J Bras Patol Med Lab, v. 53, n. 4, p. 261-269, August 2017

10.5935/1676-2444.20170041

Tissue microarray: physical and chemical parameters involved in the construction of recipient blocks

Microarranjo de tecidos: parâmetros físicos e químicos envolvidos na construção dos blocos receptores

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ABSTRACT

Introduction: Tissue microarray (TMA) is considered an innovative method in several fields, with a great diversity of applications and advantages over traditional histomorphometric techniques. The most important advantage that TMA offers is the simultaneous evaluation of a large number of specimens from a limited source of material. However, TMA exhibits a high rate of non-viable samples in the final stages of the process, which compromise their use in analyzes that can not be repeated. **Objective:** Considering this disadvantage, the objective of this study was to optimize the methodology to maximize the viability of the samples, as well as to increase the efficiency of the technique. **Material and methods:** For this purpose, several variables involved in the construction of the recipient blocks, including paraffin composition, diameter, spacing distance, localization and type of the tissue samples in the block were tested in order to establish correlations between the quality of the values and the parameters studied. **Results:** The results showed that the blocks built with polymer-enriched paraffin, subjected to the fusion protocol at 37ºC, associated to a tempering, and constructed with one millimeter diameter samples and 1000 µm spacing between tissues, produced slides whith superior features. **Conclusion:** The data obtained from the physical and chemical adjustments of the TMA recipient blocks provided vital information that, when applied in TMA research projects, may reduce the losses associated with the method.

Key words: histological techniques; paraffin; quality improvement.

INTRODUCTION

Since it was first reported in 1998, the tissue microarray (TMA) technique has been essential in hundreds of research projects involving diseases such as cancer, inflammatory and neurodegenerative diseases[1]. The construction of TMA blocks is based on the extraction of cylindrical punches of paraffin-embedded tissues from a diverse set of donor blocks, which are later reintroduced in the same recipient paraffin block in specific positions. This method allows the construction of blocks comprising nearly of 1,000 different samples. After assembling the TMA blocks, histological slides containing all the samples are obtained, enabling the simultaneous analysis of molecular targets in rigid and standardized conditions[2, 3].

TMA represents an important technological innovation in several different fields that use histomorphometric analysis as a research tool, including pathology, histology and immunology[3]. In the field of oncology, different TMA models embedding optimized tissues of interest have been used to test markers associated with the prevalence, progression or prognosis of malignant neoplasms[4]. The TMA method has also been of great importance for studies of inflammatory diseases and neurodegenerative diseases, such as Alzheimer's disease. In this case, TMA slides embedded with different samples of the brain were used to determine the number of amyloid plaques present in the affected organ. TMA can also be used for studies of healthy tissues. This approach is vital to allow for understanding of the expression of target genes in normal tissues[5].

TMA presents a range of advantages in comparison with other techniques that use slides derived from paraffin blocks, such as the

First submission on 14/03/17; last submission on 14/03/17; accepted for publication on 22/05/17; published on 20/08/17

amplification of a limited source of material, the simultaneous analysis of a large quantity of specimens, experimental uniformity, and a reduction in the time and costs required for laboratorial procedures[3]. The large-scale production of data by this method is possible due to the number of samples that can be processed simultaneously, a number which, depending on the apparatus used for construction, may reach 1,000 spots per block[6, 7]. In addition, TMA allows cylindrical specimens with a small diameter to be removed from the donor block without causing any significant damage to the donor tissues. This prevents the depletion of the original tissues present in the donor blocks, maintaining them interpretable for future morphological and/or molecular analysis[8]. Despite its advantages, however, the TMA method presents a high rate of non-viable samples at the end of the process. This is caused by losses, scratches or folds in the tissue during the stages of the cutting and transferring of the sections from one block to the other[2]. Previously reported data estimated that tissue damage at the end of the TMA process can reach 10% to 30% of the original material used[9-13]. This drawback can compromise the use of the method in the laboratory experiments where repetitions are not possible due to the use of limited or scarce samples[14].

Variables such as type of tissue, paraffin characteristics of the recipient block, paraffin block tempering protocol of both blocks (donor and recipient), dimensions of the cylinder punch of the sample, spacing and positioning between the punches in the block, may act as determinant factors affecting the quality of the product. Despite their importance, these variables have not been studied in a systematic and controlled way. Therefore, the aim of the present study was to analyze the physical and chemical parameters involved in recipient block construction to optimize the TMA method and to reduce the high rate of non-viable samples produced.

MATERIAL AND METHODS

Embedded paraffin blocks containing a variety of healthy mice organs were used to analyze the physical and chemical parameters employed in the construction of the recipient blocks. The experimental protocol followed the ethical principles in animal research indicated by the International Guiding Principles for Biomedical Research Involving Animals[15, 16] and was approved by the Commitment to the Ethical Use of Animals in Research at the Universidade Federal de São Carlos (CEUA 7669010815).

The variables studied were: block tempering protocol (37ºC, 40ºC and 64ºC), tissue type (dorsal skin, heart, small bowel, lung, liver, spleen, stomach, kidney and tongue), recipient block composition (pure paraffin, pure paraffin with increasing percentages of beeswax 5%, 10% and 15%, paraffin enriched with Histotec® polymers from Merck Millipore, Darmstadt, Germany), punch sample diameter (0.6 mm, 1.0 mm and 1.5 mm), spacing between samples in the recipiente block (400 µm, 700 µm and 1000 µm), and positioning in the block (upper right, upper left, center left, center right, lower left and lower right). The punch diameter values and the spacing between them were based on publications on TMA methodology and the options enabled by the TMA Master equipment (3-D Histech®, Budapest, Hungary). This equipment is a drilling and punching machine for the construction of the TMAs. The operator has a range of possibility to control parameters such as size of punch, layout of distribution and size of the samples.

Animals

The mice used in the experiment were healthy, three-month-old male Swiss mice that had not undergone any type of previous treatment or procedure. The mice were sacrificed with an overdose (150 mg/kg) of Ketamine (Sespo, Paulínia, SP, Brazil)[17]. Tissues such as the dorsal skin, heart, small bowel, lung, liver, spleen, stomach, kidney and tongue were removed and immediately fixed in 10% buffered formalin (Sigma-Aldrich, St. Louis, MO, USA) for 24 hours at 25ºC. After the fixation period, the tissues were washed in running water and kept in a 70% ethanol solution at 4ºC for 24 hours. Subsequently, the samples were prepared for inclusion in paraffin using an automatic tissue processor (Lupetec, São Carlos, Brazil).

Construction of the TMA blocks

Five different reagents were used for the construction of the recipient blocks: pure conventional paraffin, a mixture of pure conventional paraffin with 5%, 10% or 15% of beeswax, and paraffin enriched with polymers – Histosec® (Merck Millipore, Darmstadt, Germany). Only the blocks constructed with Histosec® did not suffer scratches after trimming of the surface with a microtome, and for this reason, the paraffin enriched with polymers was used for the final construction of all the TMA blocks employed in the subsequent tests.

Using the TMA Master device, three identical recipient blocks were constructed for analysis of the block tempering protocols, together with nine different blocks for the process of the analysis and optimization of the other variables. This construction was performed by the successive repetition of three basic steps: 1) performance of a small puncture in the recipient block; 2) removal of one cylindrical sample from the donor block, and 3) insertion of the cylindrical sample of the tissue removed from step (2) into the puncture performed in step (1).

Experimental design of the recipient blocks used for analysis of the block tempering protocols

For analysis of the block tempering protocols, the recipient blocks received 1.5 mm diameter cylinders made up of samples from different tissues, with a spacing between cylinders of 1000 µm. The arrangement of the tissue samples was identical among the three recipient blocks, although the samples were distributed to occupy the different regions of the block (**Figure 1**).

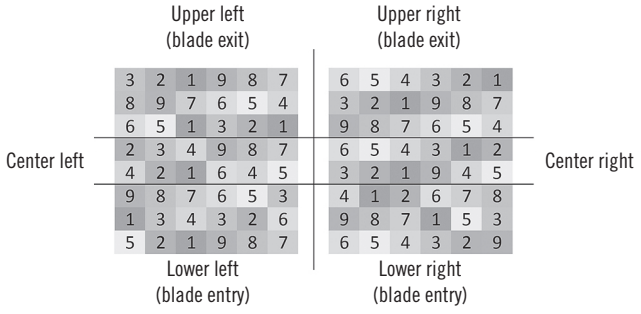

FIGURE 1 – *Example of the experimental design of the recipient blocks*

The numbers represent the location of each tissue distributed throughout the block: 1) skin; 2) heart; 3) bowel; 4) lung; 5) liver; 6) spleen; 7) stomach; 8) kidney; 9) tongue.

Experimental design of recipient blocks used for analysis of the remaining variables

The TMA were produced with cylindrical punches of the sample, with three different diameters (0.6 mm, 1.0 mm and 1.5 mm), positioned with a spacing between samples of 400 µm, 700 µm or 1000 µm. The blocks were designed so that each tissue would be distributed uniformly throughout each recipient block. Additionally, the tissues were located at least once in the first line of the entry and exit point of the blade, receiving the first and last contact between the blade and the block during the cutting of the histological section (Figure 1).

Performing the block tempering protocols

The block tempering protocol is used to incorporate the tissue sample present in the cylinder obtained from the donor block in the recipient block. Three different block tempering protocols currently described in TMA experiments were tested.

• Protocol 1: recipient blocks containing 1.5 mm diameter tissue samples with spacing between punches of 1000 µm were submitted to a temperature of 64ºC in the oven for 10 minutes. The blocks were allowed to cool at room temperature (25ºC) for 10 minutes. The procedure was repeated three times so the recipient blocks were tempered, and the blocks were then allowed to stand overnight at room temperature (25ºC)[18].

• Protocol 2: the recipient blocks were submitted to a temperature of 37ºC overnight, placing them in the stove with the surface facing downwards, with a clean slide as support. Subsequently, the blocks were submitted to a temperature of -12ºC for 10 minutes, followed by two heating-cooling (37ºC/-12ºC) cycles of one hour each[19].

• Protocol 3: the recipient blocks were submitted to a temperature of 40ºC for 10 minutes, with the surface of the block facing downwards using a clean slide as support.

Preparation of slides for quality analysis – block tempering protocols

After the qualitative verification of the integrity of the respective recipient blocks, those submitted to block tempering protocols at temperatures of 37ºC and 40ºC were used for obtaining sample sections of 5 µm thickness, as they did not suffer dimensional distortions. Subsequently, three sections from each block were extended on glass slides and analyzed under light microscopy for quality evaluation. Five µm thickness was the lowest value that allowed the performance of cuts in all the extensions of the block (3 cm × 2 cm) and the attainment of the slides without loss of tissues.

Preparation of the slides for quality analysis – diameter and spacing

The block tempering protocol that presented the best result was used for the construction of the other nine recipient blocks from the combination of the three diameters of tissue (0.6 mm, 1.0 mm or 1.5 mm) and the three spacing values (400 µm, 700 µm or 1000 µm). The blocks were cut using a microtome loaded with a new blade. Three sections from each block were again extended on slides for later analysis of the quality of the sections through light microscopy.

Establishing the level and quality indicators

Each tissue sample was analyzed individually, with qualitative value described based on an index of quality comprising the values 0, 1, 2, and 3. Value 0 was attributed for samples that presented complete absence of tissues (**Figure 2A**) in the section. Value 1 indicated that the tissue was present (totally or partially) in the section, but presented bends and scratches (**Figure 2B**). Value 2 meant the tissue was present in a complete form, exhibiting slight spacing between the borders and the paraffin of the recipient block (**Figure 2C**). Finally, value 3 represented the complete and integral presence of the tissue without exhibiting any spacing (**Figure 2D**).

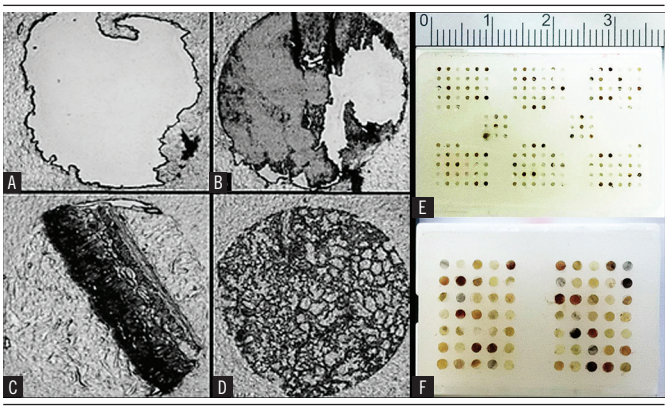

FIGURE 2 – *Quality levels established for quantitative analysis of tissue samples*

The levels were organized in an increasing order of quality: A) value 0; B) value 1; C) value 2; D) value 3. TMA blocks built with polymer-enriched paraffin, submitted to the 37ºC block tempering protocol, associated with a tempering process and filled by 0.4 (E) and 1.5 mm diameter samples (F) with 1000 µm spacing between the tissues.

TMA: tissue microarray.

To improve the optimization of the TMA technique, the three quality values of each sample analyzed in triplicate were merged into one value by multiplying them together, so that zero represented the worst quality condition and the value 27 stands for the best possible situation. For the dichotomization of the quality indicators, values equal to or greater than nine were considered acceptable. This type of sample did not receive zero values (worst quality) at any stage but achieved a score of three on two occasions (best quality).

Quality analysis of slides exposed to hematoxylin and eosin (HE) staining

After the quality analysis, the block with the best result was used to obtain four sections with 6 µm thickness, which were immediately positioned on histological slides. The sections were maintained at 37ºC in a drying stove for 24 hours to keep the tissue dry and to attach it to the slide before the staining process. Subsequently, the sections underwent deparaffinization and HE staining based on the standard protocols. The slides were scanned and digital images were obtained using the Panoramic Desk (3D Histech®, Budapest, Hungary). Each tissue was qualitatively assessed using the quality indicator values created in this study.

Statistical analysis

The experimental variables were analyzed using the Mann-Whitney, Kruskal-Wallis and Dunn Test with an adjusted *p*-value. The adjusted *p*-value was adjusted by Benjamini-Hochberg correction. Statistical tests were performed using the IBM SPSS Statistics (SPSS Inc.®) and R (version 3.2 – www.r-project.org) software packages, with $p \leq 0.05$ considered significant in all tests.

RESULTS

Structure of the recipient blocks

The blocks constructed with pure conventional paraffin did not endure the entry of the blade, releasing them from the cassette. They also suffered scratches and breaks along their entire length. The blocks constructed from the mixtures of pure paraffin and different percentages of beeswax endured the microtomy phase. However, the cuts performed by the blade were carried out under great friction, resulting in low quality of the sections. These characteristics were found in all mixtures, regardless of the percentage used. The sections constructed with paraffin enriched with polymers presented the best results, and so this material was used in all the other steps.

Block tempering protocol

The selection of the block tempering protocol was a determinant for the quality of the experiments. The recipient block submitted to a temperature of 64ºC (protocol #1) did not retain its integrity, melting during the first heating phase. However, protocols #2 and #3 produced blocks without distortions, however, the quality of the samples from the blocks submitted to protocol #2 with tempering [index of quality (IQ) = 12] were significantly better than those undergoing protocol #3 without tempering (IQ = 4) (**Table 1**; $p < 0.01$, Mann-Whitney *U* test). Since tempering protocol #2 was the most efficient, this treatment was used as the basis for testing the others variables.

Diameter of sample inserted in the recipient block

Statistical analysis showed that the quality values of the samples inserted in the recipient blocks varied in relation to the diameter of the punch ($p < 0.01$, Kruskal-Wallis statistical test). Samples with a diameter of 1.0 mm presented a median of quality which was statistically higher (IQ = 18, $p < 0.01$, Dunn's test) than the samples with diameters of 1.5 mm (IQ = 8) and 0.6 mm (IQ = 6). However, the samples with a diameter of 1.0 mm had a median value statistically higher ($p < 0.01$) than those with a diameter of 0.6 mm (Table 1). There was no statistical difference between the 1.5 mm and 0.6 mm diameter samples.

Spacing between samples inserted in the recipient block

The quality value results were statistically different depending on the spacing distances ($p < 0.01$; Kruskal-Wallis statistical test). The data showed that the median of the quality values for the blocks with 1000 µm spacing (IQ = 12) was statistically higher ($p < 0.01$;

TABLE 1 – Quality of blocks in relation to physical parameters used to construct the TMA

Variables	*n*	Quality values for testing in triplicate				*p*
		Median	Mean	SD	Standard error of the mean	
Block tempering*						
Protocol 2 – with tempering	70	12	14.93	8.916	1.066	< 0.01
Protocol 3 – without tempering	70	4	6.53	7.699	0.92	
Diameters of cylindrical punches of sample**						
1 mm[(a)]	238	18	17.62	9.145	0.593	-
1.5 mm[(b)]	166	8	9.95	8.756	0.68	< 0.01
0.6 mm[(c)]	342	6	7.68	6.469	0.35	< 0.01
Gaps between the cylindrical punches of the samples inside the block**						
1000 µm[(a)]	344	12	12.8	9.034	0.487	-
700 µm[(b)]	402	8	10.12	8.895	0.444	< 0.001
400 µm[(b)]	382	8	9.43	9.001	0.461	< 0.001
Diameter vs. sample spacing combination**						
1 mm-1000 µm[(a)]	112	18	18.25	8.337	0.788	-
1 mm-700 µm[(a, b)]	126	18	17.06	9.807	0.874	0.720
1.5 mm-1000 µm[(b)]	70	12	14.93	8.916	1.066	0.028
1 mm-400 µm[(b)]	164	18	14.36	9.006	0.703	< 0.001
0.6 mm-1000 µm[(c)]	162	8	8.1	6.861	0.539	< 0.001
0.6 mm-700 µm[(c)]	180	6	7.29	6.087	0.454	< 0.001
1.5 mm-700 µm[(c, d)]	96	4	6.31	6.618	0.675	< 0.001
0.6 mm-400 µm[(d)]	218	3	5.72	7.008	0.475	< 0.001

TMA: tissue microarray; SD: standard deviation.
**: Mann-Whitney* U *test; **: Dunn statistical test with* p*-value adjusted by the false discovery rate according to the correction proposed by Benjamini-Hochberg; a, b, c and d: show statistically significant differences between groups.*

Dunn statistical test) than the blocks with 700 µm spacing (IQ= 8) and 400 µm spacing (IQ = 8). No significant difference was found between the blocks with spacing of 700 µm and 400 µm (Table 1).

Correlation among sample spacing and diameter in relation to quality values

After identifying the existence of an individual correlation between diameter and the spacing between samples in relation to the quality values, analysis was performed to check whether the spacing-diameter combination could influence quality values ($p < 0.01$; Kruskal-Wallis statistical test). The data revealed an interaction between the two variables. The combinations of 1.0 mm diameter and 1000 µm spacing (IQ = 18); and 1.0 mm diameter and 700 µm spacing (IQ = 18) presented the best quality values in comparison to the other combinations ($p < 0.01$; Dunn statistical test). The samples with diameter of 0.6 mm regardless of spacing, displayed the worst quality values (1000 µm, IQ = 8; 700 µm, IQ = 6; 400 µm, IQ = 3) (Table 1).

Tissue type

Analyzing the influence of the tissue type with the quality indicators, the results showed that the values of quality of the lung (IQ = 12), tongue (IQ = 12), bowel (IQ = 9) and kidney (IQ = 9) were significantly superior to the others ($p < 0.05$; Dunn statistical test). Among these tissues, there were no statistical differences. The tissues: skin (IQ = 4), spleen (IQ = 6) and stomach (IQ = 4) showed the worst results. Liver (IQ = 8) and heart (IQ = 8) showed intermediate values of quality (**Table 2**).

Samples positioning

Assays were performed to verify the existence of a correlation between the positions of the samples in the recipient blocks and their respective quality values. When results were analyzed, it was verified that, except for the upper right position (IQ = 6), which was considered the worst, there was no statistical difference in the quality of samples based on the position of samples in the recipient blocks (Table 2).

Correlation between tissue types and sample diameters and quality indicators

When the effect of the association between tissue type and sample diameter on the quality of the indicators was analyzed, it was found that there were interactions between both variables which impacted the results. The best quality values obtained were

TABLE 2 – Quality of blocks in relation to the type of tissue used to construct the TMA

Variables	*n*	Median	Mean	SD	Standard error of the mean	*p*
Quality values for testing in triplicate						
Type of tissue*						
Lung[a]	124	12	14.26	8.756	0.786	-
Tongue[a]	122	12	12.35	9.24	0.837	0.4048
Bowel[a]	128	9	11.89	9.368	0.828	0.1794
Kidney[a]	124	9	11.65	8.378	0.752	0.1164
Liver[b]	129	8	11.19	8.319	0.732	0.0382
Heart[b]	126	8	11.04	8.922	0.795	0.0268
Skin[b, c]	127	4	9.02	9.671	0.858	< 0.001
Spleen[c]	124	6	8.07	8.187	0.735	< 0.001
Stomach[c]	124	4	6.84	8.635	0.775	< 0.001
Position of the samples in the block*						
Lower left[a]	202	12	12	9.295	0.654	-
Center left[a]	157	8	11.5	8.96	0.715	0.981
Center right[a]	165	8	11.25	8.855	0.689	0.896
Lower right[a]	199	9	10.88	9.201	0.652	0.609
Upper left[a, b]	202	8	10.31	8.825	0.621	0.217
Upper right[b]	203	6	8.55	8.959	0.629	< 0.001

TMA: tissue microarray; SD: standard deviation.
**: Dunn statistical test with p-value adjusted by the false discovery rate according to the correction proposed by Benjamini-Hochberg; a, b and c: show statistically significant differences between groups*

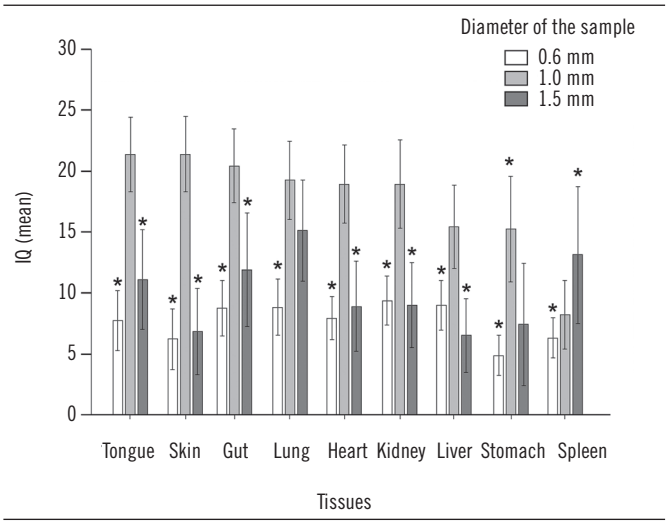

FIGURE 3 – *Influence of the combination of the type of tissue and the diameter of cylinders in the IQ of samples inserted in the recipient blocks, considering the size of gaps between them*

The values of quality were represented as mean ± confidence interval (95%). Each combination of tissue and diameter was compared with the value from the best sample (tongue – 1.0 mm). The results were analyzed by the Kruskal-Wallis and Dunn statistical tests. The p-value were adjusted using the false discovery rate proposed by Benjamini-Hochberg and the (*) p-value < 0.05 was considered significant.

IQ: index of quality.

through the combinations of tongue/1 mm (IQ = 27), skin/1 mm (IQ = 27), bowel/1 mm (IQ = 27), lung/1 mm (IQ = 18), lung/1.5 mm (IQ = 15), heart/1 mm (IQ = 18), kidney/1 mm (IQ = 18) and liver/1 mm (IQ = 18). Overall, all the tissues presented the best quality values when the 1 mm diameter was used, with the exception of spleen and lung, which did not exhibit statistical differences between the 1 mm (IQ = 18) and 1.5 mm (IQ = 15) diameters. Regarding the spleen, an improvement in the mean quality value was obtained when the 1.5 mm diameter was utilized (**Figure 3**).

DISCUSSION

Experiments showed that physical and chemical parameters related to the construction of the receptor block can affect the quality of the histological sections. The structure of the blocks constructed with pure paraffin was too rigid so that the recipient blocks were not capable of supporting the action of the blade during the microtomy phase, releasing from the cassettes and suffering fractures in their structures. When 5%, 10% or 15% of beeswax was incorporated into the pure paraffin, the process of microtomy was achieved. The beeswax increased the tenacity of the block, allowing the paraffin to withstand the shear pressure without breaking. The blocks constructed with paraffin enriched with polymers were the only ones that endured cutting with the blade without great resistance or additional problems. In this case it is likely that the mixture of various polymers of different densities enriched the paraffin, resulting in a lubricant characteristic and a greater malleability/tenacity, ensuring that the block could receive a great quantity of energy (impact) without suffering fractures.

Regarding the block tempering protocols, the block submitted to a temperature of 64ºC could not withstand such heat, and its structure melted. The other block tempering protocols tested (tempered and not tempered) used temperatures below the point of fusion of the paraffin enriched with polymers (37ºC and 40ºC, respectively) and thus, the integrity of the block was preserved.

Regarding the tempering process, a significant difference in the quality of the samples was found between the two protocols, although proximal temperature values were used in the block tempering process. This difference can be explained by two main factors, the exposure time of the recipient block to the heating temperatures and the absence or presence of the tempering step. In the case of the protocol without tempering, the blocks were exposed to a temperature of 40º for only 10 minutes, and were subsequently stored at room temperature (25ºC). In this context, probably the exposure time was not sufficient for the

samples to be properly allocated in the holes of the recipient block, preventing proper adhesion between the tissue and the paraffin on the recipient block. For the tempered block, the time of exposure (overnight) at 37ºC of the recipient block was significantly higher. In addition, the block underwent two phases of tempering with heating-cooling cycles. This could have allowed more time for the samples to be properly allocated in the recipient block and enabled the embedding of the paraffin based on the contraction and expansion cycles of the materials.

Regarding the relationship between sample size and quality, blocks constructed with tissues with 1.0 mm diameter reached better results than those with 1.5 mm or 0.6 mm diameters. The low quality of the group comprised of 0.6 mm diameter samples could be explained by the small area of contact between the tissue and the paraffin. Although the recipient blocks constructed with 1.5 mm diameter samples also had a performance worse than those of 1.0 mm, in this case, as the sample diameter increased, the area of the sample submitted to cutting force also increased, impairing the effect produced by the perimeter increase. Therefore, samples with 1.0 mm diameter presumably present the best relation between area and perimeter to resist the forces produced during the path of the blade. Regarding the combinations of diameter and spacing, the construction of the blocks with 1.0 mm diameter with 700 µm or 1000 µm tissues spacing was enough to obtain the resistance required for the histological sections.

On the other hand, the influence of the two types of tissue on the mean quality can be characterized as a function of the intrinsic nature of the tissues, as well as the effect of the structure after the stages of fixation and processing for paraffin inclusion. Tissues that present a structural composition with pores or villi, such as the lung, tongue, bowels and kidney could have benefitted during the tages of infiltration and final insertion in the liquid paraffin. In this case, the paraffin could have penetrated such cavities and due to a greater stability to the tissue-paraffin complex of the final recipient. In contrast, tissues with a highly dense or fragile structure such as the spleen, heart, or liver tend not to adhere correctly to the paraffin of the recipient block, or to withstand the action of the blade during the cutting of the histological sections, resulting in the lower quality of the final sample. Some tissues such as the skin and stomach mucosa may have had their fine structures modified greatly by the fixation, dehydration, clarification and infiltration process, which could have negatively influenced the annealing of the tissue to the paraffin, and the microtomy stage.

The fact that the best mean quality values for most of the tissues were obtained with a diameter of 1.0 mm can be explained by an increase in the total perimeter (internal and external) between the tissues and the paraffin of the recipient block. For the spleen, a diameter of 1.5 mm was capable of overcoming, at least partially, the low quality of the tissue samples. The impact suffered by the spleen during the puncturing process of the donor block was probably lessened by the greater area of the tissue. In this case, the puncture pressure was better distributed throughout the section than the punctures performed in smaller areas, due to a greater stability of the spleen structure helping to maintain its integrity.

The position of the samples in the recipient blocks did not influence the quality values presented, and the entry and exit point of the blade were not correlate to the final result. The only significant difference during the tests involved the upper right position on the recipient block, which corresponds to the exit point of the blade. This result could indicate a potential influence during the process of microtomy, or related to the construction of the histological slides.

For the slides that underwent HE staining, there was a clear degradation of around 40% of the samples (data not shown). The cause may be due to the conditions they were exposed during the deparaffinization process which requires the use of aggressive reagents that decrease tissue quality. This result enforces the importance of the optimization of the parameters to produce high-quality TMA slides which are submitted to staining procedures to limit the failure rate.

CONCLUSION

The physical and chemical adjustments of the recipient blocks of the TMA method provided vital information which, when applied in TMA research projects, can reduce the losses associated with the method. Based on the analysis of the variables tested, the construction of the recipient blocks with polymer enriched paraffin, assembled with samples of 1.0 mm diameter, 1000 µm spacing, and submitted to a 37ºC block tempering protocol associated with a tempering process, presented the best quality values. Although highly probable, the optimal block design/spacing specifications obtained from this instrument may be extrapolated to the other instruments that have been designed and marketed for the construction of TMAs.

ACKNOWLEDMENTS

This work was funded by grants from the Fundo de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Research Support Fund of the State of São Paulo) (grant no. 14/50256-4). Any

opinions, findings, conclusions or recommendations expressed in this article are those of the authors and do not necessarily reflect the views of funding bodies. The funding bodies played no role in the study design; collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

RESUMO

Introdução: *O microarranjo tecidual (MAT) é considerado um método inovador em vários campos, com uma vasta diversidade de aplicações e vantagens em relação às técnicas histomorfométricas clássicas. A vantagem mais importante que o MAT oferece é a avaliação simultânea de um grande número de espécimes de uma fonte limitada de material. Contudo, ele apresenta uma taxa elevada de amostras não viáveis nos estádios finais do processo, o que compromete sua utilização em análises que não podem ser repetidas.* ***Objetivos:*** *Considerando essa desvantagem, o objetivo deste estudo foi otimizar a metodologia para maximizar a viabilidade das amostras, bem como aumentar a eficiência da técnica.* ***Material e métodos:*** *Para tanto, foram testadas várias variáveis envolvidas na construção dos blocos receptores, como composição da parafina, diâmetro, distância de espaçamento, localização e tipo das amostras de tecido no bloco, a fim de estabelecer correlações entre a qualidade dos valores e os parâmetros estudados.* ***Resultados:*** *Os resultados mostraram que os blocos construídos com parafina enriquecida em polímero, submetidos ao protocolo de fusão a 37ºC, acoplados a ciclos de aquecimento e resfriamento e construídos com amostras de um milímetro de diâmetro e espaçamento entre os tecidos de 1000 µm, produziram lâminas com características superiores.* ***Conclusão:*** *Os dados obtidos dos ajustes físicos e químicos dos blocos de receptores de MAT forneceram informações vitais que, quando aplicadas em projetos de pesquisa de MAT, podem reduzir as perdas associadas ao método.*

Unitermos: *técnicas histológicas; parafina; melhoria de qualidade.*

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