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Short Communication

Creation of Efficient Pathology Research Pipelines for Discovery: Tissue Microarray Construction Coupled with Digital Image Analysis

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Abstract

Pathology translational research pipelines are complex, as many studies rely on analyses of tissue immunohistochemistry. High-throughput methodologies such as tissue microarrays (TMAs) have been critical for many recent advancements as large datasets are necessary to make detailed disease and biologic discoveries. Currently, TMA molds can be purchased from online vendors or constructed in house with complex machinery. However, there are certain limitations to these molds, such as cost per mold and inability for selection of core slot patterns. Thus, we propose a practical and cost-effective method for constructing a silicone-based rubber TMA mold that can be used to create a paraffin template block. Our results show that these self-manufactured molds are durable, effective and easy to customize, reducing operating costs and creating flexibility for users. Furthermore, in leveraging this technique, we describe coupling TMA-prepared slides with a rapid research-focused digital analytic pipeline that allows for a streamlined method of quantitative and qualitative companion discovery.

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Introduction

Translational pathology research relies heavily on tissue specimens, histologic analyses, immunophenotypic studies, and molecular analyses. A bottleneck frequently exists for studies due to the lack of an efficient method for high-throughput acquisition and analysis of data, in particular histologic and immunophenotypic data. For more than 20 years, pathology researchers have attempted to address this bottleneck in their studies by using tissue microarrays (TMAs).

TMAs are a collection of formalin-fixed paraffin embedded (FFPE) tissue sample cores that are subsequently embedded into a prepared blank recipient paraffin block. Utilizing this method, samples from multiple tissues can be investigated simultaneously.[1] This concept was introduced in 1986 by Hector Battifora, who proposed the initial method of multitumor tissue blocks (MTTBs) or "sausage" blocks. MTTBs were made to enable immunohistological staining for over 100 tissue samples on one slide.[2] Battifora and Mehta later improved on this method by developing the checkerboard tissue block. In this method, the tissue samples are evenly distributed in a checkerboard arrangement, which makes them readily identifiable when the block is sectioned.[3] In 1998, Kononen *et al*. developed an array-based technique that resembles the form of current TMAs. It is a high-throughput, high volume technique that facilitates gene expression and copy number surveys of as many as 1,000 different tumor tissues.[4] TMAs are considered powerful tools for high-throughput analyses and have been used extensively in various aspects of cancer research. Additionally, TMAs have been used as a method to quantify biomarkers present in cancer patient tissues and allows for molecular profiling and large-scale analyses at lower cost, both monetary and in donor tissue needed, and less time than conventional full-tissue approaches.[5,6]

However, TMAs have certain drawbacks, particularly in that preparation and construction on a larger scale is difficult. Currently, TMA molds are often purchased from vendors but can be expensive and custom designs would be more prohibitive in their cost, if offered at all. Alternatively, TMAs can be made in pathology laboratories but typically require complex and expensive machinery for construction. These roadblocks to TMA preparation can be restrictive to prospective researchers as the financial or physical resources to produce them as desired may not be readily available. Thus, we illustrate a method to manufacture a durable TMA mold that can be made for the specific needs of the user. This method aims to minimize the handicaps and possible errors that could arise from TMA construction, making the procedure more accessible and customizable.

Once constructed, TMA slides are generated using appropriate staining protocols, such as hematoxylin and eosin (H&E) and immunohistochemistry (IHC). Although procurement of slides may occur in a rapid fashion, the final steps of data analyses (dozens, hundreds or thousands of tissues

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Abbreviations: FFPE, formalin-fixed paraffin embedded; H&E, hematoxylin and eosin immunohistochemistry; IHC, immunohistochemistry; MTTB, multitumor tissue blocks; TMA, tissue microarray; WSI, whole slide imaging.

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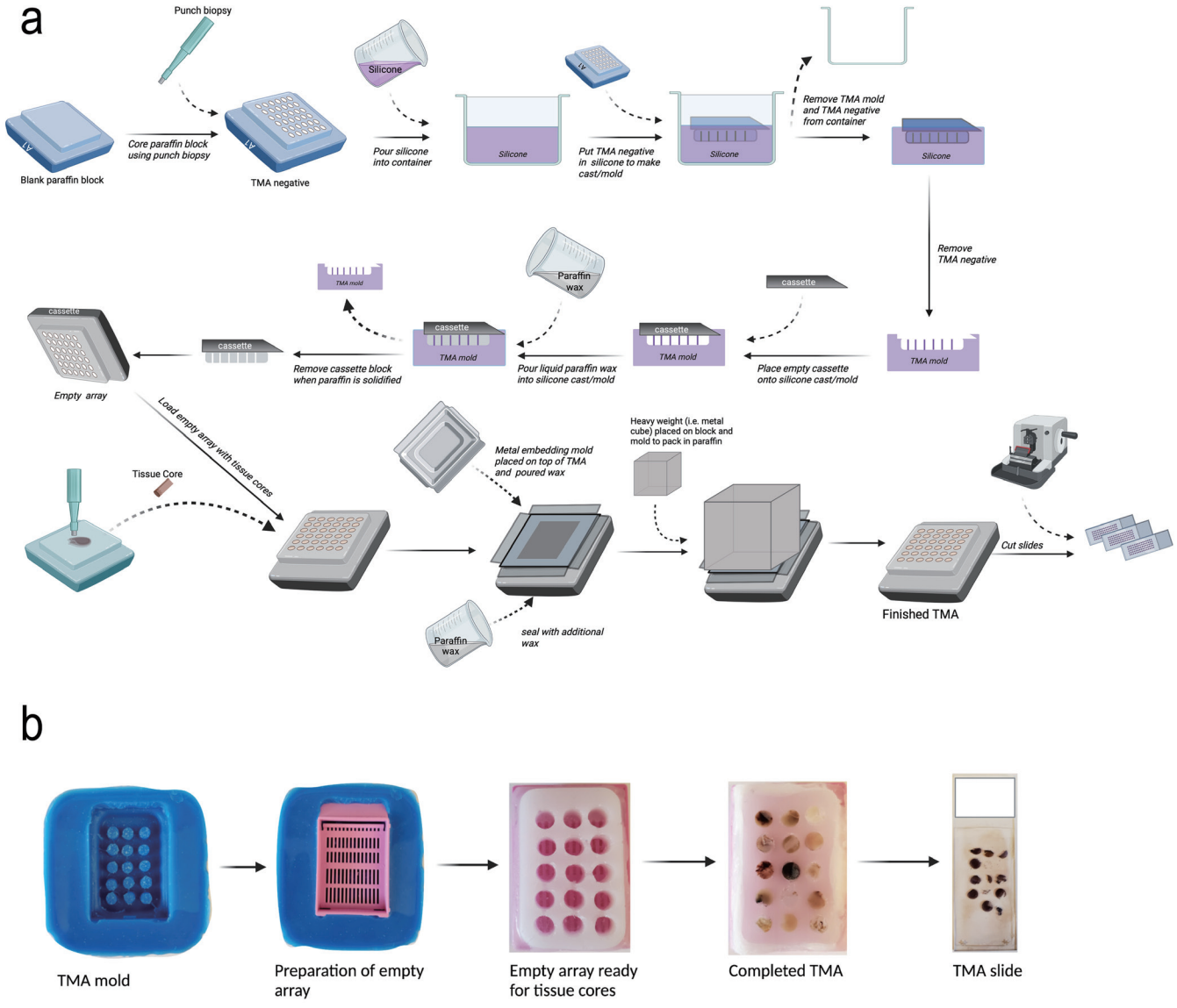

Fig. 1. Tissue microarray mold development. (a) Graphic illustration of the full tissue microarray array (TMA) process, from mold creation to slide production, (b) Full images of the silicone mold and its products in successive stages (5 × 3, arrangement; core diameter, 4 mm).

to be analyzed) may be delayed as manual review of large slide datasets can be cumbersome and time-consuming. Thus, we additionally discuss and share a broadly adaptable digital analytic pipeline, which allows researchers to couple cost-effective and efficient TMA construction, with digital image analyses for high-throughput discovery.

Methods

Construction of a silicone TMA mold

The construction of the TMA mold begins with creating the TMA negative (Fig. 1a). The TMA negative is a blank paraffin block with a core punch pattern. The blank paraffin block is made by pouring liquid paraffin (Paraplast X-tra, Product #39503002; McCormick Scientific, St. Louis, MO, USA) at a temperature of 60°C into a stainless-steel mold (Sakura Tissue-tek Base Mold, Product #4165, 37 mm × 24 mm × 5 mm; Sakura, Torrance, CA, USA), much like the standard FFPE embedding procedure. Once the blank is prepared, the core slots can be made by inserting biopsy punches (1–5 mm in diameter; Disposable Biopsy Punches, Product #B007TVKSPU; Robbins Instruments, Chatham, NJ, USA) into the wax block.

A silicone rubber base solution (Smooth-On MoldStar 30; Smooth-On, Inc., East Texas, PA, USA) for mold construction is prepared by mixing 20 mL of the A and B components each with a small metal spatula until the solution color is even, taking care to avoid bubbles. The silicone solution is then poured into a 4 oz OXO plastic container (Product #61130000; OXO, Chambersburg, PA, USA), and the prepared negative is submerged into the silicone solution. The cassette holding the paraffin should be facing up and visible, while the paraffin should be completely submerged. The holding container is agitated lightly to remove air bubbles, particularly within the areas of cored-out wax, and the solution and TMA negative block sits overnight (12 h or more) to solidify. The now-solid-

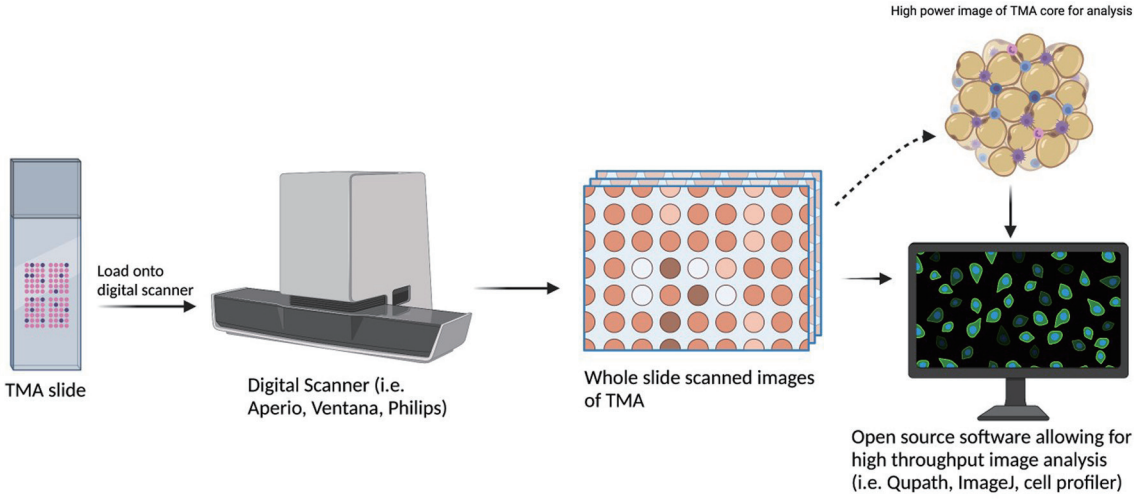

Fig. 2. Digital image analysis pipeline. Graphic illustration of digitization and high-throughput analyses of generated TMA slides.

ified silicone TMA mold is extracted from the container, and then the TMA negative is removed from the TMA mold.

Once the TMA mold is completed, an empty TMA array can be made by pouring paraffin at 60°C into the TMA mold, affixing a cassette on top of the mold and allowing the filled TMA mold to sit and cool as the paraffin solidifies (at least 15 min on a cold plate at −6°C). Once the empty paraffin TMA array is extracted from the mold, tissue cores from tumor specimens with the same diameter as the core slots can be loaded into the empty TMA array. In some cases, the depth of the core slots in the recipient block are greater than the depth of the cores from the donor blocks; these can be corrected by adding additional paraffin to the bottom of the recipient block before tissue core insertion. If unused core slots are present after loading, they can be loaded with empty paraffin wax cores. The wax block is sealed by gently pouring a layer of additional liquid paraffin at 60°C that covers the face of the block, and compressed using a stainless-steel metal base mold. A heavy weight (i.e., 840 g metal cube) is then placed on top of the stainless-steel metal base mold to seal the surface and fill gaps between the inserted cores and the TMA wax block. The weight is applied for 15–20 min. The sides of the block can also be compressed slightly to correct the shape and ensure potential gaps are minimized. Excess paraffin on the block is removed with a heated paraffin trimmer (Thermo Scientific Shandon Para-Trimmer; Thermo Fisher Scientific, Waltham, MA, USA). Tissue sections can be produced by using a microtome, and slides can be made as per routine pathology sectioning.

Digital image acquisition and analysis pipeline

The pipeline utilizes the methodology of constructing TMA slides described above (Fig. 1), which are then stained either using protocols for H&E or IHC. Then image data is acquired by loading the prepared slides into either a large-scale automated commercial scanner (i.e., Ventana iSCAN HT) or mechanical arm automated microscope scanner (i.e., Aperio VERSA). The generated WSI can be either stored on local hard drives or cloud-based storage. Of note, file formats are dependent on the scanner used, with the most commons extensions being Tag Image File Format (.tif, .tiff), Video Studio Installation (.vsi) and/or Scanscope Virtual Slide (.svs). After generation and storage of WSIs, readily available open-sourced desktop digital pathology analytical software as mentioned before can be used for viewing and analysis. Additionally, certain bioimage analysis platforms allow for customizable analytical tools and batch-processing (i.e., QuPath).

Results and discussion

A manually derived silicone TMA mold

Using the TMA mold construction methods noted above, any pattern of the user's choice can be punched into the blank paraffin block, resulting in the TMA negative. The process from TMA mold creation to TMA array slide generation is shown in Figure 1a. A completed TMA mold, in a 5 × 3 arrangement with core sizes at 4 mm diameter, is shown in Figure 1b. The silicone mold is customizable, can be made with core sizes as small as 1 mm without impacting performance, and will retain shape after at least 30 uses. The cost of the materials needed to make the mold are overall < $100 and endpoint construction takes less than 3 days.

Digital image analysis pipeline

The use of whole slide imaging (WSI), which involves the ability to acquire high-resolution scans of preprepared histological slides, has revolutionized tissue biomarker and companion diagnostic discovery within the educational, research and clinical settings over the last few years.[7,8] Currently, WSI can be generated using commercial scanners (e.g., Aperio, Ventana, Philips) to rapidly generate ultra-large two-dimensional images or Z-stacks which allows for high-throughput digital image analytics, quantitation and screening of tissue samples.[7] This in turn can be used in a mass scale setting to elucidate high-quality, reproducible and objective data.

Prior to the utilization/adoption of digital pathology WSI workflows, clinical and biological research was heavily reliant on manual review of either static microscopic images or prepared histologic slides via microscopes. The time constraints and workforce required due to these techniques can hamper high-volume analyses and innovation. Additionally, manual review introduces the possible interference of interobserver biases due to subjectivity of scoring and/or visual assessment. Thus, we describe a researched focused objective digital analytic pipeline (Fig. 2) that can be

used in multiple settings, which leverages the creation of simple silicone-based TMA molds, automated digital scanners and widely available open-sourced desktop software, such as QuPath,[7] Cellprofiler,[9] and ImageJ,[10] allowing for streamlined quantitative and qualitative analyses in a rapid manner.

The advantages of a digital workflow pipeline, coupled with our TMA array construction, is that it allows researchers to access powerful cost-effective and efficient tools to perform high-throughput and mass-scale studies that can lead to innovative and novel discoveries. However, certain caveats do exist as processing time is dependent on the hardware (CPU, GPU, RAM, etc.) that is readily available. Nevertheless, the underlying primary applications of this digital analytic pipeline can be utilized regardless of computing power and/or workforce.

The TMA is a high-throughput method that has been used in diagnostics and research for several decades, but the molds that are needed to mass-produce FFPE TMAs are expensive and customized designs would be difficult to procure. Here, we outlined a simple method to manufacture TMA molds that are effective and customizable to the needs of the user. In addition, leveraging this technique, we emphasize an efficient analytical research pipeline that incorporates widely available open-sourced digital pathology tools to further empower innovation and discovery. In summary, our proposed analytical pipelines allow for high-throughput analyses of tissue specimens for novel translational pathology research.

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Conflict of interest

One of the authors, Dr. Robert S. Ohgami has been an editorial board member of *Journal of Clinical and Translational Pathology* since May 2021. The authors have no other conflicts of interests to note.

Author contributions

Study concept and design (SG, KS, RSO), acquisition of data (SG, CS), analyses and interpretation of data (SG, KS, RSO), drafting of the manuscript (SG, KS, RSO), critical revision of the manuscript for important intellectual content (SG, KS, CS, SM, RSO), administrative, technical, or material support, and study supervision (RSO).

Data sharing statement

No additional data are available.

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