SHORT REPORT

A simple method for the construction of small format tissue arrays

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Tissue arrays can evaluate molecular targets in high numbers of samples in parallel. Array construction presents technical difficulties and tissue arrayers are expensive, particularly for small and medium sized laboratories. This report describes a method for the construction of 36 sample arrays using widely available materials. A blunted 16 gauge needle for bone marrow aspiration was used to extract paraffin wax cylinders and manually define a 6 × 6 matrix on a blank paraffin wax block. Tissue cores from 36 paraffin wax embedded premalignant lesions and invasive cervical carcinomas were injected into the matrix using a 14 gauge needle. This tissue array was sectioned using a standard microtome and used for the immunodetection of CD44 variant 9 and interleukin 18 with satisfactory results. This method can be applied in any laboratory, without the need of specialised equipment, offering a good alternative for the wider application of tissue arrays.

Tissue arrays make possible the high throughput validation of novel tumoral markers.[1] The parallel analysis of large numbers of tissues under the same experimental conditions represents savings both in time and resources.[2] This technology offers a way to fill the gap between the discovery of new molecular markers, derived from high throughput genomic analysis, and their application in the clinical setting.[3] Despite the flexibility and benefits of this method, its broader use has been hampered because of the technical difficulties of array construction and the costs of available arrayers. Tissue microarrays are available from commercial and academic sources (http://www.nhgri.nih.gov/DIR/CGB/TMA/TARP.html), but the construction of custom arrays is still necessary, especially if the tissue of interest is not present in the available arrays. To allow a broader application of this technology, we developed an easy method for the construction of small format tissue arrays containing 36 samples. These arrays can be constructed using materials available in every hospital and do not require specialised equipment.

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MATERIAL AND METHODS

Both 16 gauge (16G) and 14G bone marrow aspiration needles were blunted and sharpened using a grinding machine. The 16G needle was used to punch paraffin wax cylinders, which were 3 mm in height and 2 mm in diameter, defining a 6 × 6 cylinder matrix on a 2.5 × 2.5 cm paraffin wax block. The 14G needle was used to obtain tissue cylinders from 36 paraffin wax embedded tissues, which were injected into the blank paraffin wax block. We used tissues derived from preinvasive lesions and invasive cervical carcinomas for array construction. Tissues were obtained from the histopathological archives of the pathology departments, Hospital General de México, S.S. and Hospital de la Mujer, S.S. Once the array was completed, a small amount of hot liquid paraffin wax was poured over the array surface and the tissue cylinders were levelled with the block using a glass slide. To blend together the paraffin wax from the blank block and the tissue cylinders, the array was incubated on the slide at 60° C for 15 minutes. After incubation, the array was chilled on ice and 5 µm sections were obtained using a rotatory microtome. Array sections were mounted on sylanised slides and incubated overnight at 40°C. Immunohistochemical analysis was performed using antibodies against interleukin 18 (IL-18; 1/200 dilution; Santa Cruz Biotechnologies, Santa Cruz, California, USA) and CD44 variant 9 (CD44V9; 1/1000 dilution; US Biological, Swampscott, Massachusetts, USA), using the Dako Envision system (Dako, Carpinteria, California, USA), according to the manufacturer's instructions. Antigen retrieval was done using the Vector antigen retrieval solution (Vector Laboratories, Burlingame, California, USA) in a standard microwave oven for 10 minutes at power level 1.

RESULTS

The smaller diameter (16G) of the needle used to punch the blank paraffin wax block allowed the bigger tissue cores (14G) to fit exactly into the blank. Because of this, core injection has to be done carefully to avoid the possible breakage of the tissue cylinder. The combination of this tight fitting, the use of hot liquid paraffin wax, and incubation in the oven resulted in a solid, homogeneous paraffin wax surface, thereby avoiding possible tissue loss during array construction or sectioning. A 6 × 6 sample matrix (fig 1A) was the most appropriate for the size of the blank block that we used, but the number of arrayed tissues could possibly be increased, depending on the type of blank block used. The array sectioning was the most difficult step of the process. The use of new disposable blades and cooling the array on ice improved the sectioning process. Tissue spots were lost, especially during block orientation and positioning, but once the array was oriented with the blade, the average number of losses was two to three tissue spots every six to 10 sections. Depending on the height of the original donor tissue, we were able to get 40 to 60 complete array sections from each tumour array. However, these numbers might be an underestimate because the original tissue blocks used for array construction were quite old and have been sectioned many times before.

We successfully performed IL-18 and CD44V9 immunodetection without substantial tissue cylinder loss during antigen retrieval or washes. Figure 1 shows the different tissues after immunohistochemistry.

Abbreviations: CD44V9, CD44 variant 9; G, gauge; IL, interleukin

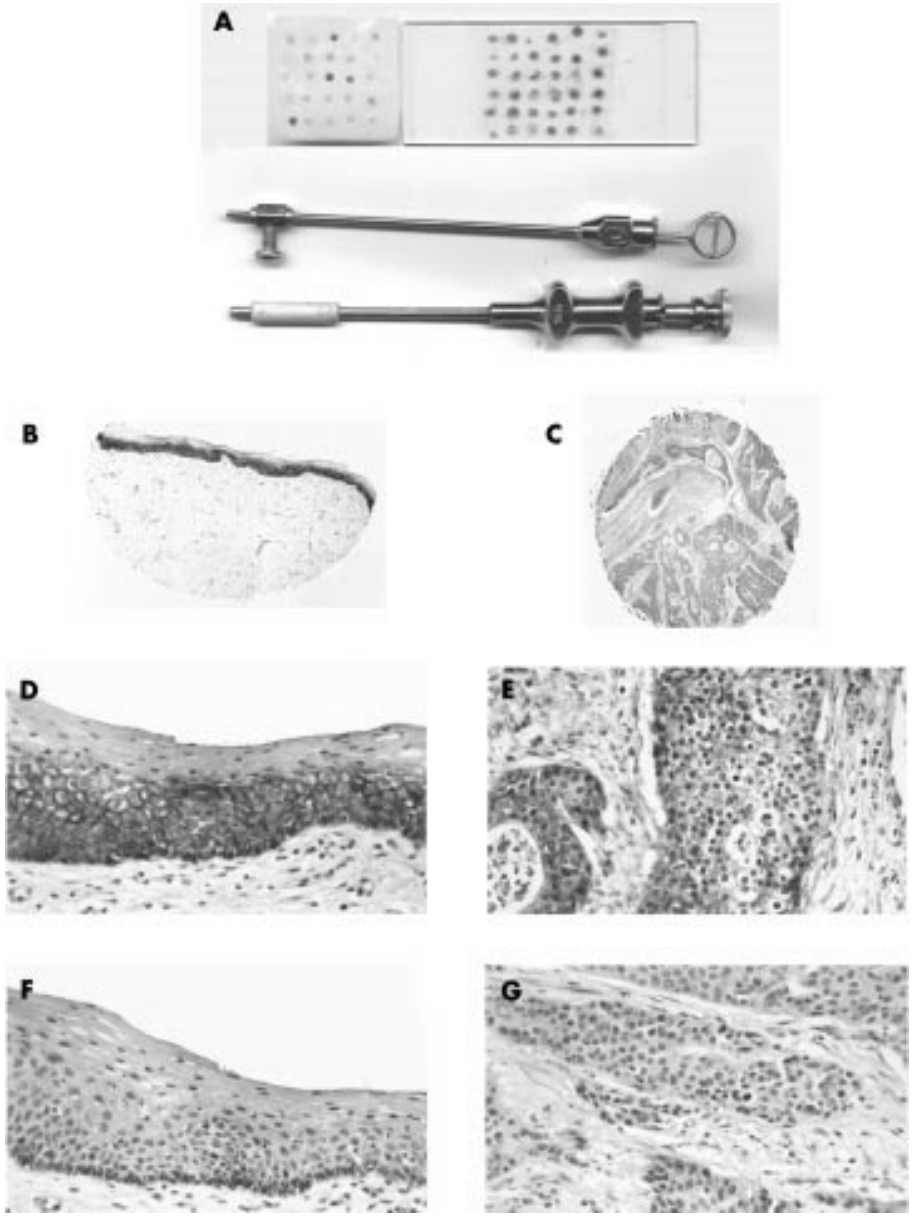

Figure 1 Small format tissue array construction. (A) The equipment needed for array construction: the 16 gauge and 14 gauge needles, the array block, and a haematoxylin and eosin stained array section. CD44 variant 9 immunostaining on (B) a cervical premalignant lesion and (C) an invasive cervical carcinoma cylinder. (D, E) Enlarged areas from (B) and (C), respectively. Interleukin 18 immunostaining in (F) a premalignant lesion and (G) an invasive tumour.

DISCUSSION

Manual tissue array construction using the method described here is a cheap and easy alternative for small laboratories. The 2 mm diameter of the tissue cylinders maintained recognisable histological features of the arrayed tissues, and offered more tissue surface to evaluate immunostaining. This increased cylinder size also makes the correct sampling of premalignant cervical lesions easier and more accurate. Normally, tissue microarray sections are obtained using an adhesive tape that prevents the array sections from breaking apart. However, the tape system needs several accessories, and its cost is quite high. Manual microtome sectioning is also used with tissue microarrays, but this demands that the histotechnologist sectioning the array should be very experienced. Once complete sections of our array were obtained, it was necessary to cut as many sections as possible, because proper orientation of the array with the microtome blade might result in important loss of tissue spots. A very similar approach for manual tissue array construction has been described previously.[4] That method used a standard R A Lamb processing cassette as a 34 hole template and needles with different diameters for punching. However, the paper did not fully described the complete array construction method, the number of tissue spots lost during sectioning, or the number of sections that can be obtained from the array.[4] The use of the cassette as a template allows an even and ordered distribution of the cores in the array, but it obviously limits the number of arrayed samples. This situation does not arise with the method that we describe.

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Even though the arrays that we describe contain a small number of samples compared with other tissue microarrays,

Take home messages

- We have developed a method that offers an economical alternative for the construction of small format tissue arrays (36 samples)
- This method can be used to validate emerging molecular markers in small or medium sized laboratories

which can contain up to 600 samples, they are still a good alternative when time and money need to be conserved, if a tissue arrayer is not available, or if a limited number of samples needs to be tested.

In summary, the method described here offers an economical alternative for the construction of small format tissue arrays, which can be used to validate emerging molecular markers in small or medium sized laboratories.

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