ABSTRACT

The assessment of the margin of the surgical cuts is crucial for the success of oncoplastic surgeries. We show a rapid approach to assess the presence of tumoral cells at the margin of tumor resections using a microfluidic tissue processor. We optimize the staining protocol to reduce the staining time to 8 minutes.

KEYWORDS: Rapid immunohistochemistry, Microfluidics, Intra-operative, Tumor resection margins

INTRODUCTION

During oncoplastic surgery of breast carcinomas, the location of cancer cells at the borders of the excision is indicative of probable recurrence. Several intra-operative margin assessment techniques have been described in literature, which make use of magnetic resonance [1], ultrasound [2] or impedance spectroscopy [3] to create an image or detect a signal due to the presence of cancer cells. Staining of cryo-fixated surgical specimens using hematoxylin and eosin remains the most common technique due to the simplicity and speed (5 minutes) of the assay. The major common drawback these techniques share is the lack of cancer cell-specificity, preventing the pathologist to spot a small number of infiltrating tumor cells and eventually resulting in late positives [4]. A tool that helps the surgeons assess the proximity of tumoral cells to the cut within surgical times is therefore highly desirable. In a previous study, we have showed that our MTP can perform fast fluorescence immunostaining of tissue samples of Her2 antibodies [5]. However, fast staining using chromogenic techniques using cytokeratins as markers of epithelial cells was never demonstrated. We developed a microfluidic protocol that allows performing rapid IHC assays on the surface of tumorectomy samples and, therefore, help localize cancer cells at the margins of the cut. We optimized a chromogenic protocol that allowed us to perform an IHC staining in 8 minutes, compared to the 70 minutes required with classical methods.

EXPERIMENTAL

Breast carcinoma samples of blocks located in close proximity to the surgical margins of the tumorectomy were obtained from the bio-bank of the Institute of Pathology. The sample preparation protocol, was done off-chip following the guidelines given by the pathology laboratory. Once the samples ready to be stained, they were loaded on the microfluidic setup and the protocol was launched. Figure 1A is a cross-section schematic of the microfluidic device. By clamping a histological glass slide to the MTP via an elastomer gasket, a thin (100 μm) chamber is formed that allows fast delivery and washing of the reagents across the tissue section. A schematic of the reaction chamber is depicted in Figure 1B, where the IHC steps are shown: (i) washing, (ii) primary antibody incubation, (iii) secondary antibody incubation and (iv) chromogen substrate incubation. Figure 1C shows the microfluidic setup used to interface the sample-containing slide, the MTP and the delivery system. Upon finalization of the staining protocol, the histological glass slides are unclamped and mounted using a coverslip to be imaged by bright-field microscopy.
RESULTS AND DISCUSSION

We optimized the chromogenic step by performing a flush of the substrate during a multiple of fixed short incubation times of 1 minute (figure 2A). On a further optimization step we increased the delivery flow rate from 10 µL/s to 25 µL/s, showing an increased uniformity in the final staining across the chamber (figure 2B). In figure 2C we show a typical breast carcinoma tissue section stained with anti-cytokeratin antibodies, where cancer cells are found in close proximity to the surgical margin. The arrows show the margins of the surgical cut, which is marked with a special ink administrated to the sample. In brown, the specific staining of epithelial cells can be observed. Pathologists can recognize the presence of tumoral cells at the margins of the resection, which determines whether further excision is necessary.

CONCLUSION

Fast IHC staining enabled by the MTP has the potential to increase the quality of intra-operative margin assessment by providing cancer cell-specific information, reducing in this manner the rate of re-excision.

REFERENCES


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