ESI – Semi-automated Preparation of Fine-needle Aspiration Samples for Rapid On-site Evaluation

Supplementary information

Title:

Semi-automated Preparation of Fine-needle Aspiration Samples for Rapid On-site Evaluation

Authors:

Filipe Marques[a], Janosch Hauser[a], Emre Iseri[a], Igor Schliemann[b], Wouter van der Wijngaart[a], and Niclas Roxhed*[a,c]

[a] Div. of Micro and Nanosystems, KTH Royal Institute of Technology, 10044 Stockholm, Sweden.
[b] Pathology and Cytology Department, Karolinska University Hospital, Stockholm, Sweden.
[c] Medtech Labs, Karolinska hospital, Stockholm, Sweden.

Correspondence to:

Niclas Roxhed (Roxhed@kth.se)

Table of contents

ESI – Semi-automated Preparation of Fine-needle Aspiration Samples for Rapid On-site Evaluation

1. Microfluidic Chamber Layout

The microfluidic chamber was designed using Adobe Illustrator (Adobe Inc. 2022). Figure 1 shows the different materials color-coded and respective layout for 3 devices. During fabrication, adhesive layers a-i), a-ii) and a-iii) were stacked forming the walls of the chambers and creating a height gradient. The chamber lid (hydrophilic sheet) was laminated to the latter stack, sealing the top side of the chamber. Following Giemsa stain drop casting to the chamber ceiling, the blotting unit was installed by laminating the adhesive a-iv) to the chambers outlets. Paper c-i) was placed inside the outlets, while paper c-ii), carrying a water-soluble PVA film, was laminated to the adhesive layer a-iv).

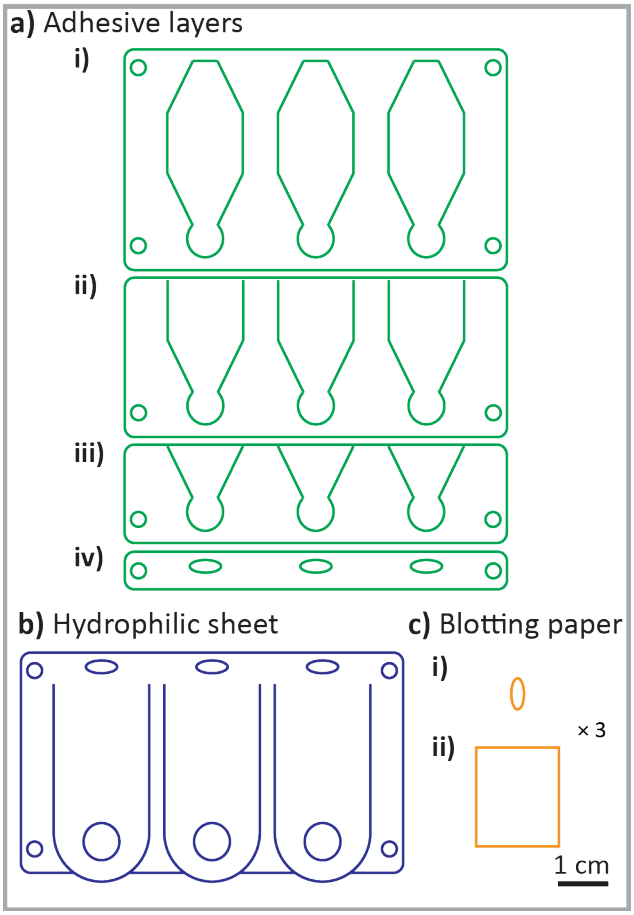

Figure 1: Layout of three microfluidic chambers indicating: **a) i)**, **ii)**, **iii), iv)** the adhesive layers; **b)** the hydrophilic sheet; **c) i)**, **ii)** the blotting papers.

2. Experimental

2.1. Cell Staining Concentration

Cell staining concentration was assessed by staining an FNA sample model at different Giemsa stain concentrations. The FNA sample model was generated from a porcine small intestine retrieved immediately post-mortem and placed in a 0.9 % saline buffer solution during transport for three hours before use. A 3 cm section was separated from the organ and its lumen exposed with a scalpel. This section was cleaned with tap water for 1 min and placed in a hemispherical holder with a diameter of 5 cm. With the lumen facing upwards, 1 mL of DMEM was placed on top of the section creating an open cyst model. The tissue was brushed with a Dentalux® rubber interdental brush (Apotea, Stockholm, Sweden) with a side-to-side movement for 1 min. A 500 µL of sample was aspirated with a pipette and placed in an Eppendorf tube. Giemsa stain was mixed with water at concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL and 20 mg/mL for 5 minutes and filtered with a 70 µm strainer (431751, Corning®, Netherlands). For each stain concentration, 10 µL of sample were manually smeared on a glass slide and stained by submerging the glass slide in the stain solution for 30 s. Each glass slide was imaged at 5× and 20× magnification (Figure 2). Staining quality was deemed sufficient for all concentrations except 5 mg/mL, where not all cells had nucleus colored. We chose 15 mg/mL when producing the stain film as to guarantee cell staining on the device.

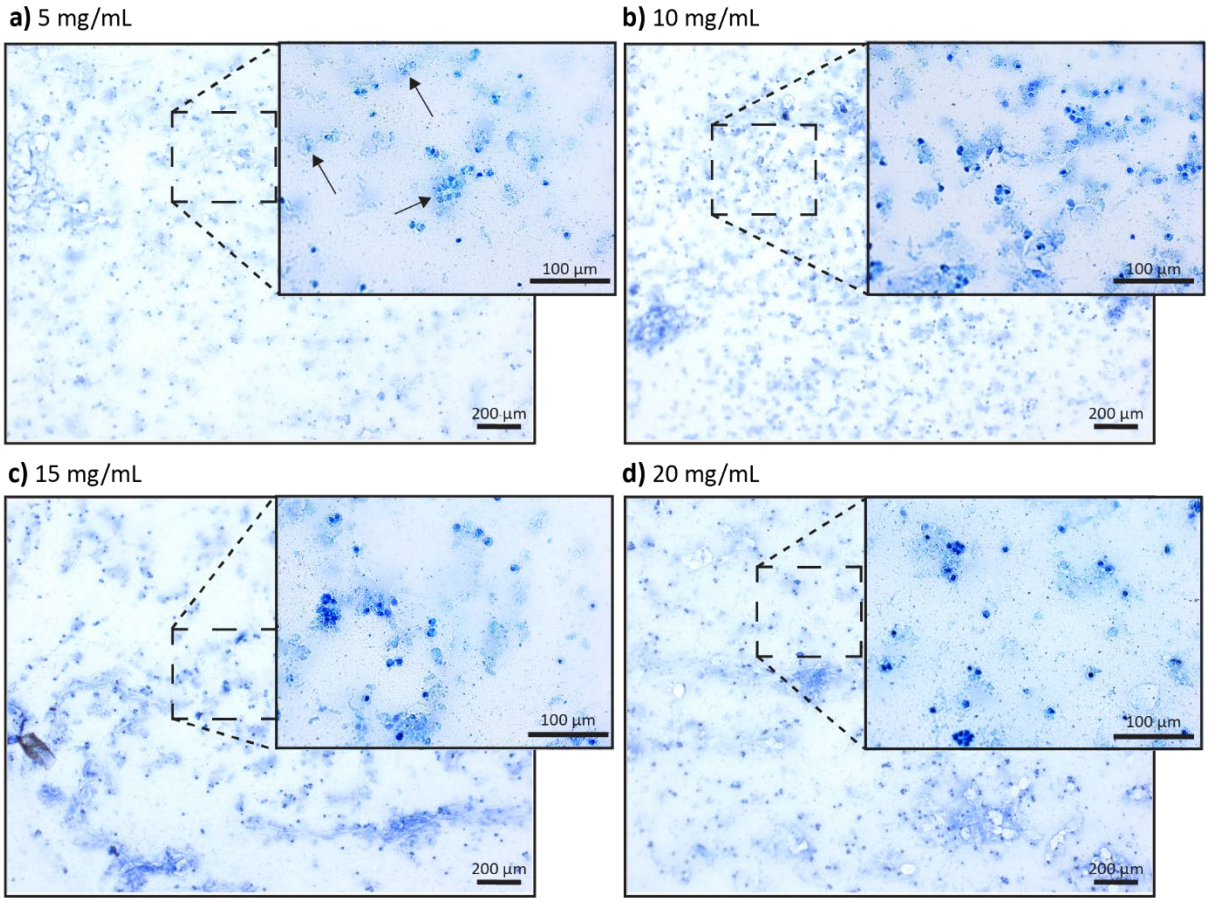

Figure 2: 5× and 20× magnification images of small intestine samples prepared at: **a)** 5 mg/mL with arrows indicating cells with unstained nucleus; **b)** 10 mg/mL; **c)** 15 mg/mL and **d)** 20 mg/mL Giemsa stain concentrations.

2.2. Blotting unit – Water-soluble Polymer Film

Water-soluble polymer films for the blotting unit were fabricated from granular polyvinyl alcohol (PVA, 360627, Sigma-Aldrich), as described earlier[1]. A 20% (w/v) aqueous PVA solution was prepared on a hot plate stirrer (Fisherbrand™ Isotemp™ Advanced Hot Plate Stirrer) at 80°C. After filtration with a Whatman® Anotop® 10 Plus syringe filter, the solution was spread out on laminating pouches (3385694, Office Depot, LA Venlo, Netherlands) and dried under ambient conditions. A color dye was added to facilitate an assessment of film thickness variations. Film thicknesses were measured using a thickness gauge with 1 µm graduation (2109L Metric Dial Gauge, Mitutoyo).

[1] Hauser, J. *et al.* "A microfluidic device for TEM sample preparation." *Lab on a Chip* 20.22 (2020): 4186-4193.

2.3. Stain Film Fabrication

The stain film was produced in a two-part mixture. A 4% (w/v) PVA was prepared in a hot plate stirrer (Fisherbrand™ Isotemp™ Advanced Hot Plate Stirrer) at 500 rpm and 50° C for 1 h. Another pre-mix of 45 mg/mL of Giemsa stain in absolute ethanol was prepared in the hot plate stirrer at 500 rpm and 40° C for 1 h. Both pre-mixes were combined in a 2:1 ratio, establishing a 3% PVA and 15 mg/mL Giemsa stain solution. The latter solution was passed through a Whatman® Anotop® 10 Plus syringe filter to remove all unsolved particles. The microfluidic staining chamber was inverted in a Petri dish and 300 µL of the stain solution were carefully placed with a pipette into the chamber ceiling. The solution was dried by placing the latter Petri dish in the hot plate at 40° C for 1 h.

2.4. Cell Culture and FNA Viscosity Model Preparation

Cells were cultured in adhesion promoted cell culture flasks. Cell media mixture containing of DMEM (high glucose) media with 10% FBS, 0.5% PS, and GLUTAMAX supplement was used for the experiments. PANC-1 cells were split, and cell media changed two times a week. Cells were washed with PBS and treated with trypsin for further cell suspension splitting. Cells suspended in 1-2 mL fresh media were collected after every cell splitting for sample preparation device testing.

As a sample model with low viscosity we used the original PANC-1 sample with a viscosity of 1 mPa·s. A medium viscosity solution of 57 mPa·s was created according to previous literature[2], by manual mixing of 60% w/w sugar in water at 22°C and adding PANC-1 cells in a similar concentration as in the low viscosity sample. Then, 500 µL of the 1 mPa·s sample was placed in a new Eppendorf tube and centrifuged using a Micro Star 17R centrifuge (VWR, Sweden) at 11000 rpm for four minutes. The latter supernatant was replaced by 500 µL of the medium viscosity solution, following gentle homogenization with a pipette, by aspirating and dispensing liquid in 2-second cycles for 30 seconds, finalizing a 57 mPa·s sample.

2.5. Robotic Stage

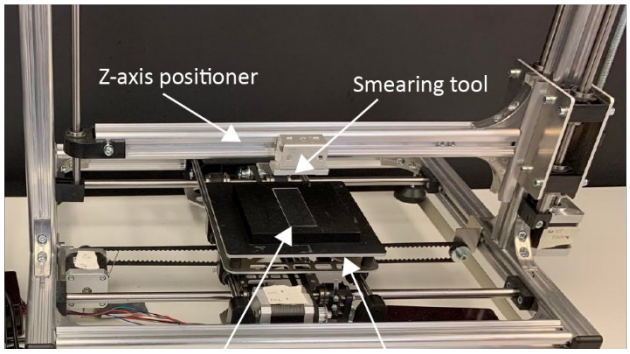

Figure 3: Picture of the robotic stage displaying the smearing tool attached to the Z-axis positioner and the glass slide on top of the XY-axis platform.

A custom-built robotic stage was used during the "Effect of Smearing Speed and Sample Viscosity" to control the smearing of different samples (Figure 3). The software-controlled stage consists of a motorized XY-axis platform and a Z-axis positioner. The platform transported the glass slide while the positioner moved the smearing tool. During smearing, the stage movement went as follows: positioner moved 2 cm at 5 cm/s until there is contact between the glass slide and the smearing tool, positioner halted for 5 s during sample spread, platform moved 4 cm in Y at 1, 3,

[2] Telis V, Telis-Romero J, Mazzotti H, Gabas A. Viscosity of Aqueous Carbohydrate Solutions at Different Temperatures and Concentrations. International Journal of Food Properties. 2007;10(1):185-195.

5 or 7 cm/s to smear a sample, positioner moved -2 cm at 5 cm/s and the platform moved -4 cm at 5 cm/s in Y to return to the original position.

3. 10× and 20× Cell Images

10× and 20× magnification images during the "Applicability to Different Cell Types" experiment (Figure 4). Liver, thyroid, lymph node and PANC-1 cells are showed in no particular distribution.

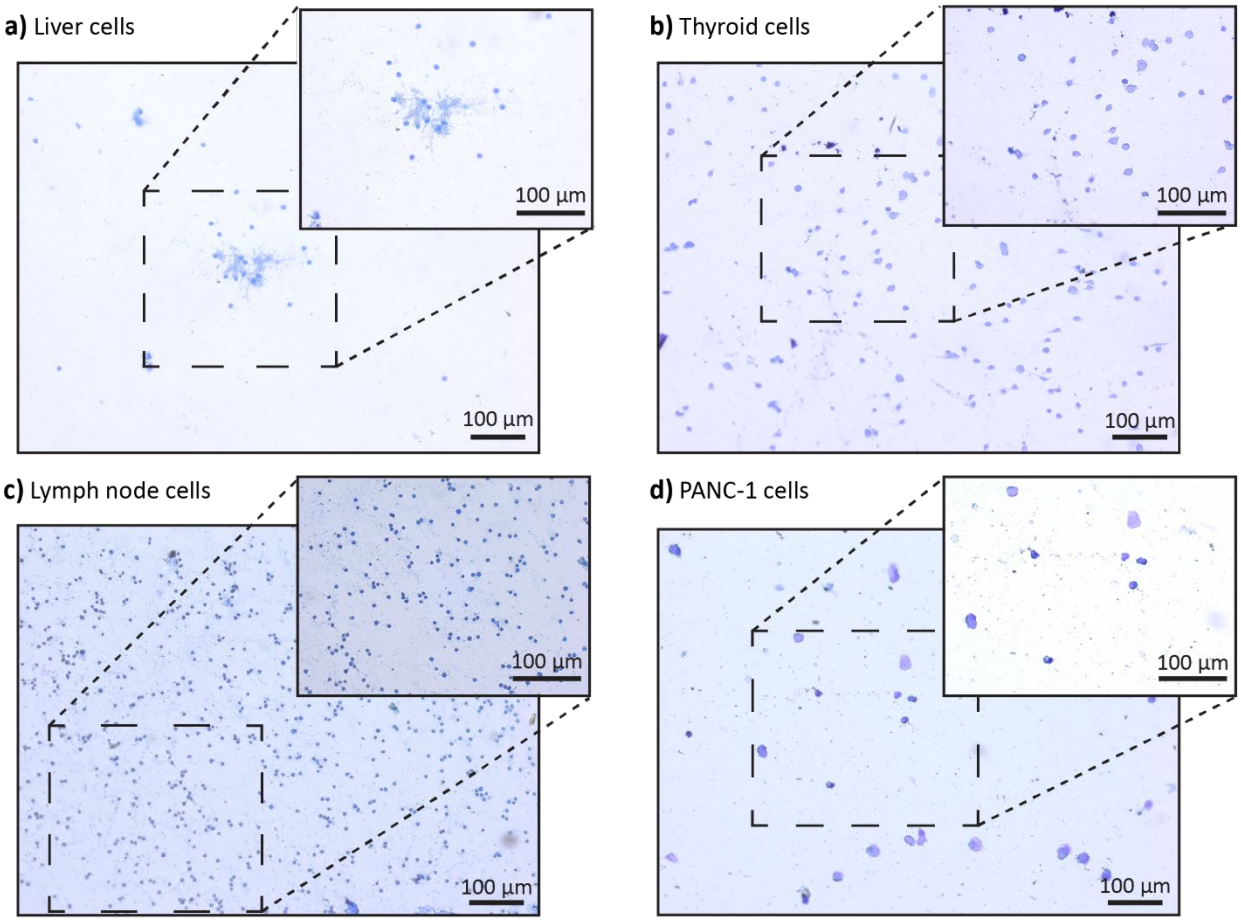

Figure 4: 10× and 20× magnification images of different cell types prepared with the ROSE sample preparation device: **a)** liver cells; **b)** thyroid cells; **c)** lymph node cells; **d)** PANC-1 cells.

4. Sample preparation with tissue fragment

5× and 20× magnification images during the “Sample Preparation with Tissue Fragment” experiment. Images show that tissue was stained uniformly with insets depicting stained cells.

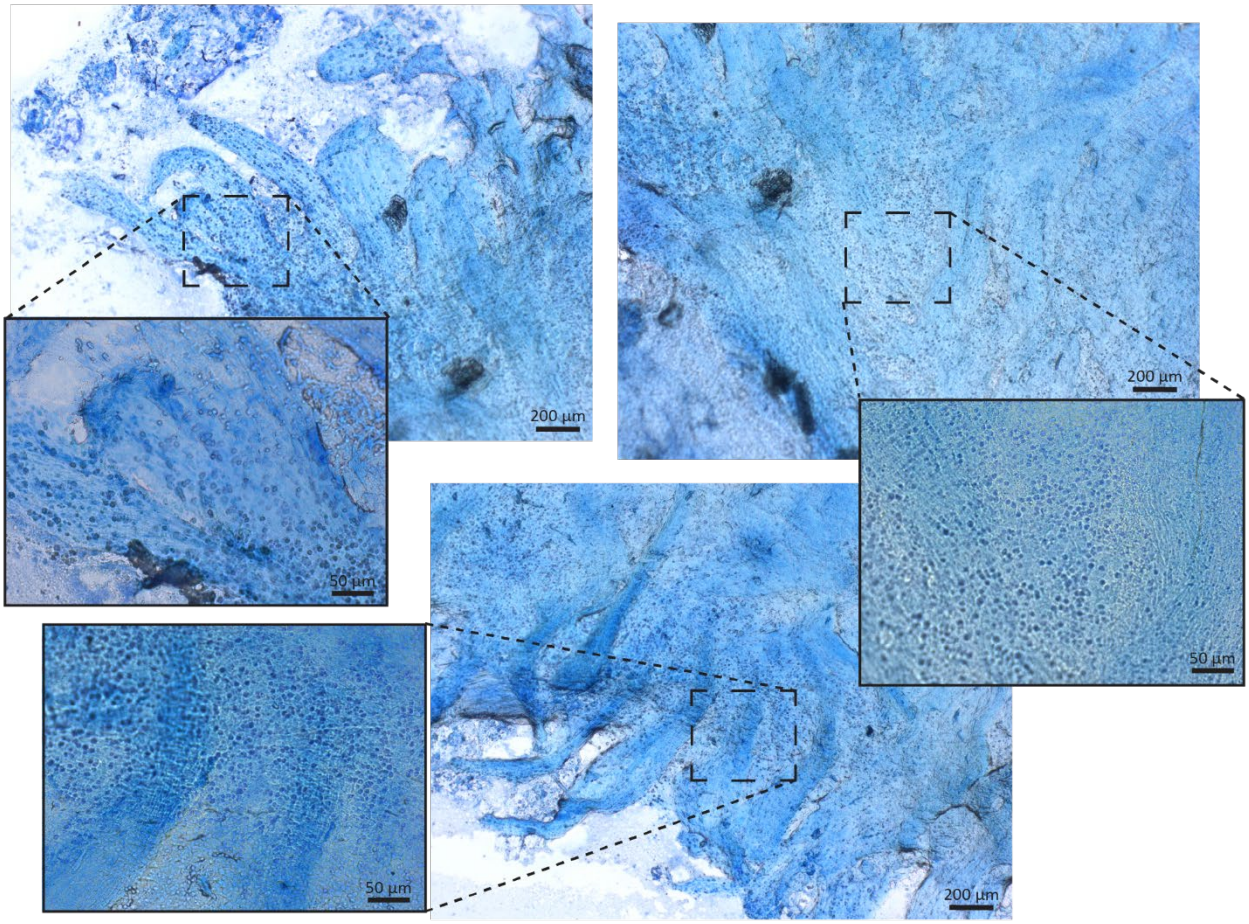

Figure 5: 5× and 20× magnification images of small intestine tissue fragment samples prepared with the ROSE sample preparation device.