. (D) A combination of sampling needle, v2.0 auxiliary device and recipient block.

Construction of a recipient block by v2.0 auxiliary device

The blank paraffin block was prepared the same as described above. Unlike the v1.0 auxiliary device, the holes were punched directly with the v2.0 auxiliary device on the paraffin block (figures 2C and 1D). The recipient block (figure 2D) with 77 holes was accomplished in about 10 min.

Construction of TMA block

The recipient block was placed back in the mould (figure 2A), and the tissue rods were inserted into the holes, respectively. It was necessary to ensure that every tissue touched the bottom of the mould. The mould was heated on the heating plate of the tissue embedding console system (Tissue-Tek TEC-5, Sakura, Japan) until the paraffin began to melt. The process took about 10 s. The recipient block was pressed gently to the bottom by tweezers to remove the bubbles between the block and the mould. Then, so did every tissue rod. If some tissue rods were too short to fill up the holes completely, additional paraffin

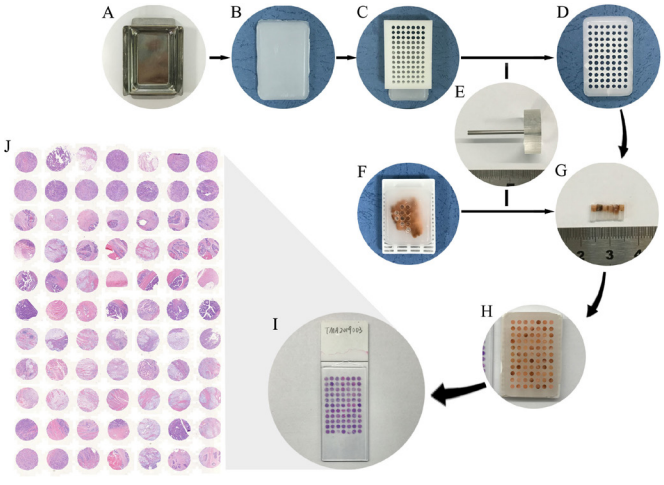

Figure 2 The process of making a tissue microarray (TMA) block by v2.0 auxiliary device. (A) Mantel mold. (B) Blank paraffin block. (C) A paraffin block with v2.0 auxiliary device on it. (D) Recipient block. (E) Sampling needle. (F) A donor block. (G) Tissue rods from the donor block. (H) A TMA block. (I) hematoxylin and eosin (HE) slide. (J) The digital scan picture of a HE slide.

rods were recommended to put in as supplements. Otherwise, the remainder space would form cavities and lead to retraction of the tissue rods, since the liquid paraffin was unable to feed into it. The additional paraffin rods were redundant materials for constructing the recipient block. In the last step, the mould was filled up with liquid paraffin, and a plastic embedding box was put on it. After cooling down, the construction of the TMA block was considered complete.

Slide, IHC and image processing

A 4 µm thick slide was sectioned on a microtome machine (Leica RM2235, Germany). Hematoxylin and Eosin (HE) stained slides were obtained and processed by an automatic staining machine (Dako, Denmark). IHC was performed by an automatic IHC staining machine (Dako Omnis, Denmark). The slides were scanned as digital images by Pannoramic DESK (3DHistech, Hungary).

Quality evaluation

The spots of a high-quality TMA block were in a straight line and the spacing between each spot was the same. In order to verify the location of each spot in the manual TMA, the area coincidence rate (ACR) of the actual spot and the datum spot was calculated. The datum array had the same capacity, length and width with the evaluated actual array, and in which the area of every spot and the spacing between each spot were the same.

The specific steps were as follows: (1) The image of a TMA to be assessed was opened by Photoshop CS3. The actual spot array was selected by the Wand and Lasso tool, and named as Layer 1. (2) In Layer 1, a grid of ruler lines was presented according to the TMA capacity by the Slice tool. (3) A round datum spot was drawn by an Ellipse tool with the closest area to the first spot in the TMA in a new layer named Layer 2. (4) The datum array in Layer 2 was drawn by copying the datum spot and snapping them to the ruler lines. (5) The out-of-spot area in Layer 1 was selected by the Wand tool. Then, by choosing Layer 2 and deleting the selected out-of-spot area, the remaining area in Layer 2 was the coincidence area. (6) The number of pixels was used as the unit of area measurement. ACR was obtained by dividing the coincidence area by the datum area.

Statistical analysis

Statistical packages for data analysis were IBM SPSS Statistics Base V.25.0 and Microsoft Excel 2010, and we used descriptive statistics and independent sample t-test analysis to test any differences among the TMA blocks. A value of $p<0.05$ was considered statistically significant.

RESULTS

Tissue rods

The tissue rod was obtained from the donor block, which consisted of specimen and paraffin with a total length of about 5 mm (figure 2G). The length of the specimen part varied, and was determined by the thickness of the donor tissue. As the number of slices that can be made by one TMA block was influenced by the length of the specimen, those rods with the specimen part shorter than 1 mm were eliminated.

Recipient block

Two recipient blocks were made by v1.0 and v2.0 auxiliary devices, respectively (figure 1Ab). The v1.0 block had a 6×11 hole array, and the v2.0 had a 7×11 hole array.

Table 2 The calculated area coincidence rates (ACRs)

Method	Area coincidence rate (mean±SD)	Sample capacity	P value
Frosina [4]	0.82±0.07	24	0.097
Shi [5]	0.77±0.12	70	<0.001
Kim [6]	0.57±0.22	176	<0.001
HE	0.85±0.09	77	–
TMA	0.97±0.03	77	<0.001

HE: hematoxylin and eosin slide in this paper.
Tissue microarray (TMA): the TMA block in this paper.
The p value was a comparison to HE.

TMA block

A 7×11 TMA block was constructed as described above (figure 2H). The diameter of each tissue rod was 2 mm, and the spacing between each rod was 0.8 mm. The rows and columns were fixed in straight lines. No specimen was lost during the process of embedding.

Slide

The hematoxylin and eosin (HE) slide was shown in figure 2I and the digital scan pictures were shown in figure 2J. In the HE slide, the shapes of the tissue appeared round, and the nucleus and cytoplasm of each cell appeared clear with no mechanical damage.

Quality evaluation

The ACRs of the HE digital image and the TMA paraffin block in our research were calculated, and also the ACRs with evaluable images in the references.[4–6] The results are as shown in table 2 and figure 3.

Immunohistochemistry

IHCs of caudal-type homeobox 2 (CDX2) and human epidermal growth factor receptor 2 (HER2) were performed on representative slides (figure 4). CDX2 was positive in the nucleus with varying degrees in the colorectal cancer specimens (figure 4B1–4), and was negative in the gastric cancer specimens (figure 4B5). The expression of HER2 of each specimen varied. One of the five samples showed a moderately positive change in the membrane (figure 4C3).

DISCUSSION

For decades, researchers have never stopped the formulation of new TMA techniques owing to its advantages, which were as follows: (1) The slides from TMA blocks should be capable of performing IHC, FISH and RNA-ISH. (2) The microenvironment should be the same on a slide, reducing the experimental deviation. (3) The time and economic costs were greatly reduced when dozens of experiments were condensed into one. (4) A TMA block can be sectioned into nearly 1000 slides, meaning that hundreds of experiments can be done once and for all. In one word, construction of a TMA block is considered as a good start in the research area.

However, normal laboratories cannot afford to buy a commercial TMA instrument, such as TMA Master (3DHISTECH), MTA-1 (Beecher), and so on. This study aimed to raise a standardised TMA preparation technique that is easily operated and is economical. Based on the original principle of the TMA technique, the two key procedures included donor tissue extraction and recipient block preparation. To construct the recipient block, the v1.0 auxiliary device was established at first.

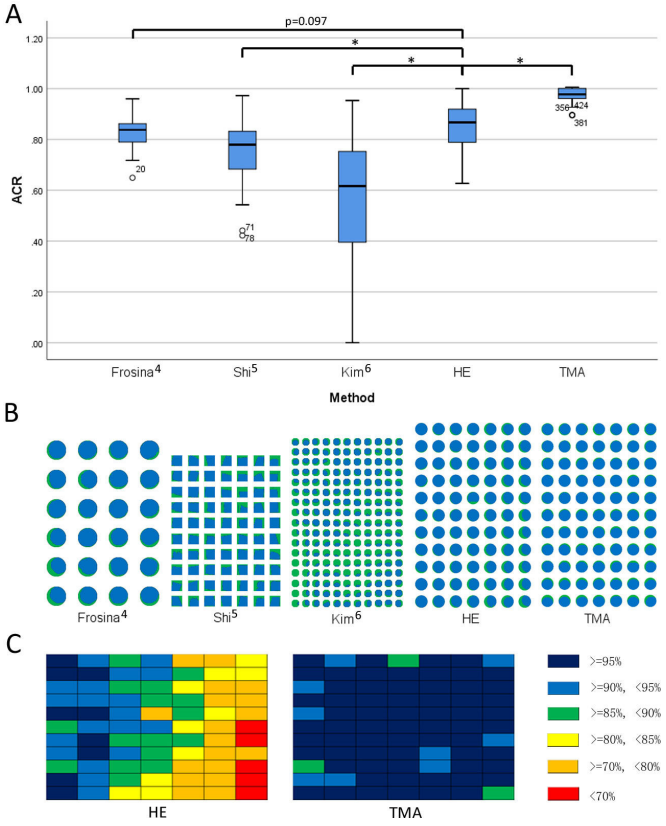

Figure 3 The ACR distribution. (A) The box plot of ACRs in different methods for TMA block preparation. *p<0.001. (B) A diagram of ACRs in the different methods. The blue color shows the coincidence area, and the green colour shows the datum array area. (C) A diagram of ACR distribution in He and TMA. A trend seen was that the greaer the distance from the first point, the lower the ACR value. ACR, area coincidence rates; He, hematoxylin and eosin. TMA, tissue microarray.

However, practically fixing only the entry point cannot guarantee the quality of the recipient block. The holes are irregularly placed at the bottom side of the block (figure 1Ac, v1.0). Non-vertical punching resulted in the chaos of tissue arrangement, leading to irregular spots on the slide eventually (figure 5). The shapes of the samples could be round or oval and the positions

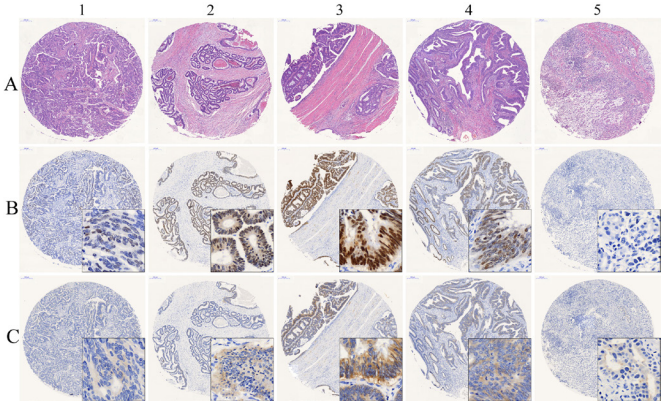

Figure 4 Scanned pictures of five samples. (1–4) Colorectal cancer. (5) Gastric cancer. (A) He. (B) CDX2. Negative: (5) Weekly to moderately positive: (1, 2, 4). Strongly positive: (3). (C) HER2. Weekly positive: (1, 2, 4, 5). Moderately positive: (3). CDX2, caudal-type homeobox 2; HER2, human epidermal growth factor receptor 2.

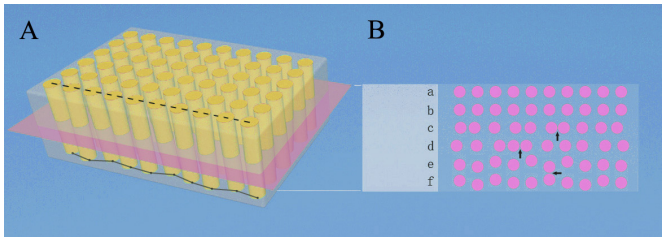

Figure 5 A possible situation of a tissue microarray (TMA) block without the assistance of an auxiliary device. (A) A virtual three-dimensional (3D) model. The tissue rods were regular (dotted line) in the beginning, but were irregular (solid line) in the end, as they were not perpendicular to the surface. (B) A section of the 3D model on which the spots were in a disordered manner (line CD: lateral migration, line EF: longitudinal migration), and some of the spots were nearly touched (black arrows).

may not be in a straight line. Neighbouring points can even come into contact with each other (figure 5B).

Therefore, a second-generation (v2.0) model of the auxiliary device was updated. Details are presented in figure 1. With the help of the v2.0 auxiliary device, the array is observed to be regular whether on the surface (figure 1Ab) or at the bottom (figure 1Ac) of the recipient block. An additional advantage of this is it can be easily operated and it saves time. It is simple and convenient to punch a hole along the model. No targeting, alignment or movement of paraffin blocks has been considered. A TMA block of 77 specimens can be constructed in about 2 hours by an experimenter.

ACR was used for evaluating the quality of the TMA arrangement. An average ACR was 0.85±0.09 in the HE slide, which was significantly higher than some of the previous manual methods[5 6] (p<0.001). These differences may be attributed to the application of the v2.0 auxiliary device. Moreover, the ACR of the TMA paraffin (0.97±0.03) was much higher than that in the HE slide (p<0.001). According to an analysis of the ACR distribution (figure 3C), it can be seen that lower ACR spots on the TMA block are scattered, and those on the HE slide have a certain pattern. The farther away from the first spot, the lower the ACR, which indicates that slide processing has a negative impact on array arrangement. Therefore, a high-quality TMA paraffin block is a prerequisite for a high-quality slide.

IHC was used to justify the applicability of the TMA block. Two antibodies were chosen for IHC: CDX2 and HER2. CDX2 is positive in the nucleus, and expressed with different degrees in most colorectal cancers. HER2 is positive in the cytoplasm and membrane, and is an important target for drug selection in targeted cancer therapy. The expression of the two antigens varied on the TMA slide from totally negative to strongly positive. The positive locations varied: nucleus, cytoplasm and membrane. Clear and specific staining indicated a successful experiment.

This study provided an alternative for TMA construction. The advantages of our method are: it is extremely low cost (sampling needle US$13, auxiliary device US$4 and a total cost of US$17), easy to operate (punch and insert) and standardised (sampling needle creates uniform cylindrical tissue rods, and auxiliary device ensures same adjacent spacing of each rod).

There are also points that need improvement: (1) The capacity of our TMA block was 77. High output TMA blocks are under research. (2) Manual measurements are never as accurate as professional fully automated devices.[12] The ACR of our TMA block is 97%, and there is still a gap till 100%, for improvement.

CONCLUSIONS

We presented an alternative method for the construction of TMA blocks that is extremely low in cost, easy to prepare and standardised. It meets the basic requirement of many laboratories and can be performed effortlessly.

Take home messages

- We presented an alternative method for the construction of TMA blocks for general laboratories.
- The method is low in cost (sampling needle US$13, auxiliary device US$4 and a total cost of US$17), easy to operate (punch and insert) and standardised (sampling needle creates uniform cylindrical tissue rods, and auxiliary device ensures same adjacent spacing of each rod).
- A novel auxiliary device introduced in this study, made using commercial 3D printing technology, indicates the spot's position and ensures vertical punching.

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Contributors Y-JC was responsible for the design of the 3D auxiliary device, TMA block construction, and the written of the paper. C-MY and J-SH were responsible for tissue rods extraction and recipient block construction. PW was responsible for language and format editing. Y-HL and CT were responsible for giving informed consent to participate and donor block construction. WD was responsible for experimental design and technical guidance.

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REFERENCES

1 Battifora H. The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing. *Lab Invest* 1986;55:244–8.
2 Kononen J, Bubendorf L, Kallioniemi A, *et al*. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998;4:844–7.
3 Srinath S, Kendole RK, Gopinath P, *et al*. Economic methods used in fabrication of tissue microarray: a pilot study. *J Oral Maxillofac Pathol* 2016;20:86–90.
4 Frosina D, Jungbluth AA. A novel technique for the generation of multitissue blocks using a carrier. *Appl Immunohistochem Mol Morphol* 2016;24:668–72.
5 Shi Y, He D, Hou Y, *et al*. An alternative high output tissue microarray technique. *Diagn Pathol* 2013;8:9.
6 Kim KH, Choi SJ, Choi YI, *et al*. In-House manual construction of high-density and high-quality tissue microarrays by using homemade recipient Agarose-Paraffin blocks. *Korean J Pathol* 2013;47:238–44.
7 Shebl AM, Zalata KR, Amin MM, *et al*. An inexpensive method of small paraffin tissue microarrays using mechanical pencil tips. *Diagn Pathol* 2011;6:117.
8 Vogel UF, Bültmann B. Application of a novel and low cost technique to construct paraffin tissue microarrays out of paraffinised needle biopsy specimens from patients with breast cancer. *J Clin Pathol* 2010;63:640–3.
9 Pires ARC, Andreiuolo FdaM, de Souza SR. TMA for all: a new method for the construction of tissue microarrays without recipient paraffin block using custom-built needles. *Diagn Pathol* 2006;1:14.
10 Dan H-L, Zhang Y-L, Zhang Y, *et al*. A novel method for preparation of tissue microarray. *World J Gastroenterol* 2004;10:579–82.
11 Vogel U. Overview on techniques to construct tissue arrays with special emphasis on tissue microarrays. *Microarrays* 2014;3:103–36.
12 Zlobec I, Suter G, Perren A, *et al*. A next-generation tissue microarray (ngTMA) protocol for biomarker studies. *J Vis Exp* 2014.