Microorganisms, particularly bacteria and yeast, are used by many scientists as a tool for molecular biology research. In contrast to mammalian cell systems, the speed of bacterial growth allows isolation of DNA and vast amounts of proteins within hours. Yeast cells are often used in the isolation and function of proteins using fluorescent reporter genes.

I. A single-cell suspension is loaded into the input chamber of the cartridge. The cartridge is placed into the MACSQuant Tyto Instrument. Pulsed air coming from the instrument enters through a 0.1 µm filter within the air inlet port at the bottom of the cartridge and flows through the air pressure line towards the input chamber. The air enters the input chamber through another 0.1 µm filter, dries the cells through a microchannel and into the microsystem, allowing for sorting of delicate cell types such as the yeast cells (2×10⁶ cells/mL) were transfectected to induce expression of c-Myc protein. From a target cell frequency of 35% in the original fraction, the c-Myc+ yeast cells were sorted to a purity of 95.3%. Figure 5 indicates the gating strategy and designated sort gate.

II. Within the microsystem, the cells are interrogated by three lasers. Once a target cell is identified, a magnetic field is applied. Based on their fluorescent and scatter light signatures, target cells are redirected by a sort valve located within the collection chamber. After a positive cell has passed through, the sorting principle of the MACSQuant Tyto Cartridge guarantees a smooth sorting process.

III. Once a target cell is identified, a magnetic field is applied. Based on their fluorescent and scatter light signatures, target cells are redirected by a sort valve located within the collection chamber. After a positive cell has passed through, the valve returns the sort channel. The sorting principle of the MACSQuant Tyto Cartridge guarantees a smooth sorting process.

To investigate the tightness of the MACSQuant Tyto Cartridge, a system mimicking the sorting conditions within the MACSQuant Tyto Instrument was established, which allowed for pressurizing and stirring of the input chamber and running fluid through the microchip. Two MACSQuant Tyto Cartridges were loaded with 7×10⁶ yeast cells/mL, and a mock sort was executed. Adjacent to these cartridges, two cartridge containing only MACSQuant Tyto Running Buffer were positioned (data and figure 3). To test for potential cross-contaminations, the running buffer of these cartridges was subjected to a bioburden test after the mock sort. No yeast cells could be detected after 9 days (<1 CFU), suggesting maintained viability of the sporozoites (fig. 8). Microscopic images recorded with a time delay between fluorescent and epifluorescence were taken of sporozoites directly after sorting on the MACSQuant Tyto, suggesting maintained viability of the sporozoites (fig. 8).

Results

1. Sorting of bacteria without cross-contamination, no need to completely sterilize the instrument

2. Tight cartridge allows complete containment of yeast cells

Conclusion

- We present a novel microchip-based sorting technology that allows for isolation of high-risk organisms such as bacteria, yeast, and parasites in a fully closed system.
- Cross-contamination and tightness testing performed using yeast, showed that all cells were retained in the closed MACSQuant Tyto Cartridge. No sample-to-sample carry over occurred.
- The gentle sorting conditions of the MACSQuant Tyto allow for sorting of delicate cell types such as the sporozoite stage of Plasmodium parasites. Sorted parasites were viable, which indicates that viability was maintained.

Methods

The MACSQuant Tyto Cartridge

Figure 4 and 5 show the side view and bottom view of the cartridge, respectively.

The sort principle of the MACSQuant Tyto Cartridge

Isolation of the sporozoite stage of the Plasmodium parasite is problematic on conventional droplet sorters due to the harsh sorting conditions. Using the gentle sorting conditions of the MACSQuant Tyto, 29.55% of the P. berghei (GFP+) and 37% of the yeast, and parasites in a disposable and fully closed cartridge system using the MACSQuant™ Tyto™ Sorter

Microchip-based sorting of high-risk material such as bacteria, yeast, and parasites in a disposable and fully closed cartridge system using the MACSQuant™ Tyto™ Sorter

Introduction

Figure 2A and B show the side view and bottom view of the cartridge, respectively. Another group of organisms labeled as high-risk material are parasites. A commonly known example of parasites is Plasmodium, which causes malaria. The human stages of Plasmodium parasites are the sporozoites, which infect liver cells, and infect red blood cells more merozoites are formed, leading to a continuous cycle of malaria symptoms. Isolation of sporozoites has been proven problematic on conventional droplet sorters due to the harsh conditions such as high-shear sorting pressure, the charge that is applied, and decomposition. Therefore, flow cytometric facilities have to conduct sorts of high-risk material on their conventional droplet-based systems due to the harshness. Yeast cells are often used to study the localization and growth allows isolation of DNA and vast amounts of proteins within hours. Yeast cells are often used in the isolation and function of proteins using fluorescent reporter genes.

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