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Modelling and prototyping of a microfluidic cartridge for extracting immune cells from small volume blood samples

Abstract: Within the human blood immune cells assume specific functions to modulate and control the human immune response. Consequently, they are used for the diagnosis and treatment of autoimmune and hematologic diseases. Traceless Affinity Cell Selection Technology (Fab-TACS® technology) is a new method for selective isolation of specific immune cells from blood samples. Currently, the technology is used in a 60 mL syringe-like cartridge, which is operated by a control unit (Fabian®). Nevertheless, diagnostic applications require the reduction of the sample volume to several microliters. Therefore, we developed a cartridge for the purification of small blood volumes with the aid of suitable scaling and simulation methods. By implementing a multi domain physical systems model of the established cartridge we could simulate the separation process. Based on that a microfluidic cartridge was designed and several prototypes were manufactured. To operate the microfluidic cartridge a setup and software for fluidic control were created. Afterwards the prototype cartridge and the microfluidic control were used to isolate lymphocytes from a buffy coat. By flow cytometric analysis we could show that it is possible to isolate lymphocytes with a purity that is comparable to the established 60 mL syringe-like cartridge.

Keywords: TACS, microfluidic, in-vitro diagnostics, simulation, Cell separation

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1 Extraction of immune cells

1.1 Motivation

The human immune system is composed of different cell populations, which develop from a common stem cell by cellular differentiation. Within the human blood the fraction of immune cells is called peripheral blood mononuclear cells (PBMC) including lymphocytes (T cells, B cells, NK cells), monocytes and granulocytes. They all assume specific functions to modulate the human immune response. Consequently, they are used for the diagnosis and treatment of autoimmune and hematologic diseases [1]. Nowadays the targeted use of specific immune cells is becoming increasingly important for the adoptive transfer of T cells (ACT) as a new field of transfusion medicine. This includes the infusion of lymphocytes to mediate anti-tumor, anti-viral or anti-inflammatory effects with the aim of combating diseases such as multiple myeloma [2]. Thus, new technologies are arising to isolate such cell populations from whole blood. Up to now complex multi-stage procedures are required to specifically select these from the cell mixture of the blood. Traceless Affinity Cell Selection Technology (Fab-TACS® technology) is a new method for the selective, gentle and highly pure isolation of specific immune cells from whole blood or buffy coat. Currently, the technology is used in a 60 mL syringe-like cartridge which is operated by a control unit (Fabian®). This fits well to ACT applications. Nevertheless, for applications in laboratory diagnostics or pediatric oncology the amount of blood is limited to less than 100 µL. Thus TACS® technology has to be transferred to a microfluidic cartridge that ensures purification results similar to the established cartridge.

1.2 TACS technology

In contrast to conventional methods, non-magnetic cell affinity chromatography, or TACS® technology (traceless affinity cell selection), does not use fluorescent markers or

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magnetic beads, which cause long-term damage to the target cells [3]. Instead it is based on affinity purification with weakly binding antibodies [4]. The method uses the effect of low but numerous binding cell-specific antibodies, also called avidity effect. These so-called Fab-fragments are those elements of an antibody that only contain the binding region. They are produced recombinantly with an affinity tag, the Strep-Tag®. The Strep-Tag® is capable of binding to another specific protein, Strep-Tactin®. At the beginning of the purification process, the Strep-Tactin® is placed on the agarose matrix (compare figure 1).

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

If the Fab-Strep antibodies are now added and bind to the matrix (A, B). Afterwards whole blood is loaded into the matrix. Those cells that have expressed the specific antigen on the cell surface are bound within the matrix. The remaining unbound blood cells are rinsed from the column by a washing procedure (C, D). Finally, biotin, also known as vitamin H, is added. It acts as a competitor and releases the binding between Strep-Tag® and Strep-Tactin® and attaches itself irreversibly to the matrix (E). Besides its attachment, biotin causes the target cells to detach from the column particles. By conformational change of the Fab-fragments the binding is

released, and they separate from the antigens of the cells and provide residue-free target cells (F).

2 Simulation aided prototyping

2.1 Approach

To transfer biochemical processes, like the Fab-TACS® technology, from a high-volume cartridge into microfluidic applications model based approaches have become indispensable [5]. These include several steps (compare figure 2). The first step is to establish a multi domain physical systems model. The technical processes that are implemented in the model are determined by measuring relevant process parameters. Based on these data the separation process was simulated. Within the next step simulation data were used to specify the design of the novel microfluidic cartridge. Afterwards several prototypes were manufactured by layer object manufacturing [6] and the performance of the cell selection was characterized.

Figure 2: Step by step downscaling process

2.2 Modelling and simulation of the established cartridge

For the modelling of the established cartridge the following systematic methodology has been established for a structured approach:

1. definition of the subsystems
2. definition of the interfaces
3. reduction to simple elements
4. compiling key effects
5. model notation

In the first step of modelling a multi domain physical systems model of the entire system, including the control unit Fabian® and the disposable cartridge, was established by using SimulationX® (ESI ITI GmbH, Germany). Therefore, logical subunits of the system were created and correlated to corresponding predefined model equivalents of the software (e.g. syringe like cartridge is modelled as plunger cylinder). Afterwards the interconnection of these system components was described by specific predefined interfaces (e.g. cartridge and control unit are connected by mechanical force). Physical properties of the model were determined by tribology measurements of friction forces and the resistance above the agarose matrix during the separation process in the established cartridge. By simulating the separation process within the physically determined cartridge the system pressure as a major influence for the separation process could be extracted from the model. Subsequently a multi domain physical model of the microfluidic cartridge was generated using a model library for microfluidic applications [7].

2.3 Design and prototyping of a microfluidic cartridge

The basic design properties of the microfluidic layout were extracted from the model of the microfluidic cartridge and its sub models from the microfluidic library. This included the length of the channels as well as the size of the separation camber. Within the downscaling process the size and geometry of the strep activated agarose compartment was the most crucial part as acts as the major binding site for the cells. The volume of the chamber was calculated by an allometric scaling approach [8] to fit the requirements of the affinity binding of cells to the matrix. Nevertheless, the geometric dimensions that fit this volume were determined mostly by microfluidic mixing processes. To ensure that sample and buffer media did not mix during the separation process, pneumatic control valves were integrated that regulate the opening and closing of sample and buffer tanks. After the design was finished several prototype cartridges were manufactured using layer object manufacturing technology [6].

3 Development of the flow control setup

To run the separation process within the microfluidic cartridges a flow control platform has been developed. It combines a pneumatic control unit (PCU) and a syringe pump for the regulation of media flow within the microfluidic

cartridge. Thus, the whole setup can be divided into the microfluidic application and the control unit that are shown in figure 3. For valve switching, the pneumatic control unit (4), which can switch between defined overpressure and vacuum, was first connected to the inputs of the chip (1). The syringe pump (5) was connected to the output of the microfluidic cartridge. To control the operation of the pneumatic control unit, the syringe pump and the downstream two-way valve (6) a graphical user interface was developed and implemented (7). Thus, it was possible to switch between the output storage tanks for the negative fractions (2) and the isolated cells (3).

Figure 3: Schematic illustration of the developed flow control setup and the implemented setup below: 1) Microfluidic cartridge; 2) Waste tank; 3) Storage of the isolated target cells; 4) pneumatic control unit (PCU); 5) Syringe pump; 6) Changeover valve; 7) Pump controller.

4 Performance test

The selectivity of the manufactured prototypes was characterized by isolating lymphocytes from a buffy coat (acquired from DRK Spendedeservice, Institute of Transfusion Medicine Dresden). Sixty microliters of the buffy coat were pipetted into the inlet of three cartridge prototypes. Buffers and samples were drawn by the syringe pump through the cartridges according to the regimen specified by the pneumatic

control unit. The eluate of the last rinsing step, after the addition of the elution buffer, was collected separately for each cartridge. These samples and the initial buffy coat were characterised by flow cytometric analysis to determine the distribution of PBMCs and erythrocytes. To distinguish between nucleated PBMCs and erythrocytes, cells were stained with Höchst 33342 (compare figure 4). The diagrams

Figure 4: Scatter plots of single cell signals of Höchst 33342 (H33342) vs. forward scatter (FSC): A) Buffy coat; A) Purified sample

show the gated populations in red, green and violet representing the lymphocytes, granulocytes and monocytes. The black gate shows the number of erythrocytes. Compared to the buffy coat the distribution of cell populations after purification showed a significant increase in the proportion of lymphocytes in the sample with a mean of 80,1% (compare table 1) and a maximum value of 93.2 %. Thus, the selectivity of the microfluidic cartridge is comparable to the established TACS cartridge (selectivity > 90%) [9], which was the initial benchmark. Nevertheless, the repeatability of the experiments has to be further improved by geometric changes of the cartridge outlet to minimize erythrocyte accumulation during the rinsing of the negative fraction.

Table 1: Mean amount of selected cells from three microfluidic cartridge tests: L – lymphocytes; E – erythrocytes; M – monocytes; G - granulocytes

	L [%]	E [%]	M [%]	G [%]
Buffy coat	38,7	55,4	2,5	3,6
Microfluidic cartridge	80,1 ± 10,01	19,7 ± 10,0	0,0 ± 0,01	0,1 ± 0,1

5 Conclusion

This work shows that simulation based down scaling is a suitable method to transfer a medium volume cartridge for blood cell separation from millilitre to microliter scale. The prototype of the microfluidic cartridge yields to similar purity in the isolation of lymphocytes compared to the established

high-volume cartridge. Further developments have to address the repeatability and technologies for mass production of the microfluidic cartridge to transfer the technology to point of care applications.

Author Statement

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References

- [1] Kaufmann SHE. Basiswissen Immunologie. Berlin: Springer 2014.
- [2] Ali SA, Shi V, Maric I, et al. T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. *Blood* 2016; 128: 1688–1700.
- [3] Grützkau A, Radbruch A. Small but mighty: how the MACS-technology based on nanosized superparamagnetic particles has helped to analyze the immune system within the last 20 years. *Cytometry. Part A : the journal of the International Society for Analytical Cytology* 2010; 77: 643–647.
- [4] Weiss R, Gerdes W, Leonhardt F, Berthold R, Sack U, Grahner A. A comparative study of two separation methods to isolate monocytes. *Cytometry. Part A : the journal of the International Society for Analytical Cytology* 2019; 95: 234–241.
- [5] Laurien E, Oertel H. Numerische Strömungsmechanik. 5th ed. Wiesbaden: Springer Vieweg 2013.
- [6] Sonntag F, Grünzner S, Schmieder F, Busek M, Klotzbach U, Franke V. Multilayer based lab-on-a-chip-systems for substance testing. In: Klotzbach U, Washio K, Arnold CB, editors. *Laser-based Micro- and Nanoprocessing IX*: SPIE; 2015. 93510C (SPIE Proceedings).
- [7] Busek M. Modellierung und Regelung der Nährstoffversorgung in mikrofluidischen Zellkultursystemen. Dresden: TUDpress 2018.
- [8] Sung JH, Wang Y, Shuler ML. Strategies for using mathematical modeling approaches to design and interpret multi-organ microphysiological systems (MPS). *APL bioengineering* 2019; 3: 21501.
- [9] IBA GmbH. Isolation Kit for CD3+ cells from Whole Blood (human) or Buffy Coat (human) with FABian®.