## Supporting Information

## Multi-layer assembly of cellulose nanofibrils in a microfluidic device for the selective capture and release of viable tumor cells from whole blood

Tharagan Kumar<sup>a+</sup>; Ruben R. G. Soares<sup>a+</sup>; Leyla Ali Dholey<sup>a</sup>; Harisha Ramachandraiah<sup>a#</sup>; Negar Abbasi Aval<sup>b</sup>; Zenib Aljadi<sup>a</sup>; Torbjörn Pettersson<sup>b\*</sup>; Aman Russom<sup>a\*</sup>

<sup>a</sup> Division of Nanobiotechnology, Department of Protein Science, Science for Life Laboratory, KTH Royal Institute of Technology, Solna, Sweden

<sup>b</sup> Department of Fibre and Polymer Technology, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, Stockholm, Sweden

<sup>#</sup>Current address: Biopromic AB, Tomtebodavägen 23A, Solna, Sweden

\*Contacting authors: torbj@kth.se (T. Pettersson) aman.russom@scilifelab.se (A. Russom)

## **Table of Contents**

Figure S1. Specificity of Carbotrace <sup>TM</sup> 680 for CNF detection	3
Figure S2. CNF digestion time using cellulolytic enzymes	3
Figure S3. Non-specific adsorption of cells to the PAH layer	4
Figure S4. Release efficiency at different input cell concentrations	5
Table S1. Cell capture efficiency using the P-Chip and HB-Chip	6



**Figure S1** Comparison of Carbotrace<sup>TM</sup> 680 fluorescence on a P-Chip modified with (A) PAH or (B) PAH + CNF (0.23 g/mL CNF stock, 30 min flow time). A signal to non-specific signal ratio of >7-fold was obtained.



**Figure S2** Real-time monitoring of enzymatic digestion after coating a single layer of CNF on a P-Chip using the conditions optimized in Figure 2. The cellulolytic enzyme mixture was continuously flowed at 5  $\mu$ L/min. Washing was performed with PBS according with the methods section.



**Figure S3** Impact of the presence of a 4% BSA protein matrix combined with the cells on minimizing non-specific adsorption of the cells to the PAH layer. The devices were coated with PAH and 10<sup>5</sup> cells/mL were flowed through the channel suspended in PBS (10 mM sodium phosphate, 140 mM NaCl, pH 7.4) (top) or the same PBS formulation containing 4% BSA (bottom).



**Figure S4** Enzymatic cell release efficiency after trapping cells with different initial concentrations. Number of cells refers to total number of counted cells in the same ROI (or multiple ROIs in the case of concentrations <  $10^5$  cells/mL) before and after treatment with cellulolytic enzymes. Cell release efficiencies of 94.5%, 92.3%, 80% and 86% were obtained for  $10^5$ ,  $10^4$ ,  $10^3$  and  $10^2$  cells/mL input, respectively, flowed at 5 µL/min for 10 min.

**Table S1** Cell capture efficiency estimated for two independent P-Chips and HB-Chips calculated as C=(I-(O+W))/I. Input cell titer refers to the solution flowed into the microfluidic device. Output cell titers were measured on the collected flow-through after flowing the cells into the device, i.e. cells which were not captured on the device. Wash cell titers refer to cell titers collected at the outlet while washing the device after the capture step. In the case of the output cells being undetectable (N.D) this value was assumed zero to calculate the capture efficiency.

Chip	Input (I) (cells/mL)	Output (O) (cells/mL)	Wash (W) (cells/mL)	Capture (C) efficiency (%)
HB-Chip (1)	- 5.96x10 <sup>4</sup>	N.D.	1.11x10 <sup>3</sup>	98.1
HB-Chip (2)		1.67x10 <sup>3</sup>	5.6x10 <sup>2</sup>	96.3
P-Chip (1)		1.67x10 <sup>4</sup>	2.23x10 <sup>3</sup>	68.2
P-Chip (2)		3.39x10 <sup>3</sup>	8.90x10 <sup>3</sup>	79.4