TISSUE MICROPROCESSING

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ABSTRACT

We recently reported on multiplexed and adaptive staining of tissue sections in the micrometer-scale using the vertical microfluidic probe. With this approach, in addition to staining tissue sections precisely for high-quality information, the paraffin embedded tissue sections can now be locally dewaxed and processed for subsequent staining. This will ensure the re-use of valuable tissue sections in the future.

KEYWORDS

immunohistochemistry, tissue processing, microfluidic probe and open microfluidics.

INTRODUCTION

Immunohistochemistry (IHC) on tissue sections is central to pathology and research. This method is based on specific antibody–antigen binding and is widely used to detect one disease marker per tissue section. It requires numerous steps involving exposure of the entire section to reagents without *a priori* knowledge of the optimal processing conditions. A method for multiplexed and adaptive staining that would be conservative of tissue and provide increased information is needed.

Our recent work focused on local staining of tissues using a vertical microfluidic probe (vMFP).[1] We demonstrated multiplexed tissue analysis and called the method µIHC.[2] Here, we use this concept to dewax and rehydrate locally paraffin embedded sections for conservative sample analysis, which can be equally, if not more important than µIHC. We termed the processing and consumption of small amount of tissue as tissue microprocessing. Tissue microprocessing can likely meet these needs by precisely localizing chemical/biochemicals on samples for increased flexibility and multiplexed analysis.

To improve IHC, Park *et al.*[3] and Gijs *et al.*[4] used sealed microchannels to flow reagents over dewaxed tissues for multiplexed staining. These approaches are restrictive and lack flexibility. Another technique, laser capture microdissection (LCM), excises areas of tissues for analysis but tissue integrity, important for histology, is lost.[5] In contrast; our approach of using the vMFP is flexible, interactive and preserves tissue integrity. In conventional IHC, the whole section is dewaxed and stained. Using the vMFP allows microscale removal of paraffin, followed by conventional IHC staining. This ensures that the remainder of paraffin embedded tissue stays intact for additional analysis or archiving, Fig. 1.

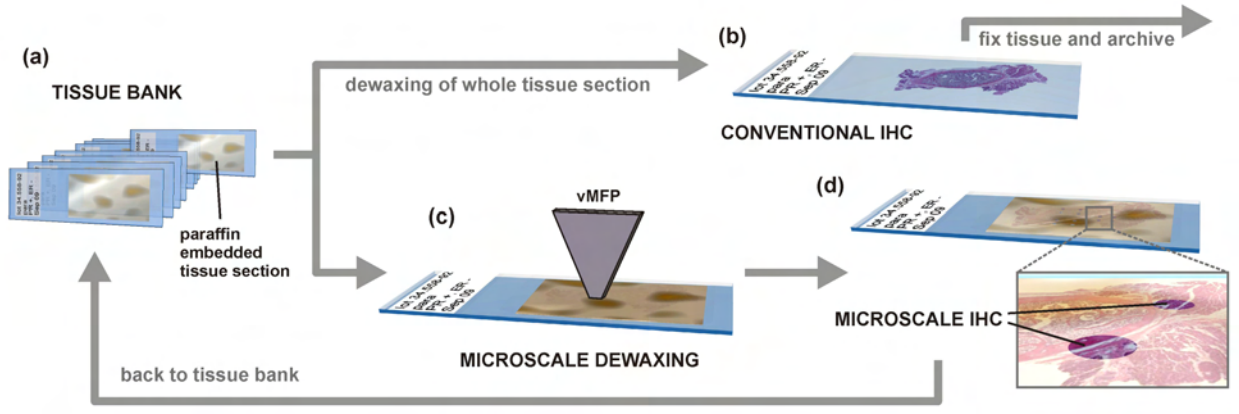

***Figure 1**. Workflow for tissue microprocessing. (a) Conventional IHC involves dewaxing of the entire paraffin-embedded tissue section followed by IHC staining (b); the tissue is then fixed and archived. (c) With the vMFP, microscale dewaxing is performed on selected areas of the tissue, followed by conventional IHC staining (d), resulting in analysis of selected regions. The processed section can be archived in the tissue bank for future analysis.*

EXPERIMENTAL SECTION

The vMFP confines locally picoliters of liquids on a substrate. Its central component is a microfluidic chip (vMFP head), which is mounted on precision stages for scanning. The head is brought close to the substrate in the presence of an immersion liquid. A processing liquid is injected via an aperture, and simultaneously aspirated with some immersion liquid, resulting in hydrodynamic confinement of the processing liquid, Fig. 2a. This principle was used to locally dewax and rehydrate tissue sections, followed by conventional IHC, Fig. 2b.

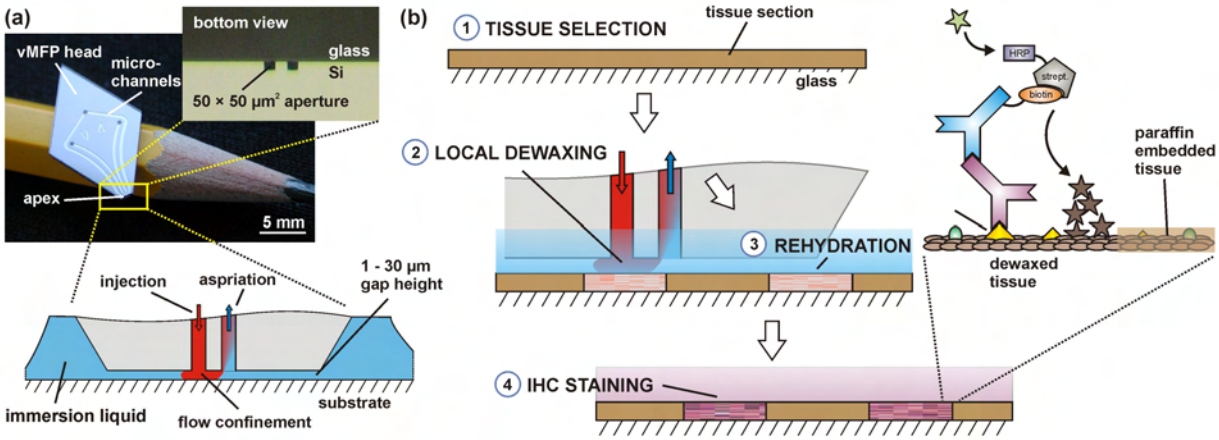

***Figure 2**. Tissue microprocessing using a vMFP resulting in µIHC. (a) Photograph of a Si/glass vMFP head comprising microchannels etched in Si, and terminating at a 1 mm² apex. The head operates 1-30 µm above a substrate in the presence of immersion liquid. (b) Microscale IHC protocol for processing paraffin embedded tissue with the vMFP. A standard IHC method is used for visualization of the antigens involving an enzyme-mediated chromogenic detection. (drawings not to scale)*

PLATFORM, vMFP HEAD FABRICATION AND PROCEDURE

The vMFP platform used was an assembly of four functional components: (1) A vertically mounted microfluidic chip, the vMFP head, (2) syringe pumps for liquid handling (Cetoni GmbH, Korbussen, Germany), (3) a computer controlled X-Y-Z-stage built from motorized linear drives having 0.1 µm resolution (Lang GmbH, Huettenberg, Germany), and (4) an inverted fluorescence microscope (Nikon Eclipse TE300, Egg, Switzerland). The vMFP head was housed within a holder mounted to the Z-drive. The vMFP holder was equipped with ports for liquids to be pumped from or to the vMFP head via capillaries. A custom-made frame, attached to the X- and Y-drives was used to mount standard microscope glass slides (1 × 3 inches) and for enabling the movement of the sample relative to the head in X and Y directions.

The vMFP heads were fabricated using standard microfabrication techniques (photolithography, deep reactive ion etching and anodic bonding). The vMFP head is a two layer (Si/glass) microfluidic device comprising microchannels, vias for fluidic connection and a polished edge (apex) through which the microchannels exit and form apertures. The apex physically supports the flow confinement and has an area of approximately 1 mm^2. The microchannels are typically 50 µm deep and taper from 200 µm width at the vias to 50 µm at the apertures. Further details are presented elsewhere [1].

The vMFP was operated using the following procedure: The vMFP head was leveled relative to the glass slide comprising a tissue section and the zero position established. The vMFP head was elevated to ~50 µm above the glass slide and after pipetting a few mL of immersion liquid (buffer) onto the surface, the aspiration was started with a flow rate of 5 µL min^{-1}. After approximately 30 s, the injection with the antibody solution (1 µL min^{-1}) was initiated. A stable flow confinement was formed within less than a minute.

RESULTS

In adapting the vMFP for dewaxing, we developed techniques to confine immiscible liquids needed for wax removal and gentle hydration of the tissue by gradually altering the ethanol concentration. Confining immiscible liquids is particularly challenging due to the liquid-liquid interfacial tension. Aspirating xylene in an aqueous environment was unsuccessful having aspiration apertures ($50 \times 50\ \mu m^2$) originally used for µIHC. We developed a range of strategies using different aperture dimensions and geometries, and found the approach of shielding xylene within ethanol, to be most suitable, Fig. 3a. Local dewaxing and gradual rehydration of a human normal thyroid tissue section was performed within 2 min. We attribute this short time in dewaxing and rehydration to improved convection compared to conventional methods. Subsequently, IHC against thyroglobulin and counterstaining with hematoxylin was performed on processed tissues, Fig. 3b.

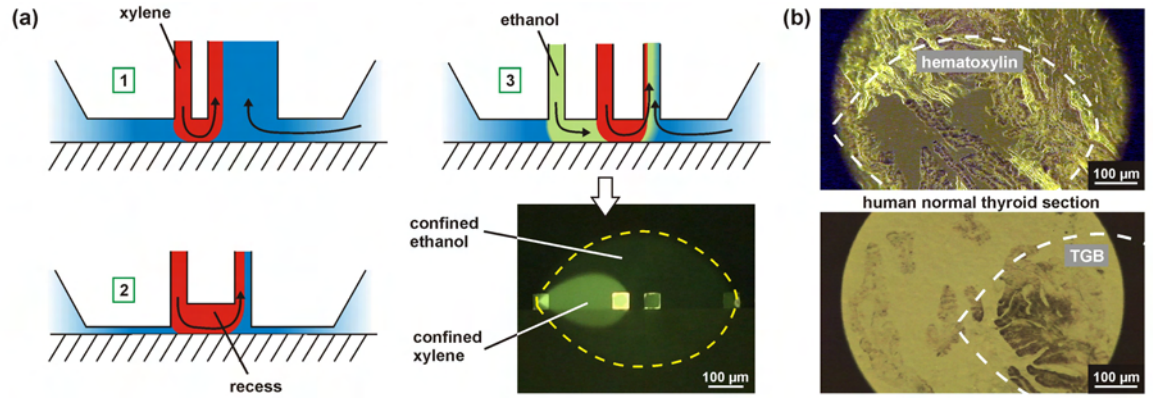

Figure 3. *Confinement of immiscible liquids and staining results.* ***(a)*** *Strategies to confine immiscible liquids such as xylene within water. Large aspiration apertures for improved aspiration (1), a recess for liquid guidance (2), and multiple liquid confinements for shielding liquids (3) were tested. Bottom right is an image of confined xylene within an ethanol flow confinement surrounded by water. For visualization, both liquids were spiked with fluorescein.* ***(b)*** *Locally dewaxed 4-µm-thick human normal thyroid section stained using hematoxylin (top) and analyzed with 700 µg/µL anti-thyroglobulin, TGB (bottom).*

OUTLOOK

Tissue microprocessing with the vMFP is flexible, interactive and conservative of samples. It may become a method of choice in tissue banks to "back-up" and minimize depletion of valuable samples enabling their reuse for validation of new scientific findings.

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