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Adipose Stem Cells (ASCs) isolation by on-chip pre-treatment of biological samples

Marion Valette, Mathias Bouguelmouna, Mélanie Mariotte, Rémi Courson,
Marie-Charline Blatché, Amandine Girousse, Coralie Sengenés, Karine
Reybier, Anne Marie Gué

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Context

Early diagnosis or therapeutic follow-up (circulating tumour cells – CTC), regenerative medicine (mesenchymal stem cells) or the control of bacteriological risks (pathogens detection) are major topics of cell isolation. The techniques currently dedicated to cell isolation, a miniaturized system integrating several laboratory functions, achieves quick analysis while consuming a small volume of sample. This cost-effective tool is easy to use and can be used in any area. It represents an attractive alternative to other common cell isolation techniques.

The goal of this study is to **develop a miniaturized analysis system (lab-on-chip) able to isolate and identify different cell types present in very small quantity in biological samples**. It potentially concerns a large variety of cell species : mesenchymal stem cells, bacteria, etc. Cells of interest are **stem cells from the adipose tissue** (Adipose stem cells – ASCs). These cells are relevant for regenerative medicine and may be a key in the prevention of type 2 diabetes.

To isolate them, we propose an original approach combining two complementary steps:

- 1) Passive cell sorting based on a physical criterion: the size**
Based on hydrodynamic filtration, the goal is to eliminate (by filtration) cells with $d < 10 \mu\text{m}$
→ red blood cells, lymphocytes and blood platelets (more than 99% of the blood)
- 2) Separation by immunological specificity : exclusion of the remaining blood cells**

Cells	Red Blood Cells	Monocytes	Lymphocytes	Blood Platelets	ASCs
Diameter	$5 < d < 7 \mu\text{m}$	$15 < d < 20 \mu\text{m}$	$2 < d < 5 \mu\text{m}$	$7 \mu\text{m}$	$10 < d < 40 \mu\text{m}$

This presentation is focused on the first part of the device.

3D Microfabrication

Legend:
 ● Lamination
 □ Glass substrate
 ▭ Uncrosslinked resist
 ▭ UV exposure
 ▭ Photolithography mask
 ▭ Crosslinked resist
 ▭ Bonding

Microfluidic chip

Step 1: Hydrodynamic Filtration

Size determination

The size of target cells is an essential criterion for the choice of cell sorting techniques. Up to now, we studied samples of adipose tissue from mice. The size of cells has been determined by using a FACS (Fluorescent Activated Cell Sorting) calibrated with beads of different diameters : 5, 10, 15 and 20 μm . A sample of Stromal Vascular Fraction (SVF = white blood cells, blood cells and ASCs) from a mouse has been analysed via FACS → $10 < \text{diameter}(\text{ASCs}) < 40 \mu\text{m}$.

ASCs are not distinguishable from leucocytes by their size

Characterization of different cell populations size with a FACS calibrated with beads of diameter 5, 10, 15 and 20 μm .

- ASCs
- Lymphocytes
- Cells of diameter 5 or 10 or 15 or 20 μm

Hydrodynamic filtration

Introduced for the first time by Yamada and Seki in 2005. The principle is to pick a certain amount of liquid from the principal channel: the diameter of aspirated particles in side channels depends on ratio of the flow rate in the main and in the side channels.

$$Q^*(r_c) = \frac{Q_{lat}}{Q_{in}} = cte = \frac{r_c}{2w} \frac{1 - \sum_{n=1}^{\infty} \frac{96}{(n\pi)^2} \frac{h}{r_c} \left[\tan h\left(\frac{n\pi w}{2h}\right) \left(1 - \cosh\left(\frac{n\pi r_c}{h}\right) + \sinh\left(\frac{n\pi r_c}{h}\right)\right) \right]}{1 - \sum_{n=1}^{\infty} \frac{192h}{n^2 \pi^2 w} \tan h\left(\frac{n\pi w}{2h}\right)}$$

Hydrodynamic filtration depends on the cut-off radius and the dimensions of the device

Fouet et al., 2016
Scheme of the principle of hydrodynamic filtration

Scheme of the principle of hydrodynamic filtration

Validation with synthetic samples

Final design of the device with 30 side channels (CieWin5)

- Cut-off radius: $r_c = 5 \mu\text{m}$
- Made in Clean Room
- 30 side channels
- 2 Inputs (A: Sample / B: Buffer)
- 3 Outputs (C: Particles $d > 10 \mu\text{m}$ / D1-D2: Particles $d \leq 10 \mu\text{m}$)

- ✓ Filtration of 5 μm diameter beads
- ✓ No filtration of 15 μm diameter beads
- ✓ Beads of 10 μm diameter partially filtered

Fluorescent polystyrene beads ($d = 5 \mu\text{m}$) filtered by the system

Tests on biological samples

Efficiency of filtration

Zone 1: solution of RBCs and THP-1
Zone 2: solution of RBCs and ASCs

- ✓ Filtration does not depend on pressure
- ✓ Filtration of 1 mL of diluted blood (1/5)
- ✓ Low times of filtration:
 - ✓ 30 min to filter 1 mL at 800 mbar
 - ✓ 1 h to filter 1 mL at 500 mbar
 - ✓ 1 h 30 min to filter 1 mL at 300 mbar
- ✓ No cell lysis
- ✓ 100 % of cells of interest collected in the main output (C)
- ✓ Good proliferation of cells after filtration

Cell viability Cells: THP-1

Cell viability Cells: THP-1

Filtration of Red Blood Cells < 100%

? Side channels Saturation

? Margination of Red Blood Cells
High deformability of RBCs → flow to the middle of the channel
Platelets and leukocytes less deformable stay close to the walls

RBCs margination
Q. M. Qi and E. S. G. Shaqfeh, Physical Review Fluids, 2017

Resume and Prospects

- ✓ Validation of the device with synthetic samples:
 - Particles $d \leq 10 \mu\text{m}$ filtered
- ✓ Validation of the device with biological samples:
 - Cells of interest recovery: 100%
 - Exclusion of cells with $d \leq 10 \mu\text{m}$ (red blood cells, lymphocytes)
- ✓ Cell viability : low impact on proliferation
- ✗ Red Blood Cells filtration \neq 100%

→ Promising results with the new design with 100 side channels:

- ✓ Filtration of non diluted blood
- ✓ Few remaining red blood cells in the sample

Last channels: few Red Blood Cells remaining
First channels which filter the sample

Step 2: Exclusion by Cell Rolling

No ASCs specific antibody known
Specific leucocytes antibodies well known : some of them are not compatible with ASCs
We work with specific leucocytes / non-specific ASCs **anti-CD45**

Principle of « cell rolling » :
Alternate areas on which specific antibodies are grafted (target cells are leucocytes) with areas without antibodies. Cells bind to the functionalized surface (affinity antibody/antigen). Hydrodynamic forces prevent the cells from binding completely to the surface and force them to move along the functionalized areas.

Cell Rolling in SPRI

- ✗ 100% AC
- ✗ 50% 50% AC/Albumine
- ✗ 20% 80% AC/Albumine
- ✗ 5% 95% AC/Albumine

✓ Promising results: Cell rolling of T Lymphocytes

Principle of cell rolling: leucocytes are separated from the rest of the sample → they roll on the functionalized surface

Bose, S. et al.