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A Novel Technique for the Generation of Multi-Tissue Blocks employing a Carrier

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Abstract

Paraffin blocks containing several tissues have become a major tool in surgical pathology. As multi-tissue blocks, they usually consist of few rather large samples and as tissue micro-arrays they may contain up to several hundred small sized tissue cores. We developed a novel approach to generate multi-tissue blocks employing a carrier in which tissue samples are inserted. Normal tissues with homogeneous consistency such as liver, spleen and lung appear to be ideal carriers. Carrier tissue size can be freely chosen to accommodate number and size of sample tissues as desired. Since the carrier tissue serves as a scaffold for the inserted tissue, even small sized tissues will stay exactly as placed in the carrier. This makes carrier-based multi-tissue blocks (CBMTBs) an ideal approach when exact orientation of layers is important, for example in normal GIT tissues. The carrier tissue approach can also be used for few large sized sample tissues or to generate classical TMAs with sample cores of <1mm. Since the newly generated carrier-based multi-tissue block (CBMBT) or carrier-based TMA (CBTMAs) is completely re-embedded after assembling, sectioning of CBMTBs is comparable to sectioning a normal solid tissue block producing virtually no sample loss and requiring minimal trimming and consequently vastly increasing yield.

INTRODUCTION

Paraffin blocks containing several tissues have become a major tool in surgical pathology as well as in a research setting for many years. Simple multi-tissue blocks (MTBs) can be constructed by embedding several single tissue specimens in the same paraffin block. A major modification was the introduction of 'sausage' tissue block containing an increased number of tissue samples in a singular paraffin block(1). A more recent development is the tissue-micro-array (TMAs) technology, which comprise of up to several hundred tissue specimens sampled as cylindrical cores. TMA cores have small diameters -usually less than 1mm- and are sampled with special devices(2). Due to the larger size of the tissue samples, classical MTBs contain a smaller number of tissues, which are re-embedded in a new tissue block and arranged in the desired way. This process is tedious and requires experience in order to place the single tissue specimens appropriately and efficiently. Battifora's classical

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‘sausage’ approach requires deparaffinization and sectioning of the specimens and consecutive wrapping in animal gut followed by a re-embedding procedure(1). In TMAs, tissue cores are arranged and placed into a plain paraffin block according to a pre-designed grid to enable exact localization of tissue cores. Tissue cores are usually held in place by mechanical forces by the surrounding paraffin since complete re-embedding involving melting of the paraffin is not possible.

Given the different methods and various advantages and disadvantages for constructing MTBs, we contemplated how to improve multi-tissue block approaches by incorporating several tissues in a single paraffin block, arranging them in the desired manner, while requiring little space and increasing the number of sample tissues. We finally developed a carrier-based multi-tissue block (CBMTB) technique employing a carrier, which is used as scaffolding to support the sample tissues thereby vastly enhancing the efficiency of multi-tissue approaches.

MATERIAL AND METHODS

Our initial aim was to generate multi-tissue samples which could be employed as control tissue for different immunohistochemical reagents. A multi tissue approach appeared best. We used dermatological circular punch devices of variable diameters to sample tissues from donor tissue blocks. The punch devices were commercially available from various providers (Acupunch, Acuderm; Ft. Lauderdale, FL; Biopsy Punch, Miltex, York, PA; Uni-punch, Premier Medical, Plymouth Meeting, PA). Extracted tissue cylinders were re-embedded in a new paraffin block and used as an acceptor carrier tissue (Figure 1A). We then used similar punch devices of smaller diameters to extract tissue cores from the carrier tissue. Similarly sized tissue cores, which we refer to as ‘sample cores’, were extracted from sample tissue blocks and inserted into the cylindrical holes previously created in the carrier tissue (Figure 1B, C). Depending on the overall diameter of the carrier tissue and the diameter of the sample tissue cores, variable number of tissues could be placed in a single carrier block. We experimented with different diameters of the carrier tissue as well as with the diameters of the sample cores. In order to accommodate tissues of larger diameters, we also employed large square carrier tissues. Large sized carrier tissues were cut to size during the grossing procedure and no re-embedding was necessary. Finally we generated a classical TMA employing a carrier tissue in which the TMA cores were automatically (Beecher ATA27, Sun Prairie, WI) inserted.

RESULTS

For the generation of carrier-based multi tissue blocks, various tissues can be used as a carrier acceptor tissue. This is largely dependent on the downstream application of the block but also dependent on the suitability for immunohistochemical processing. Homogeneous tissue allowing easy insertion of sample cores and smooth sectioning is mandatory. We found liver and spleen good acceptor/carrier tissues due to their consistency and homogeneity. Lung can also be used but is less suitable due to its porous nature. For the development of our technology, mostly automated stainer platforms employing non-biotin secondary detection systems were used. Nevertheless, we did test biotin-based secondaries

in combination with liver or kidney carrier tissues; once a proper blocking step was performed endogenous biotin reactivity did not pose any problem. Ideally, carrier tissues should be embedded at maximum height of the cassette to ensure high section yields. Insertion of sample cores was usually without problems. Ideally, the length of the sample cores should correspond to the height of the carrier tissue. However, in case the tissue of the sample block is thin, we were able to stack several short sample cores on top of each other. This ‘pancaking’ approach ensured the presence of sample tissue throughout the height of the carrier tissue. After the complete insertion of the sample cores, the carrier tissue including the inserted cores was placed face-down in a tissue mold and re-embedded. During the re-embedding process, carrier tissue and sample cores were aligned with the bottom of the mold by pushing carrier tissue and sample cores down. This leveling process ensured full sectioning of all tissues of the multi block starting with the first cuts (Figure 1D).

Firstly, we focused on the development of multi-tissue blocks which could be used as on-slide control or for initial evaluation and titration of new serological reagents. As an on-slide control block, a carrier tissue of 8mm proved useful as it could be easily applied in addition to even larger sized test tissues. Using an 8mm diameter carrier tissue, we were able to insert up to 5 sample tissues with diameters ranging from 1.5mm to 3mm. Diameters were adjusted to the tissue type. For layered tissues such as colon and skin 2.5 or 3mm proved to be ideal to ensure the presence of all tissue layers. We also generated a 12mm diameter carrier tissue in which we were able to insert 9 tissue cylinders of 1.5–3mm. These cylindrical shaped CBMTBs were ideal for assembling several normal tissues (Figure 1E, F). Immunohistochemical staining for various antigens such as Melan-A, CD3, CD20, and intermediate filaments were done. Immunohistochemical staining worked well on CBMTB sections as exemplified with vimentin (Figure 1G, H) and keratin (Figure 2B, E, F).

For tumor CBMTBs, we generated larger carrier blocks during the grossing procedure (Figure 2A, B). Tumor tissues were sampled as 4mm or 5mm cores. A typical tumor CBMTB would consist of 5 sample tissues, which were arranged in an asymmetrical fashion to ensure proper identification of the sample via its location in the block (Figure 2A). Those blocks were mostly used to establish tumor panels for immunohistochemical reagent validation (Figure 2B). Here, the ‘pancaking’ proved especially helpful in cases when the thickness of the tissue in the donor block was very limited.

Finally, we used a carrier tissue to generate a classical TMA with a core size of 0.5mm (Figure 2C–F). As with the other CBMTBs, the carrier-based (CB)TMA was completely re-embedded. No core was lost during the melting of the paraffin and all cores were brought easily into the level of the mold by gently pushing down the carrier tissue including cores. Sections from the carrier based TMA showed well aligned tissues (Figure 2C, D). Immunohistochemical staining revealed even immunopositivity of the inserted cores (Figure 2E, F).

DISCUSSION

The need for proper controls in immunohistochemical staining techniques has been discussed in detail previously(3;4). A multi-tissue approach appears to be the obvious and

optimal solution and can be used as controls in daily routine IHC as well as for the assessment of new serological reagents. The challenge was to generate a MTB which combines several tissues of sufficient size representing all relevant structures yet limited in overall block size. Conventional multi-tissue blocks usually require rather large sized normal tissue pieces in order to represent the desired histological structures and so they can be handled manually during the embedding process. This limits the number of tissues assembled per block, especially if a small-sized tissue block is needed. TMAs, on the other hand, use small tissue diameters which are ideal for random sampling of tumors but too small to serve as samples for many normal tissues. We initially sought to generate a MTB of normal tissues for the evaluation of novel serological reagents. For many organs such as liver, testis, kidney etc. location and size of the sample is less crucial in order to obtain a representative sample with most relevant structures. For some tissues, however, such as gastrointestinal organs and skin, exact sampling and orientation of the specimens is important. We found core sizes of 2–3mm best suited to contain all relevant structures, for example mucosa, submucosa and even parts of the muscularis propria of a colon sample. Core diameters of 2–3mm are somehow in-between classical MTBs and TMAs and the question was how to place them in a block, secure their location while ensuring easy processing. Battifora's 'sausage' approach is the classical method to gather several tissues in a single block(1). However, block assembly is cumbersome and the original technique appears to be rarely used. Our present approach of MTBs makes use of a carrier tissue thus avoiding several problems of MTB generation. A carrier medium keeps sample tissues in place in a defined location without any manipulation during the embedding process. Also, it ensures the correct positioning so that crucial structures such as epidermis or mucosa are consistently represented during sectioning. Moreover, the carrier tissue can be selected so it can serve as a proper control tissue similar to positive or negative endogenous control tissue making full use of the available space yet limiting the required size of the block drastically. Another advantage of carrier-based MTBs is the option of stacking tissue cores on top of each other, for example in case the donor block has almost been used up. The carrier tissue serves as scaffolding holding short tissue cores in place. During sectioning, the transition from one tissue core to the next was not noticeable. A key feature of our carrier-based MTBs is the fact that the newly assembled blocks are fully re-embedded. During this process, all tissues are brought into one level ensuring representation of all tissues literally from the first section. Moreover, the complete melting of the paraffin during embedding is prerequisite for homogeneous polymerization during the cooling process ensuring smooth sectioning of the block.

Circular carrier tissues proved ideal to sample several normal tissues in a confined area yet with proper representation of all relevant structures. However, for the analysis of tumor tissues, we increased the sample size to compensate for potentially heterogeneous antigen expression. Consequently, a larger carrier tissue was necessary, which could be easily generated during grossing. A typical tumor CBMTB was made up of five sample tissues of 5mm diameter asymmetrically placed for unequivocal location. The use of a carrier was even more helpful when used for generating TMAs. Conventional TMAs constructed in plain paraffin blocks are often prone to loss of cores during cutting or transfer of the section to the slide (5). Moreover, classical sections often show dislocation of cores and/or tilted

rows of cores. Special techniques employing tape sampling of the sections have been devised to circumvent these problems(6). Due to the complete re-embedding, a CBTMA block cuts like a solid tissue eliminating the loss of cores during cutting and transfer of sections to the slide. No trimming is necessary and no delicate maneuver to level the protruding TMA cores is required, largely increasing the yield of sections and improving the handling of the TMA. Immunohistochemical analysis employing various markers, showed homogeneous immunostaining of all CBTMA cores. The main features and advantages of CBMTBs and CBTMAs are summarized in Table 1.

The generation of carrier-based MTBs is straight forward and depending on the number of sample tissues takes a trained technician approximately 10–15 minutes to insert all cores.

In conclusion, the use of a carrier tissue for the construction of multi-tissue blocks greatly improves the handling, sectioning and section yield allowing for an efficient analysis of multiple tissues in an optimized space.

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Figure 1.

Generation of a Carrier-Based Multi-Tissue Block with a diameter of 12mm, A) circular newly embedded circular spleen tissue with 12mm diameter, B) extraction of tissue cylinder with dermatological punch device (3mm), C) circular acceptor block after insertion of all donor cylinders, D) multi-tissue block with spleen carrier tissue after re-embedding process demonstrating completely aligned tissues before first sectioning, E) slide with H.E. stained 5micron section of newly generated carrier-based multi-tissue block, F) higher magnification demonstrating 12mm circular spleen carrier tissue and nine sample tissues (1: carrier tissue

spleen, 2: colon, 3: pancreas, 4: kidney, 5: lung, 6: liver, 7: testis, 8: skin, 9: tonsil, 10: placenta), G) low magnification of vimentin immunohistochemical stain (DAB chromogen) displaying extensive homogeneous immunopositivity of all tissues including spleen carrier tissue and sample tissues, H) higher magnification of vimentin staining demonstrating immunonegative colonic epithelium.

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Figure 2.

Carrier-Based Multi-Tissue Block for larger sized tumor samples (A, B) and for Tissue Micro-Array (TMA, C–F); A, B) square-shaped lung tissue specimen used as carrier for five colorectal carcinoma samples (5mm diameter each), A) hematoxylin eosin stain, B) AE1/AE3 (DAKO) immunohistochemical stain displaying immunopositivity of colorectal carcinoma areas as well as of lung carrier tissue serving as ‘internal control’; C) TMA (core size 0.5mm) of colorectal carcinomas and normal kidney sampled into liver tissue as carrier, D) higher magnification of colorectal carcinoma cores (example, *) and normal kidney

(example, +) showing even alignment of cores, E) immunopositivity of colorectal carcinoma core with AE1/AE3 (DAKO) and positive staining of bile ducts of liver carrier tissue.

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Table 1

Main Features of Conventional versus Carrier-Based Multi Tissue Blocks (MTBs) and Tissue Micro Arrays (TMAs)

	Conventional MTB/TMA	Carrier-based MTB/TMA
Carrier media	None, plain paraffin	Various carrier tissues suitable (e.g. liver, spleen, lung), carrier serves as test tissue/ 'internal' control
Sample size and number	MTB: threshold size depending on tissue and need for orientation	MTB: small sample size & high sample number, yet proper orientation; TMA: similar to conventional TMA
Re-embedding	MTB: yes; orientation/location can be difficult during embedding TMA: no	MTB: yes, TMA: yes, properties like solid tissue block
'Pancaking'	MTB: no TMA: yes, but possible disruption between tissue layers	MTB: yes; TMA: yes no disruption between tissue layers
Sectioning	MTB: uneven sectioning, potential loss of tissue TMA: uneven cutting, bended rows and loss of punches during slide transfer	MTB: smooth; similar to solid tissue block TMA: smooth; similar to solid tissue block; straight rows, no loss of punches
Leveling/ Trimming	MTB: moderate trimming TMA: leveling of punches difficult, ample trimming can be necessary	MTB: minimal trimming; TMA: minimal trimming, all punches on level after re-embedding
Summary	MTB: tissue size, number, orientation negatively inter-related; TMA: Handling and leveling difficult, uneven sections, loss of punches, distorted rows, low yield	MTB: high number, small size, optimal orientation (normal tissues); precise location of (similar) tissues possible, TMA: even and continuous representation of all punches, high yield