

## Electronic Supplementary Information for

### In chip fabrication of free-form 3D constructs for directed cell migration analysis

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#### *Mix-and-match in-chip fabrication of bulk barriers*

Bulk barriers blocking the majority of the chip microchannel were fabricated to prevent cells from migrating around the in-chip produced microporous construct. In brief, the in-chip construct was exposed as described in the Materials and Methods section but without development of the structure, i.e. unexposed IP-L 780 resin remained in the chip channel. The bulk barriers were designed as open areas in a standard photolithography chrome-on-glass shadow mask. The resin-loaded chip was mounted in a Süss MA4 mask aligner (Süss Microtec, Garching, Germany) where the bulk barrier outlines on the shadow mask were aligned to the visible polymerized construct in the resin. The bulk barrier areas were exposed with a dose of  $300 \text{ mJ/cm}^2$  at 365 nm, followed by simultaneous development of both construct and bulk barriers according to the procedure described in the Materials and Methods section. Fig. S1 shows that the bulk barriers effectively blocked the channel volume next to the construct and forced the cells to migrate through the construct. Unfortunately, cells showed very limited or aberrant migration behavior that was interpreted as being caused by released of cytotoxic compounds from the bulk barriers. We did not further explore the use of bulk barriers since the cell analysis could be performed with statistical significance on the fraction of cells migrating through the construct in the absence of bulk barriers.

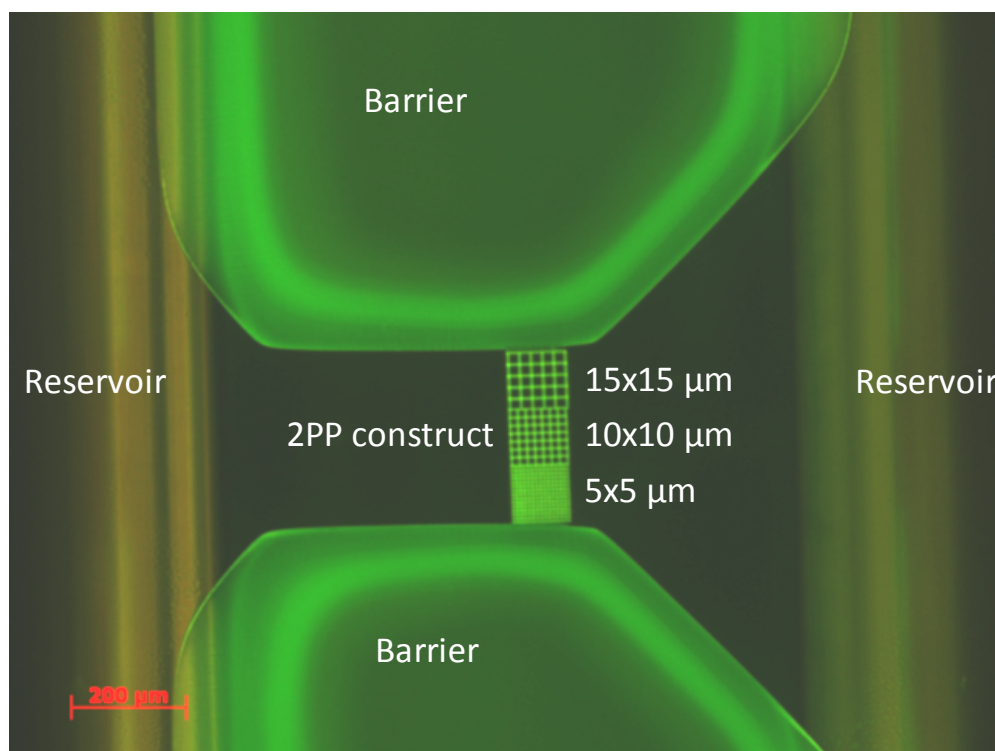
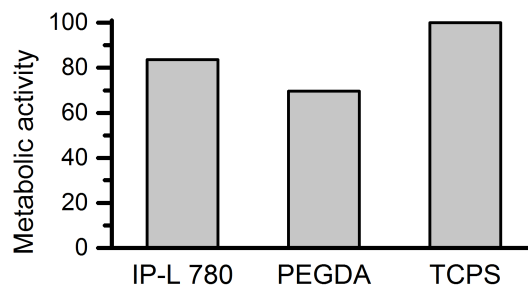


Fig. S1 Fluorescence micrograph of 2PP-written woodpile constructs with pore sizes of 5x5, 10x10 and 15x15  $\mu\text{m}$  inside the microchannel with bulk barriers blocking the remaining channel. The cells were thus forced to enter the construct in order to migrate from one reservoir to the other.

### ***Cytotoxicity test of polymerized IP-L 780 resin***

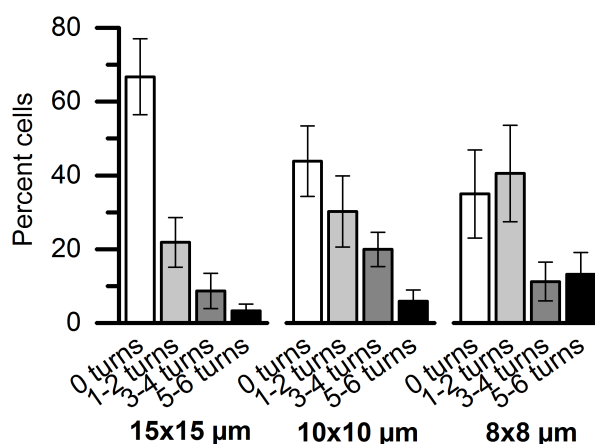
Cytotoxicity of polymerized IP-L 780 resin was evaluated using a dendritic cell (DC) metabolism assay. Cytotoxic comparison was made against Tissue Culture grade polystyrene (TCPS) as a non-toxic reference and photopolymerized poly(ethylene glycol) diacrylate (PEGDA) that is often used in biomedical applications of two-photon polymerization. Experiments were performed in a 96 well TCPS microtiter plate (Nunc grade, Nunc, Roskilde, Denmark). 50  $\mu$ L IP-L 780 was dispensed into a well and photopolymerized with a dose of 180 mJ/cm<sup>2</sup> at 365 mW in an MA4 mask aligner (Suss Microtec). 50  $\mu$ L of 1 kDa PEGDA (Laysan Bio, Arab, AL) with 0.1% w/v IrgaCure 2959 (2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone, Sigma-Aldrich) was dispensed into a second well and photopolymerized with 8.6 J/cm<sup>2</sup>. The latter high exposure dose was required due to the presence of ambient oxygen acting as radical quencher. A third untreated well was used as the non-toxic TCPS reference. Samples were prepared in triplicate on each plate.

All wells were washed 3 times with MilliQ grade water (Merck Millipore, Billerica, MA) water and left with MilliQ water for a further 72 h at room temperature. Culture medium consisted of IMDM with 10% FBS and 1% P/S. The wells were filled with culture medium for 15 min and emptied, prior to seeding 5000 – 10000 DCs in 100  $\mu$ L culture medium in each well and incubation for 24 h. 10  $\mu$ L AlamarBlue (Life Technologies) was added to each well and incubation was continued for 2 h. After incubation 100  $\mu$ L supernatant was transferred to a new microtiter plate, and the developed color was measured in a Victor3 plate reader (Perkin Elmer, Waltham, MA). The TCPS well bottom was chosen as reference material for the DC metabolic activity on the two photopolymerized materials. **Fig. S2** shows that photopolymerized IP-L 780 induces some reduction in DC metabolism compared to TCPS, but a smaller reduction than observed on the commonly used PEGDA/IrgaCure 2959 system. These results were obtained on 1.5 mm thick material layers coating the entire well bottom. Considering the extremely small volumes photopolymerized in the in-chip constructs, we do not anticipate significant cytotoxic effects.



**Fig. S2** Metabolic activity of dendritic cells cultured on different materials. Tests are performed with an AlamarBlue assay and the graph shows the activity relative to the metabolic activity on TCPS. Values are the average of 2 independent experiments.

### Detailed analysis of the cell migration pathways through woodpile constructs



**Fig. S3.** Detailed cell migration analysis as a function of construct pore size. There is a clear trend towards more cells making at least one turn in the smaller pore constructs. It is less clear if the 8x8 μm pores induce more cell turns than the 10x10 μm pores. Error bars show the standard error of the mean ( $n \geq 3$ ).

### Diffusion-based concentration gradients in woodpile constructs

The diffusivity of a compound A in a medium B can be written as  $D_{AB}$ . The diffusibility,  $Q$ , in a porous medium is then the ratio of the effective diffusivity,  $D_{eff}$ , of compound A through the porous medium to  $D_{AB}$ .<sup>1</sup> van Brakel and Heertjes expressed the diffusibility as:

$$Q = \frac{\varepsilon \delta}{\tau^2} \quad (\text{eq. 1})$$

where  $\varepsilon$  is the porosity,  $\delta$  the constrictivity, and  $\tau$  the tortuosity.<sup>2</sup> The tortuosity  $\tau$  is approximately unity in the woodpile design since the straight channels of the construct are parallel to the gradient direction outside the construct. The porosity is 0.71 for a perfect woodpile structure with 10 μm x 10 μm pores separated by bars of cross-sectional dimensions 10 μm x 4 μm. The introduction of a 1.3 μm vertical overlap between overlying bars to optimize the mechanical stability reduces the porosity slightly to a value no smaller than 0.68. The constrictivity cannot be calculated directly. However, van Brakel and Heertjes estimated that the constrictivity does not depend on the particular shape of the porous structure but only on the ratio  $\beta$  between the maximum and minimum cross-sectional dimension of the porous path through the construct. For our woodpile design  $\beta \approx 2$  corresponding to a constrictivity of approximately 0.9.<sup>2</sup> Inserting the worst case values into eq. 1 yields a diffusibility:

$$Q = \frac{0.68 \cdot 0.9}{1^2} = 0.61$$

The results shows that the effective diffusion constant is reduced by ~50% inside the construct compared to outside with a corresponding small delay in establishing a chemoattractant concentration gradient within the construct. However, the lateral extent of the construct is only up to 20% (200 μm) of the entire channel extent (1 mm) in the direction of the concentration gradient. This implies that the delay in gradient formation inside the construct will be insignificant in comparison to establishment of the channel-wide concentration gradient.

### ***Description of the time-lapse movies***

Movie 1: Migration of dendritic cells through a woodpile construct with 15x15  $\mu\text{m}$  pores (see Fig. 4 in the main text). The image sequence has been processed to highlight the cells. Images were captured every 2 minutes.

Movie 2: Migration of dendritic cells through a woodpile construct with 15x15  $\mu\text{m}$  pores. Same image sequence as Movie 1 but without any image processing. Images were captured every 2 minutes.

Movie 3: Migration of dendritic cells through a woodpile construct with 10x10  $\mu\text{m}$  pores and added barrier structures (see Fig. 7 in the main text). The image sequence has been processed to highlight the cells. Images were captured every 2 minutes.

Movie 4: Migration of dendritic cells through a woodpile construct with 10x10  $\mu\text{m}$  pores and added barrier structures (see Fig. 7 in the main text). Same image sequence as Movie 3 but without any image processing. Images were captured every 2 minutes.

### ***References for Electronic Supplementary Information***

- 1 J. Hoogschagen, *Ind. Eng. Chem.*, 1955, **47**, 906-913.
- 2 J. van Brakel and P. M. Heertjes, *Int. J. Heat Mass Transfer*, 1974, **17**, 1093-1103.