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ADIPOSE STEM CELLS (ASCs) ISOLATION BY ON-CHIP PRE-TREATMENT OF BIOLOGICAL SAMPLES

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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 are very complicated. Lab-on-chip, a miniaturized system integrating several laboratory functions, achieves quick analysis while consuming a small volume of sample. This cost-effective tool is easy of 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 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

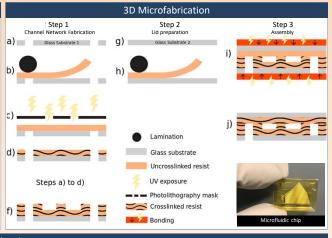
Based on hydrodynamic filtration, the goal is to eliminate (by filtration) cells with d<10um

ightarrow red blood cells, lymphocytes and blood platelets (more than 99% of the blood)

2) Separation by immunological specificity: exclusion of the remaining blood cells

This presentation is focused on the first part of the device

 Diameter
 5 < d < 7 μm</td>
 15 < d < 20 μm</td>
 2 < d < 5 μm</td>
 7 μm
 10 < d < 40 μm</td>



Step 1: Hydrodynamic Filtration

Size determination

Hydrodynamic filtration

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. 2001 A sample of Stromal Vascular Fraction (SVF = white blood cells, blood cells and ASCs) from a mouse has been analysed via FACS \Rightarrow 10 < diameter(ASCs) < 40 μ m.

ASCs are not distinguishable from leucocytes by their size

Lymphocytes Cells of diameter 5 or 10 or 15 or 20 µm Characterization of different cell populations size with a FACS calibrated with beads of diameter 5, 10, 15 and 20 un

Fouet et al., 2016

Introduced for the first time by Yamada and Seki in 2005.

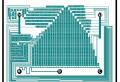
The principle is to pick a certain amount of liquid from the principal channel: the diameter of aspirated particles in side channels depends on ratio of the flow rate in the main and in the side

$$Q^*(r_c) = \frac{Q_{lat}}{Q_{in}} = \text{cte} = \frac{r_c}{2w} \frac{1 - \sum_{n=i}^{\infty} \quad \frac{96}{(n\pi)^5} \frac{h}{r_c} \left[\tanh\left(\frac{n\pi w}{2h}\right) (1 - \cosh\left(\frac{n\pi r_c}{h}\right)) + \sinh\left(\frac{n\pi r_c}{h}\right) \right]}{1 - \sum_{n=imp}^{\infty} \frac{1}{n^5} \frac{192h}{\pi^5} \tanh\left(\frac{n\pi w}{2h}\right)}$$

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



Final design of the device with 30 side channels (CleWins



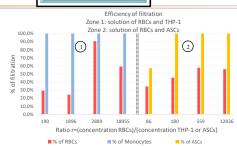
- Cut-off radius: r_c = 5 µm
- Made in Clean Room
- 30 side channels
- 2 Inputs (A: Sample / B: Buffer)
- 3 Outputs (C: Particles d > 10 um / D1-D2: Particles d ≤ 10 µm)

Validation with synthetic samples

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

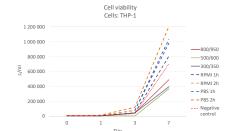


(d = 5 um) filtered by the system



Tests on biological samples

- √ Filration 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



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

- ✓ Validation of the device with synthetic samples:
- Particles d ≤ 10 μm filtered
- Validation of the device with biological samples:
- Cells of interest recovery: 100%
 - Exclusion of cells with d ≤ 10 μm (red blood cells, lymphocytes)
- ✓ Cell viability: low impact on proliferation
- X Red Blood Cells filtration ≠ 100%
- → Promising results with the new design with 100 side channels:
 - Filtration of non diluted blood
 - Few remaining red blood cells in the sample

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

Final design of the device with 100 side First channels which filter the sample



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 functionalized surface (affinity antibody/antigen). Hydrouynemic forces prevent the cells from binding completely to the surface and force them to move along the functionalized areas.

Step 2: Exclusion by Cell Rolling

No ASCs specific antibody known

Specific leucocytes antibodies well known: some of them are not compatible with ASCs



Principle of « cell rolling » :

We work with specific leucocytes / non-specific ASCs anti-CD45

- 50%/50%
 AC/Albumin
 20%/80%
 AC/Albumin
 5%/95%
 AC/Albumin
 - ✓ Promising results: Cell rolling of T Lymphocytes











