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Development of a microfluidic setup for the isolation and imaging of immune cells

Towards cell separation at the microliter scale

https://doi.org/10.1515/cdbme-2024-2121

Abstract: Specialised cells in the blood play an important role in controlling the immune responses. Thus, these cells are also valuable for the diagnosis and treatment of diseases such as autoimmune disorders and cancer. Trace-less Affinity Cell Selection technology, also known as Fab-TACS technology, is a new approach to precisely isolate specific immune cells from blood samples. Currently, this technology is used in a device called Fabian, which resembles a cassette with a capacity of 60 ml. For diagnostic purposes, however, the sample size must be reduced to a few microliters. For this purpose, a stand-alone setup for the control of a microfluidic cartridge was developed and a sequence for the implementation of the TACS technology was designed and characterized. As the combination of cell purification and subsequent cell counting in a single device can streamline workflows and save time, we also integrated an analysis platform for cell counting into the setup. Instead of using two separate devices, isolated samples can be counted immediately after isolation without interruption. This platform was initially tested with latex particles that were passed through the detection channel of the microfluidic cartridge. The functionality of the developed program sequence and cell counting was successfully demonstrated in verification experiments

Keywords: TACS, microfluidics, in-vitro diagnostics, cell separation, cell counting

1 Introduction

The human immune system is a complex network of different cells that develop from specialized stem cells. In the bloodstream, these cells are known as peripheral blood mononuclear cells (PBMC) and play a crucial role in regulating the immune response. PBMC are made up of lymphocytes (T cells, B cells, NK cells), monocytes and granulocytes, each with their own specific functions. These cells are used in the diagnosis and treatment of autoimmune and haematological diseases [1]. In recent years, there has been growing interest in using specific immune cells for targeted therapies, such as adoptive transfer of T cells (ACT). ACT involves the infusion of lymphocytes to combat diseases such as multiple myeloma by inducing anti-tumour, anti-viral or anti-inflammatory effects [2]. Currently, the isolation of specific cell populations from whole blood is a complex, multi-step process. However, a technology called Traceless Affinity Cell Selection technology (Fab-TACS technology) offers a selective, gentle and highly pure isolation of specific immune cells from whole blood or buffy coat. This technology is currently used in a syringe-like cartridge, which has a capacity of 60 ml and is particularly suitable for ACT applications. However, there is a need to adapt the TACS technology to a microfluidic cartridge that can handle smaller volumes of blood, such as less than 100 µl. This adaptation is necessary to enable its use in laboratory diagnostics or paediatric oncology, where smaller sample volumes are often required. A microfluidic cartridge to separate immune cells from samples of less than 100 µL via TACS has been developed and is described briefly elsewhere [3]. Nevertheless automated liquid handling within the cartridge as well as cell and particle tracking is still missing. Here, we aim to establish a microfluidic setup to automate liquid handling (A) and implement a setup to count particles within the microfluidic cartridge (B).

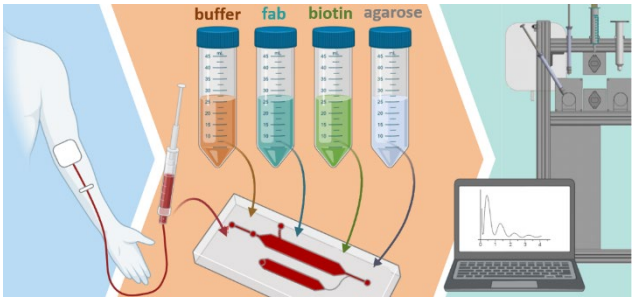

Figure 1: Graphical abstract: Development of a microfluidic setup for the isolation and imaging of immune cells (Created with BioRender.com)

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1.1 TACS technology

Unlike traditional approaches, non-magnetic cell affinity chromatography, such as TACS, moves away from the use of fluorescent markers or magnetic beads that can cause permanent damage to target cells [4]. This innovative method relies on affinity purification using weakly binding antibodies [5]. The method exploits the phenomenon of weak but abundant binding exhibited by cell-specific antibodies, commonly referred to as the avidity effect. These Fab fragments, which contain only the binding domain of an antibody, are synthetically generated with an affinity tag, Strep-Tag®, attached. The Strep-Tag® is then able to interact with another specific protein, Strep-Tactin®. For this purpose, it is first immobilised on an agarose matrix (see Figure 2) in order to start the purification protocol. The Fab-Strep

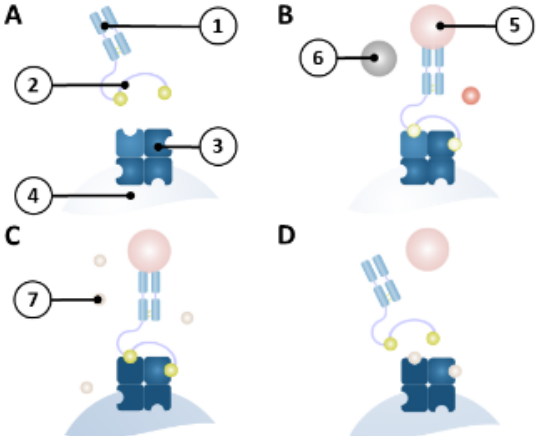

Figure 3: Process flow of TACS: 1) Fab-antibody; 2) Strep-Tag®; 3) Strep-Tactin®; 4) Agarose bead; 5) Target cells; 6) Non-target cells; 7) Biotin. A - Fab loading; B - Blood loading with connection of target cell with the Fab-antibody; C - Wash step and Biotin loading; F - Target cell elution. The illustration is purely functional and do not completely represent the real dimensions

antibodies are then introduced into the matrix where they adhere (A). Following this step, whole blood is introduced into the matrix, causing cells expressing the specific antigen on their surface to bind to the matrix (B). The remaining unbound blood cells are then removed from the column by washing. Biotin is then introduced. Acting as a competitive agent, biotin disrupts the interaction between Strep-Tag® and Strep-Tactin® by forming irreversible bonds with the matrix (C). In addition to its competitive binding role, biotin induces detachment of the target cells from the columnar particles. This detachment is facilitated by a conformational change in the Fab fragments, which leads to a loosening of their binding to the cell antigens, ultimately resulting in the isolation of target cells free of residues (D).

1.2 Tracking and counting of particles and cells

Accurate cell counting can provide important insights into biological processes and advance the development of new therapies. The Lucas-Kanade method is a widely used optical flow computation technique in image processing and computer vision. It was developed by Bruce D. Lucas and Takeo Kanade in the 1980s and is particularly useful for tracking features in image sequences [6]. The method is based on the assumption that intensity changes between successive images are due to motion in image space. By calculating the local gradient and the change in gradient over time, the Lucas-Kanade method can estimate the movement of features and thus determine the optical flow. In cell biology, the Lucas-Kanade method is used for cell counting, particularly in fluorescence microscopy. It allows cells to be tracked and quantified in image sequences [7].

2 Development of the setup

2.1 Design and prototyping of the microfluidic setup for TACS

The efficient automation of TACS on a microfluidic cartridge requires a well-planned active structure of the periphery (Figure 3). Group I of the structure includes tanks for the fluids required for the TACS, i.e. buffer, Fab solution and biotin.

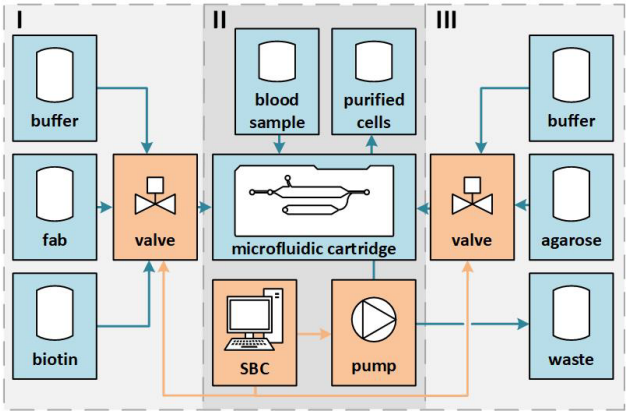

Figure 2: Schematic structure of the setup: I - storage and supply of fluids; II - microfluidic cartridge, Sampling tank, dosing unit and SBC; III - storage, supply of agarose suspension and waste container; blue arrows - fluidic tubes; orange arrows - network cable for controlling the pumps and valves

These fluids are supplied to the system via a precision fluid feeder. A rotary valve with distributor head allows the fluids to be controlled and delivered. By rotating the valve head, the

different fluids can be supplied as required. The microfluidic cartridge shown in Group II is at the heart of the system and serves as the platform for implementing the TACS. Dedicated controllers are used to control the fluidics within the cartridge. An injection pump enables precise transport of fluids within the microfluidic channels. These units are controlled by a special control software, which runs on a single board computer. This software allows precise and automated control of the fluidics within the cartridge. Group III contains additional vessels for the agarose suspension and the required buffer. This is used to homogenise the agarose suspension. The agarose is added via a rotary valve with a switching valve head, with a sample loop to ensure reproducible dosing. This allows precise control of the amount of agarose suspension added to the system.

2.2 Integration of the analysis platform for cell counting

After the above mentioned construction scheme was designed and manufactured, a cell counting platform was integrated. The result is shown in figure 4. Figure 4/I shows the left side of the set-up. The containers such as infusion bags and syringe barrels for the fluids buffer (2), FAB solution (3), biotin (4) and agarose suspension (5) are mounted on a frame made of construction profiles. A laboratory syringe pump (Cavro® XLP 6000 Modular Syringe Pump with 3-port valve) (6) is used for precise dosing of the fluids. Pinch valves (PS/PSK Series, 2/2-way normally closed) (7) and rotary valves (P201-O) (8) are used to control the flow of fluids. To create a robust setup within a laboratory environment, an enclosure (9) that houses components such as the single board computer (Raspberry Pi 4 Model B), a power board and a USB hub for control and data acquisition. Figure 4/II shows a close up view

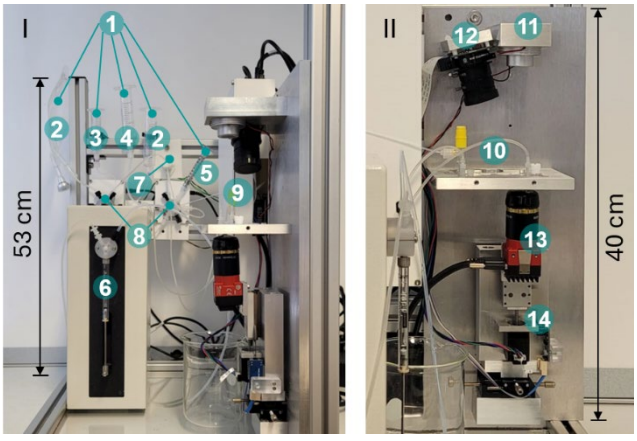

Figure 4: Final arrangement of the manufactured setup with integrated analysis platform. The components labelled with numbers are discussed in detail in the text

of the image analysis platform, which is designed to enable precise monitoring and recording of the microfluidic samples and detection of the target cells. It consists of a holder (10) for the microfluidic cartridge, which ensures stable positioning during analysis, and a light source (11) aligned with the cartridge, which provides optimal illumination during image acquisition. Hence the microfluidic cartridge for TACS contains two separate chambers for separation and cell counting, an image acquisition containing two independent cameras was integrated. A first camera (Raspberry Pi Camera) (12) is aligned with the separation chamber of the cartridge, while a second camera (Allied Vision 1800 U-500) (13) is aligned with the chamber for cell counting. A stepper motor driven lifting platform (14) allows fine adjustment of the second camera for precise focusing and image acquisition.

3 Experimental proof of function

3.1 Program sequence for TACS

The functionality of the developed cell purification program was demonstrated by staining tests. The fluids required for the TACS were stained with food coloring for visualisation. The results are shown in figure 5.

Figure 5.1 shows the empty microfluidic cartridge with the labelled areas of the separation chamber (A) and the sample chamber (S). Figure 5.2 shows the first rinse of the cartridge

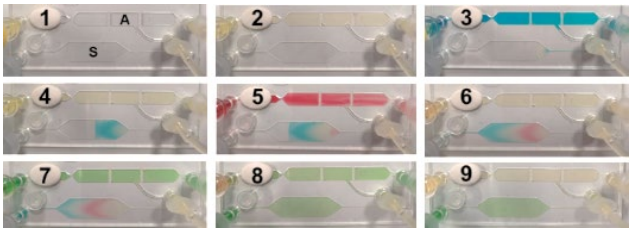

Figure 5: Proof of function of the programme sequence: A - agarose chamber, S - sample chamber; 1 - empty microfluidic cartridge; 2 - first rinsing process; 3 - addition of the FAB solution; 4 - second rinsing process; 5 - addition and mixing of the blood; 6 - third rinsing process; 7 - addition of the biotin; 8 - sampling; 9 - final rinsing process

with buffer solution, which displaces the air from the cartridge. After rinsing, the rinsed section is completely filled with buffer. The addition of the FAB solution is shown in Figure 5.3, where good mixing within the cartridge can be observed due to the oscillating movement of the fluid. Figure 5.4 shows the next rinse section where the blue color has been completely removed from the rinsed area. The addition and mixing of the blood sample can be seen in Figure 5.5, while Figure 5.6 shows the result of the subsequent rinse, which also shown in Figure

5.7, where it was also distributed evenly in the cartridge by oscillation. Then, the purified cell solution with biotin was moved to the counting chamber indicated by the continuous coloring of the sample chamber (Figure 5.8). Finally, Figure 5.9 shows the cartridge after the final rinse, with the sample retained in the counting chamber during the rinse.

3.2 Validation of cell counting

To evaluate the functionality of the analytical platform, a series of dilutions of a 10 µm latex bead suspension (C37259, Thermo Fisher Scientific) were prepared and passed through a channel at a rate of 7.5 µl/s, with image sequences (180 f/s) recorded. Figure 6/I shows an example frame of the channel.

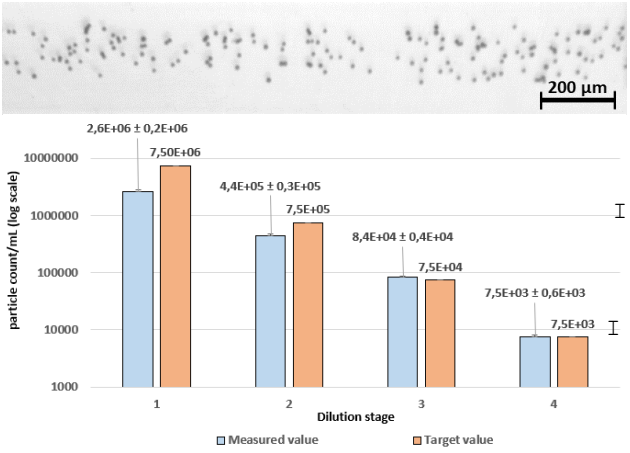

Figure 6: Top (I): example frame of the detection channel; bottom (II): Bar chart: Measured values and target values of the particlecount per mL for each dilution stage

A software program based on the Python programming language was developed for the purpose of particle counting, utilising the Lucas-Kanade method. The software employs a series of image analysis techniques to track and quantify the movement of particles. This is achieved by calculating image differences and utilising optical flow algorithms. The mean values of the measured particle counts were then compared with the target value particle count given in the manufacturer's certificate of analysis (7.5×10^7 particles/mL). Figure 6/II presents a bar chart in which the measured particle numbers are compared with the target values. The relative deviations are indicated above the bars. The particle count/mL scale has been transformed into a logarithmic scale. The chart shows significant deviations of the measured particle counts from the target values for dilutions 1 to 3. Therefore, the most reliable results were obtained at dilution 4, where the particle count could be validated.

4 Conclusion

Summing up, we have developed a setup for controlling a microfluidic cartridge to extract immune cell from samples of less than 100 µL using TACS. Moreover, an analysis platform for cell counting integrated into this setup. Experimental proof of the developed program sequence for cell extraction and cell counting was carried out by staining tests and comparison of counted versus expected particle numbers. To improve the reliability of the integrated analysis method, further experiments should be carried out to validate particle detection and counting. This can be done by using different particle sizes. In this way, the performance of the optical analysis software can be tested and possible limitations can be identified. Overall, we provide a robust setup for future research activities in the field of microfluidic cell separation and analysis. This could contribute to the development of innovative diagnostic and therapeutic procedures in medicine.

Author Statement

Research funding: This study was funded by the internal FHG Project AMBERA.

Conflict of interest: Authors state no conflict of interest.

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