Simple method for constructing and repairing tissue microarrays using simple equipment

Wei Zhang[1], Fuman Qiu[1,2], Qingping Jiang[1], Shaoyan Liu[1] and Zhongtang Xiong[1] ⓘ

Abstract

Objective: Many methods for tissue microarray (TMA) construction were described in previous reports. Because TMA-based methods are expensive and complicated, their widespread application may be restricted. This study aimed to develop a simple method for TMA construction.
Methods: High-density TMAs were constructed using simple equipment, and hematoxylin and eosin and immunohistochemical staining were performed to analyze the effect on the TMA block.
Results: A recipient block with 162 holes of 0.9 mm in diameter was prepared using a mini-drill and plastic mold. Tissue cores of 1.0 mm in diameter were obtained from multiple donor blocks with stainless-steel capillary tubes driven by the mini-drill. Under the fixation and guidance of the plastic mold, tissue cores could be easily injected into the holes in the recipient block by inserting a stainless-steel wire into the stainless-steel tube with the tissue core and then pressing using the stainless-steel wire.
Conclusion: A high-density TMA block with 162 1.0-mm cores was created. This new modified technique could be a good alternative in many laboratories.

Keywords

Tissue microarray, immunohistochemistry, colorectal cancer, hematoxylin and eosin, paraffinization, fluorescence in situ hybridization, tissue core

[1]Department of Pathology, The Third Affiliated Hospital, Guangzhou Medical University, Guangzhou, China
[2]State Key Lab of Respiratory Disease, Institute for Chemical Carcinogenesis, Collaborative Innovation Center for Environmental Toxicity, Guangzhou Medical University, Guangzhou, China

Corresponding author:
Zhongtang Xiong, Department of Pathology, The Third Affiliated Hospital, Guangzhou Medical University, Guangzhou 510150, China.
Email: zhongtang2000@126.com

Introduction

Tissue microarrays (TMAs), also called tissue chips, were first proposed in 1998 by Kononen et al.[1] They contain dozens to hundreds of tissue spots on a single slide. This powerful technology has been extensively used in fluorescence in situ hybridization and immunohistochemistry to simultaneously analyze DNA, RNA and protein expression.[2,3] Using TMAs, a molecular marker can be examined in hundreds of different specimens under the same experimental conditions.[4] The use of TMAs has advantages over conventional techniques using whole-tissue sections, such as a lower cost, more rapid procedures, less damage to donor blocks, and less experimental error.[5] The steps of traditional methods of TMA construction include obtaining tissue cores from a series of donor blocks and transferring these cores to a recipient block.[6] Recently, several novel methods that do not use a recipient block have been published.[7–9] However, these methods have some drawbacks. First, the most extensively used commercial TMA construction instruments (including automatic and manual instruments) are expensive, limiting their application in many pathology laboratories with limited budgets.[4] Second, it is difficult to insert these smaller tissue cores into the holes of a recipient block, and even these tissue cores may be broken. In addition, because traditional punch methods are used to prepare recipient blocks and obtain tissue cores, the recipient and donor blocks are easily damaged, and it may be difficult to obtain tissue cores from these hard donor blocks. Finally, these methods have complex steps, and they need special devices and professionally trained technicians.[5,6] In this study, we developed an easy method without the aforementioned disadvantages.

The number of sections cut from the TMA block without a loss of tissue spots is associated with the length of the shortest tissue core in the TMA block and the thickness of each section. These tissue cores have different lengths because they are obtained from multiple donor blocks, in which tissue specimens have different thicknesses.[10] Because of the exhaustion of these shorter tissue cores after microtomy, some tissue spots are lost on later slides. At present, strategies for quickly repairing tissue core loss are worth exploring. In this study, we developed a new method for constructing TMAs using simple tools that can readily repair a TMA block with the loss of tissue cores.

Materials and methods

Equipment

A mini-drill, drill bits (length, 30 mm; diameter, 0.9 mm), stainless-steel capillary tubes (length, 20 mm; outer diameter, 1.4 mm; inner diameter, 1.0 mm), and stainless-steel wires (length, 25 mm; diameter, 1.0 mm), all purchased from Taobao Co. (Hangzhou, China; https://www.taobao.com), were used to prepare recipient blocks and obtain tissue cores from donor blocks. A plastic mold was processed using a computer numerical control milling machine. To fix the recipient block, a hollow core, the size and shape of which matched the recipient block, was created in the bottom of a plastic board. Then, 162 holes were drilled in the top of the same plastic board. We designed and prepared the plastic mold with 162 array holes, which was used to fasten the blocks, drill bits, and stainless-steel capillary tubes to construct the recipient and TMA blocks. Each hole in the plastic mold consisted of top and bottom holes, which were 1.4 and 1.0 mm in diameter, respectively. The top hole was used to guide and fix the stainless-steel

capillary tube to ensure that capillary tube was perpendicular to the recipient block. The bottom hole was used to guide and fix the drill bit and tissue cores, and stainless-steel wires could be inserted through the bottom hole.

Selection and marking of paraffin blocks containing tissue samples

Hematoxylin and eosin (H&E)-stained slides of 160 colorectal cancer tissues were reviewed by two clinical pathologists. Interesting and representative areas were selected and marked on the slides, and their corresponding paraffin tissue blocks were labeled using a marker pen. All slides and their tissue blocks were obtained from the Pathology Department of the Third Affiliated Hospital of Guangzhou Medical University (Guangzhou, China). The clinical specimens were used for this study with the consent of the patients or their relatives according to the hospital's ethics committee. The study protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University.

Recipient block preparation

Paraffin wax was purchased from Leica Microsystems, Inc. (Wetzlar, Germany). Blank paraffin blocks prepared for the recipient blocks were made according to routine procedures. Briefly, ordinary stainless-steel embedding molds and plastic cassettes were placed in melted paraffin and warmed to 62°C before filling to avoid the formation of bubbles in the paraffin blocks. The mold was then filled with melted paraffin and covered with a plastic cassette. After pouring, the paraffin blocks were cooled to room temperature and removed from the steel molds. These blank paraffin blocks were examined, and those without air bubbles and cracks were selected for to

prepare the recipient block. The paraffin block with a plastic cassette was placed at the bottom of the plastic mold to fix the paraffin block and avoid its displacement during drilling. Holes were created in the recipient block using a mini-drill. The recipient block was removed after drilling was completed.

TMA block construction

The tissue cores were created from donor paraffin-embedded tissue blocks using a mini-drill with a stainless-steel capillary tube. Each capillary tube containing a tissue core was removed from the mini-drill. The tissue core was then removed from the capillary tube and transferred to the hole of the recipient block using stainless-steel wire. Repeating these steps, the tissue cores were inserted into the prepared holes of the recipient block to construct the TMA block.

H&E and immunohistochemical staining

The TMA block was cut and subjected to H&E staining using routine procedures. In brief, before cutting, the finished TMA block was stored in the refrigerator and chilled to −4°C for a few minutes to harden the paraffin block. Tissue sections were used for H&E staining, immunohistochemical staining, and fluorescence in situ hybridization. To verify the suitability of our TMA section, staining was performed as follows: the 3-μm-thick TMA sections were deparaffinized, rehydrated, and incubated with primary antibody at 37°C for 2 hours. Prior to incubation with anti-CK (Abcam, Cambridge, MA, USA), the sections were heated in 0.01 M sodium citrate buffer, pH 6.0, for antigen retrieval, and incubated in 3% H_2O_2 to inhibit endogenous peroxidase activity. After 2 hours, the sections were washed and incubated with the secondary antibody for 30 minutes

at room temperature. Finally, the slides were developed using a DAB chromogen kit and counterstained with Mayer's hematoxylin.[11]

TMA block repair

The method for TMA block repair is the same as that for recipient block and TMA block construction. First, the lost tissue cores of the TMA block were recorded on an array map, and their corresponding array holes in the plastic mold were marked. The TMA block was then inserted into the plastic mold, and holes were created through the recorded holes in the plastic mold using a mini-drill. Tissue cores were obtained from the donor blocks that

corresponded to lost tissue cores and re-injected into these holes of the TMA block.

Results

Recipient and TMA blocks

In total, 162 array holes were created in a 2-cm × 3-cm recipient block within 2 minutes after creating the blank paraffin block. As presented in Figure 1a and 1b, all holes were neatly arranged with uniform spacing (separation distance, 0.8 mm), size (diameter, 0.9 mm) and depth (4.0 mm). Recipient blocks were created at room temperature, and they were not broken during the drilling process.

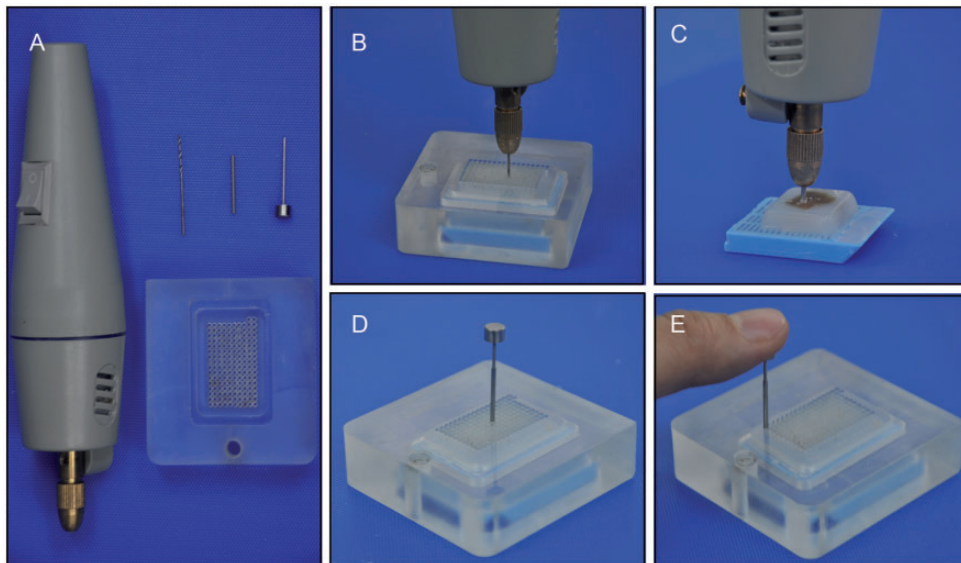Similarly as the array holes of the recipient blocks, the tissue cores in the TMA

Figure 1. Recipient block preparation and TMA block construction. (a) The self-made equipment for recipient and TMA block construction. (b) Drilling of the blank recipient paraffin block. The plastic mold was used to fix the blank paraffin block and drill bit. A mini-drill and bit were used to drill array holes in the paraffin block. (c) A mini-drill and capillary stainless-steel tube were used to obtain tissue cores from the donor blocks. (d) A capillary stainless-steel tube containing a tissue core was inserted into array holes in the recipient block. (e) The tissue core was pressed from the capillary stainless-steel tube and transferred into the hole in the recipient block using stainless-steel wire. The drilled recipient block featured 162 holes (diameter, 0.9 mm; distance, 0.8 mm)
TMA, tissue microarray.

block were arranged neatly and with uniform spacing distance between the cores. The surface of all tissue cores and recipient blocks were in the same horizontal plane. In addition, we used the plastic mold to fix and guide the stainless-steel capillary tube, and none of the tissue cores was broken during transfer to the recipient block. After all tissue cores were inserted into the recipient block, the TMA block was ready for the creation of tissue slices without any additional processing. The time of preparation for a TMA block containing 162 tissue cores was no more than 180 minutes, and none of the 162 donor samples was damaged while obtaining tissue cores (Figure 1a and e).

Sectioning and H&E staining

After slight trimming, TMA blocks were sectioned with a microtome to obtain 4-µm-thick sections of thickness. In this method, approximately 180 consecutive 4-µm sections could be excised from the TMA block, and there were few folded tissue spots in our sections during microtomy. Moreover, H&E staining illustrated that no tissue spot was detached from slides during the staining process (Figure 2a and 2b). Similarly, immunohistochemical staining for anti-CK was successfully performed without tissue spot loss during antigen retrieval and washes (Figure 2c and d).

Unrepaired and repaired TMA blocks

After 180 sections were excised from the TMA block, 5 of 162 tissue cores were lost because of the consumption of tissue cores during microtomy (Figure 3a and b). Five tissue cores were obtained from the donor blocks that corresponded to lost tissue cores and re-injected into re-drilled holes of the TMA block. According to this method, the lost tissue cores were rapidly repaired. After repair of the TMA
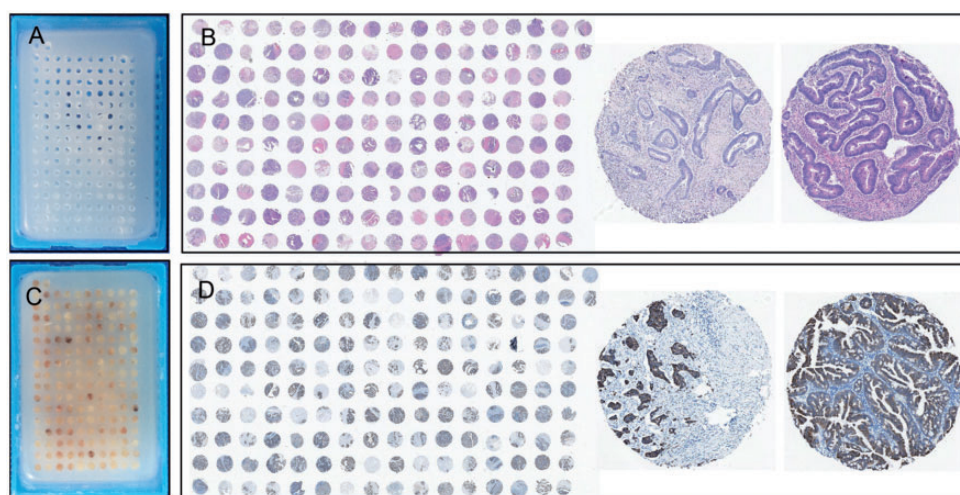

Figure 2. H&E and immunohistochemical staining of the TMA block. (a) The drilled recipient block with 162 holes (diameter, 0.9 mm; distance, 0.8 mm). (b) The finished TMA block containing 162 colorectal cancer tissue cores. (c) H&E staining of the TMA sections illustrated that no tissue spot was detached from the slides during the staining process. (d) Immunohistochemical staining of the TMA sections revealed that no tissue spot was detached from slides during the staining process
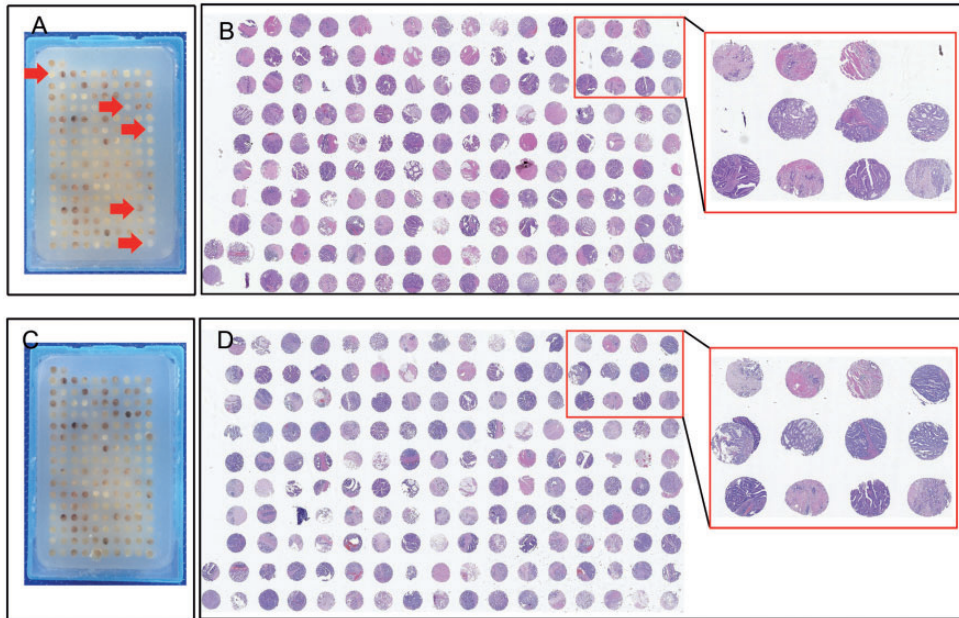H&E, hematoxylin and eosin; TMA, tissue microarray.

Figure 3. Repair of lost tissue cores. (a) Five tissue cores in the TMA block were exhausted after some sections were excised from the block. (b) H&E staining illustrated that five tissue spots were lost from slides (left). The high-power image revealed that two tissue spots were lost (right). (c) The TMA block with five lost tissue cores was repaired using the method for constructing and repairing TMAs. (d) H&E staining demonstrated that the five lost tissue spots were repaired with new tissue spots (left). The high-power image clearly presented the two repaired tissue spots (right)
H&E, hematoxylin and eosin; TMA, tissue microarray.

block, approximately 40 sections were continuously obtained from our TMA block. In addition, the TMA block could be repaired a second time in the same manner if needed. The repaired TMA block and its H&E-stained slides are presented in Figure 3c and d.

Discussion

TMAs blocks are constructed by obtaining tissue cores from a series of multiple donor blocks and inserting them into a recipient block. Sections cut from a TMA block have dozens to hundreds of tissue spots on a single slide, permitting a biomarker to be examined in different tissue specimens simultaneously on a single TMA section.[12] A series of different methods for constructing TMAs have been reported; however, these methods have some shortcomings. Our simple method for TMA construction can overcome these shortcomings.

Commercial automated and manual TMA arrayers are expensive, with a cost of $7000 to $30,000.[13–15] Because of this high cost, extensive application of the TMA technique is restricted in many small pathology laboratories with limited budgets. To reduce costs, several methods TMA construction were recently reported.[16–18] In 2011, Shebl et al.[10] described an inexpensive and simple TMA construction method in which a TMA block with 72 1.0-mm cores was successfully constructed using a pencil tip to punch holes in the recipient block and obtain tissue cores from donor blocks. In 2013, Shi et al.[19]

used a self-made blade-shaped knife to pre-pare tissue rods and then planted them in a checkerboard recipient block. Using this method, a TMA block with 70 spots was generated. In addition, other simple meth-ods without special equipment have been described.[10,17,20,21] These methods are simple and inexpensive, but they cannot be used to construct high-density TMA blocks. A high-density TMA block with up to 1363 tissue cores was constructed using a microcompound table and drill grinder.[22] However, in practice, this method is difficult to apply because of its complicated techniques and special equip-ment requirements, such as a computer numerical control (CNC) drilling machine. In this study, we designed and constructed a plastic mold that could be used to replace the CNC, permitting the creation of a high-density TMA block with 162 1.0-mm cores (Figure 1). In addition, the equipment used in our study cost no more than $30.

In previous reports, the holes of self-made recipient blocks were punched with simple tools, such as bone marrow aspira-tion needles and pencil tips,[10,23] which are inexpensive and easy to obtain by all labo-ratories. During punching, recipient blocks may be broken by these tools. In addition, it is difficult to punch holes in recipient blocks in a neat arrangement and with uni-form spacing, size, and depth.[22] To resolve these problems, we used a drill to create the holes, and other complex equipment was replaced with a plastic mold. As presented in Figure 1, the empty paraffin block was placed in the plastic mold to fix it before drilling to avoid its displacement. In addi-tion, during drilling, owing to the guiding and fixing of the plastic mold, we could ensure that the drill bit was perpendicular to the paraffin block and that its displace-ment was avoided.

Conventional needles such as bone marrow aspiration needles, ordinary biopsy punches, and hypodermic needles have been used as tissue punches to obtain tissue cores from donor blocks. These nee-dles are available for small-scale laborato-ries at lower costs than commercial tissue punches. However, during punching, espe-cially for hard donor blocks, these fine nee-dles or donor blocks might be broken. In this study, we used a stainless-steel capillary tube driven by a mini-drill to replace these conventional punching methods. Practically, none of the donor blocks was broken while drilling the tissue cores in this manner. A TMA block with 1363 0.43-mm cores was successfully constructed by Vogel using complex equipment.[22] In practice, tissue cores smaller than 1.0 mm in diameter are difficult to insert into the holes of the recipient block without other equipment. In our method, to resolve this problem, the bottom of the plastic mold was used to fix the recipient block, and its upper array holes were used to fix the stainless-steel capillary tube with tissue cores. Under the guidance and fixation of the lower array holes of the plastic mold, these thin tissues cores were easily inserted into the recipient block by pushing them with a stainless-steel wire. In a previous study, once all cores were inserted into the recipient block, the melted paraffin was poured into the TMA block followed by heating of the TMA block using a hot plate to improve the adherence between tissue cores and the recipient block.[10,24] In our method, no subsequent steps are required after organization of the chip, and the detachment of tissue cores and recipient block was avoided. The diameter of the tissue cores was slightly larger than the holes of the recipient block in our method, allowing the tissue cores to be easily inserted into the recipient block. Moreover, tissue cores were not moved by pressure or friction between tissue cores and the recipient block (Figure 2).

Moreover, tissue loss is one of the major problems in TMA construction, and many

studies have attempted to overcome this issue.[10,18,25] Our method for recipient and TMA block construction can also be used to repair the TMA block following tissue core loss. There is no doubt that these shorter tissue cores in the TMA block will be consumed after microtomy because different donor tissue blocks have different thicknesses. Consequently, some tissue spots on the section will be lost. For this shorter core, Vogel *et al.* and Shebl *et al.*[10,22] advised that a second or even third core from the same donor block could be injected in the same hole of the recipient block. However, it is difficult to avoid tissue spot loss using this method. In our modified method, when shorter tissue cores were consumed, the TMA block was placed in the plastic mold, and holes were bored in the location of core loss. Subsequently, supplementary cores were obtained from the corresponding donor blocks and re-injected into the holes (Figure 3). Using this method, the TMA block was successfully repaired.

We have developed a method for the construction of high-density TMAs using simple equipment costing no more than $30 that can be performed by any untrained technician. Compared with conventional methods, this method has advantages, such as simple operation, no damage to the donor block, and simple processes. In addition, a TMA block with lost tissue cores can be repaired using this method. This new method is a good alternative to expensive machines in many laboratories, helping researchers to perform studies involving hundreds of samples rapidly. Our modified method of TMA construction may lead to significant improvements in basic research.

Authors' contributions

XZT performed the experiments and drafted the manuscript. ZW performed statistical analysis and manuscript revision during the revision of our resubmission. QFM performed the planning study and served as a supervisor of this study. JQP and LSY assisted with data analysis. All authors read and approved the final manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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ORCID iD

Zhongtang Xiong ⓘ https://orcid.org/0000-0003-0979-4575

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