MICROFLUIDIC PROBE FOR ADVANCED STAINING OF HUMAN TISSUE SECTIONS

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ABSTRACT
A key challenge in diagnostic pathology is to provide accurate and precise diagnostics with limited excised tissue. It is also increasingly important to test the tissue samples against multiple markers to enhance the confidence in results and sub-type the diseases for personalized treatment therapies. To this end, we performed local chemical reactions and staining on tissue sections using a scanning microfluidic probe (MFP). With the MFP, human thyroid tissue sections were locally treated with anti-thyroglobulin and counterstains, enabling the possibility to optimize in real-time the staining conditions individually for each tissue/antibody pair.

KEYWORDS: Microfluidic Probe, Immunohistochemistry, Microfluidics

INTRODUCTION
Immunohistochemistry (IHC), a method central to pathology, detects antigens (disease markers) in tissue sections using antibodies. Despite IHC being widely used, pathologists are challenged by the limited ability to screen multiple markers on limited samples and the over and under-staining of tissue sections. In addition, with the ever-increasing number of markers and the need for quantification, significant improvements to IHC implementation are necessary. Ideally, IHC should test multiple markers by performing spatially separated reactions on tissue sections and enabling independent optimization of staining conditions. Along these lines, tissue microarray (TMA) has shown that few millimeter-sized tissue cores are representative of the biopsy [1]. To our knowledge, Kim *et al.* [2] is the only report on localized IHC; their approach was to flush reagents through microchannels in contact with a tissue section. For reagent localization, we recently developed a scanning non-contact microfluidic technology, called the vertical microfluidic probe (vMFP) [3] and have shown its capability to add and subtract proteins from surfaces as well as to inactivate and detach specific cells from a surface. Here, we demonstrate that the vMFP can perform local IHC on tissue sections using specific antibodies to determine healthy from diseased tissue.

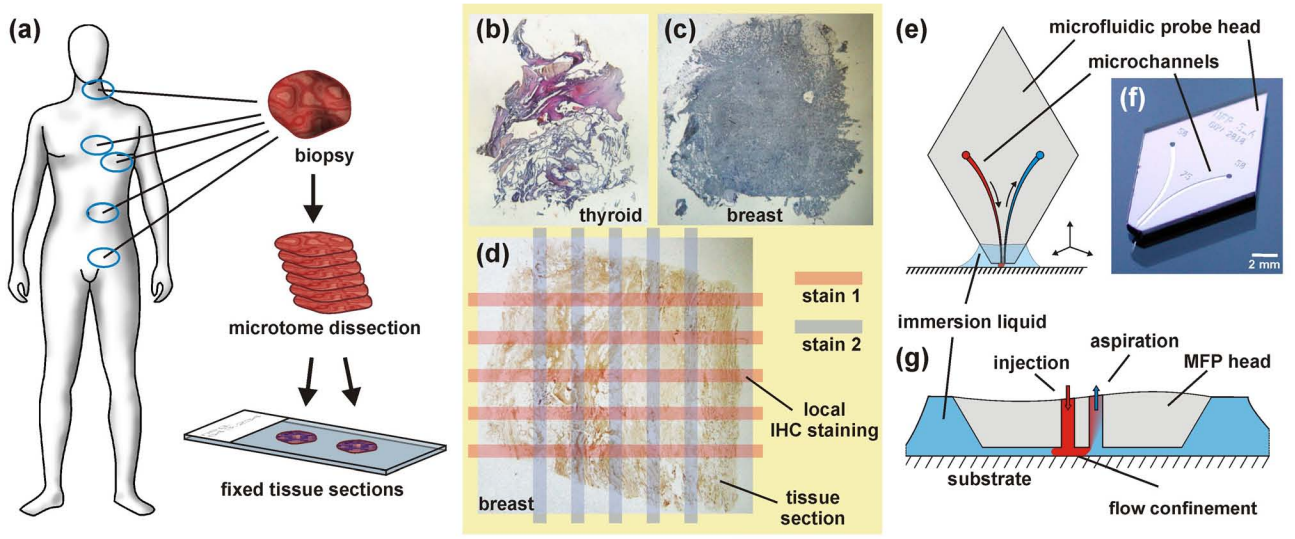

Figure 1: IHC performed on tissue sections and the concept of the vertical microfluidic probe as applied to tissue staining. (a) Diagnosis of a tumor often starts by taking a biopsy of an organ/region of the body. The excised tissue is embedded in paraffin, sliced with a microtome, and the sections placed on a glass slide. (b and c) Photographs of thyroid and breast tissue sections, stained using a conventional protocol (i.e. entire section stained). (d) Concept of local processing of a breast tissue using different stains, indicated here with intersecting lines. (e to f) Schematic and photograph of a vertical microfluidic probe (vMFP) head having vias, microchannels, and an apex into which the injection and aspiration channels open to form apertures. Typical flow rates are 2-30 nL/s for injection and 5-120 nL/s for aspiration. The head is operated at 1-30 μm from the surface.

EXPERIMENTAL

The head of the vMFP is the critical component of the platform and has apertures at its apex to inject and aspirate solutions within 1-30 μm from the tissue section. The head is mounted on a precision X-Y-Z stage which has the ability to scan in the cm-range in the horizontal plane. The vMFP localizes picoliter volumes of reagents that are hydrodynamically confined and the tissue section can be scanned with 0.1 μm resolution, Fig. 1. The confined liquid, also called the flow confinement is visualized using an inverted microscope. The translational stages, syringe pumps and the video camera were controlled using a common graphical user interface developed on LabView 2010.

The vMFP is highly versatile, enables custom patterning/scanning of a surface (here tissue section), is reconfigurable in real-time, and the staining region could be as small as a few μm^2 (in the order of 5-10 cells) and it also compensates for tissue thickness variations. To test the stability of tissue exposed to a confined flow, we placed a vMFP head ~20 μm above the tissue for 10 min and found no degradation, Fig. 2a. In other experiments (data not shown), the stability of the liquid envelop was visually monitored by a camera for extended periods (tens of minutes) with variations in the flow confinement found less than 3%. Local processing of tissue sections with different counterstains are commonly used in IHC, Fig. 2b.

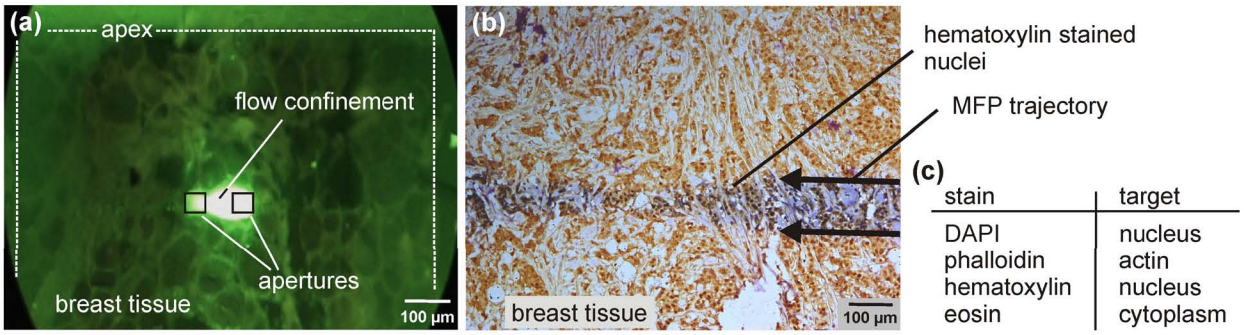

Figure 2: Interaction of the vMFP with tissue sections. (a) Photograph of a tissue section during processing with the vMFP, as seen through an inverted microscope. The apex of the vMFP head is 20 µm from the slide and comprises 50 µm × 50 µm apertures, with 50 µm spacing. (b) Image showing local staining of cell nuclei with hematoxylin along the vMFP trajectory on a breast cancer tissue section. (c) List of counter stains applied on tissue section using the vMFP.

RESULTS

IHC using the vMFP was performed by integrating this technology into a conventional protocol workflow using diaminobenzidine (DAB) as chromogen, Fig. 3a.

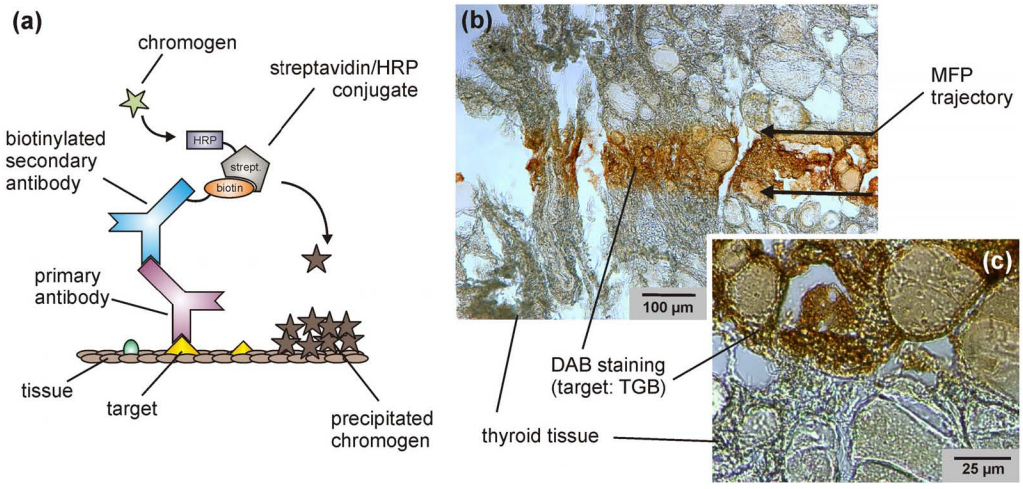

Figure 3: IHC using the vMFP on thyroid tissue sections. (a) Chemical system of a standard amplified IHC protocol. (b) The primary antibody (αTGB) is locally delivered to a thyroid tissue section using the vMFP (injection flow rate: 1 µL/min, aspiration flow rate: 5 µL/min). Post-processing was done according to the standard IHC protocol. (c) High magnification view of the tissue section, showing both stained and unstained areas.

Experiments were performed on a 4 μm thick thyroid tissue by delivering the primary antibody anti-thyroglobulin (αTGB) in ~100 μm lines across the tissue, Fig.3b. For this, the vMFP was operated in scanning mode, with the apex of the head traversing across the tissue at a constant velocity. The chemical system chosen enables post-processing of the entire tissue section using conventional protocols. In contrast to standard IHC, our approach necessitates only nanoliters of reagents rather than hundreds of microliters. Preliminary results suggest that with the vMFP staining is done ~500 times faster using 100-fold less tissue area than conventional IHC. Moreover multiplexed and quantitative staining becomes possible.

CONCLUSION

Presently, the vMFP is being applied to monoplex and multiplexed localized staining of normal and cancerous breast cancer tissue with targets such as estrogen receptors and human epidermal growth factor receptor 2. The area reduction of the samples that can be processed now suggests even the processing of individual cores of tissue microarrays, hence greatly increasing the utility of tissue microarrays. MFP-based IHC may contribute to better tumor diagnosis.

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