

## Design of a 2D no-flow chamber to monitor hematopoietic stem cells

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### Supplementary data

#### Supplementary figures

Figure S1

Figure S2

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Figure S4

#### Supplementary movies

Movie S1

Movie S2

Movie S3

Movie S4

## Supplementary figure legends

Figure S1 - Two dimensional simulation of fluid velocity field in various chamber designs using the software Comsol.

Inlet velocity (inlet of chamber line) is fixed at 2.5 mm/s. Fluid kinematic viscosity is fixed at  $10^{-6}$  m<sup>2</sup>/s.

- (A) Fluid velocity field in the design suggested by Hanry Yu and colleagues (Toh et al., Lab On a Chip, 2007).
- (B) Increasing the aspect ratio of the separating pillars can reduce fluid velocity in the chamber.
- (C) Adding some barriers perpendicular to the flow in the chambers can also reduce fluid velocity.
- (D) Reducing chamber size would allow faster solutes diffusion during medium renewal and still prevent fluid flow in the chambers.
- (E) Asymmetric disposition of inlet and outlet induces a flow in the first and the last chambers but not in the central chambers.

Figure S2 – No-flow chamber shape optimization.

The image shows water filling in square chambers. It illustrates how the square corners block fluid front progression and generate air bubbles. This problem was solved by using round chambers.

Figure S3 – No-flow chamber dimensions and chip design.

- (A) No-flow chamber dimensions preventing fluid entry and allowing a fast medium renewal by diffusion.
- (B) Chip design. A single inlet connect 24 channels containing 36 chambers each.

Figure S4 - Two dimensional simulation of fluid velocity field along the serial alignment of no-flow chambers using the software Comsol.

- (A) fluid velocity field as calculated with an inlet velocity 1500  $\mu$ m/s and a fluid kinematic viscosity of  $10^{-6}$  m<sup>2</sup>/s.
- (B) Measurement of fluid flow at the center of the chambers when inlet velocity is 150  $\mu$ m/s (left) or 1500  $\mu$ m/s.
- (C) Zoom on the velocity field in the chamber. Line fields enter the chamber from the openings but went back close by.

## Supplementary movie legends

Movie S1 - No-flow chambers loading.

The asymmetric supply allows a bubble-free chamber loading. Inlet contained water with 1  $\mu$ M of Alexa-488 (green fluorescence emission). Time is displayed in seconds.

Movie S2 - Flow observation in supply channels and no-flow chambers.

One-micron polystyrene beads were used as fiduciary markers to reveal fluid flow. Images were taken every 500 ms with 200 ms exposure time. The displayed chambers Time is displayed in seconds.

Movie S3 – Medium renewal in no-flow chambers.

The chip was filled with 1  $\mu$ M of Alexa-488 (green fluorescence emission) in water. The inlet was then switch to 1  $\mu$ M of Alexa-568 (red fluorescence emission) in water. Green and red fluorescence in the chambers was video-recorded. Time is indicated in min:sec.

Movie S4 – HSC proliferation, polarization and migration in a no-flow chamber.

HSC were loaded in the no-flow chamber during chip filling with medium. Cells were monitored in transmitted light by taking pictures every 3 minutes during 3 days. Medium was renewed every 6 hours. The video shows image acquisition every 9 minutes only in order to limit the file size. At the end of the movie, fluorescent primary antibodies against CD38 (green), CD34 (blue) and CD33 (red) were added to the medium in the side channels.