High-purity enrichment of human hematopoietic stem cells utilizing MACS® Technology

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Introduction

Hematopoietic stem cells (HSC) have the ability for self-renewal and can differentiate into all cells of the hematopoietic system. These cells are of major interest in research as well as clinical settings. In fact, HSC are used for autologous and allogeneic transplantations in the treatment of hematologic diseases, immune defects, and various types of leukemia.
CD34 and CD133 are the most prominent and well-studied surface markers that are used for the isolation and characterization of these cells. Most commonly, HSC are isolated by immunomagnetic cell separation involving magnetic nano- or microparticles coated with an antibody directed against these surface markers. MACS® Technology involving superparamagnetic iron oxide nanoparticles (SPIONs), termed MicroBeads, in combination with unique ferromagnetic cell separation columns and separators is a powerful approach to enrich human CD34+ and CD133+ cells from diverse starting materials. The CliniMACS® CD34 Reagent System, which is based on MACS Technology, was approved by the U.S. Food and Drug Administration (FDA) for selecting hematopoietic stem cells from donor apheresis, while passively depleting T cells that can cause graft-versus-host disease (GVHD). *Ex vivo* T cell depletion can be a viable option to reduce GVHD incidences in patients with AML undergoing allogeneic stem cell transplantation.
Low abundance of HSC, especially in peripheral blood, usually renders the effective magnetic enrichment to high purity and viability very challenging. Here we introduce a novel type of SPIONs and a magnetic cell separation protocol that allow for the isolation of CD34+ and CD133+ HSC to highest purities and viabilities from various sources. This protocol results in stem cell populations containing significantly diminished amounts of dead cells and cell debris.

Results

1 Enrichment of CD34+ cells with the CD34 MicroBead Kit

Typically, a standard procedure for the enrichment of CD34+ cells from PBMC using CD34 MicroBeads results in purities of ≥95% in relation to white blood cells (termed below as "gated purity"), and 10–30% in relation to the total cells (termed below as "total purity"). A typical enrichment result is shown in figure 1. Flow cytometry analysis was performed using a MACSQuant® Instrument .

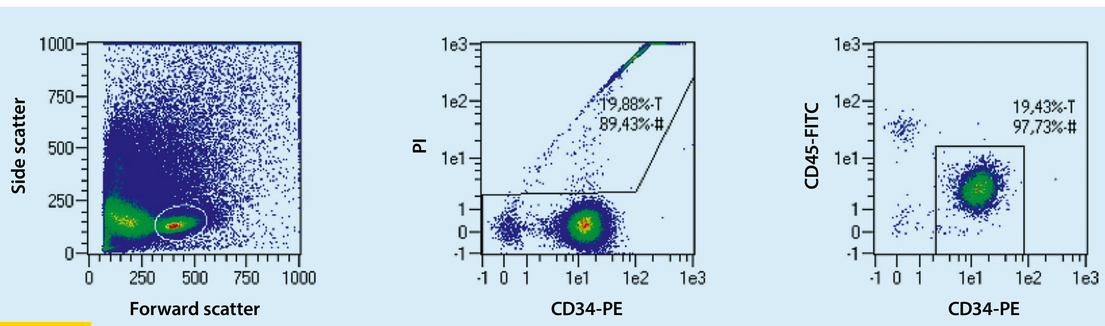

Figure 1

2 Isolation of HSC from PBMC using the CD34 MicroBead Kit UltraPure

In the PBMC the frequency of hematopoietic progenitors amounted to 0.05–0.2%. Using CD34 MicroBeads UltraPure, which are based on the novel type of SPIONs, for the isolation of CD34+ cells, we obtained a high target cell recovery and gated purity, similar to the traditional MicroBeads. However, the total purity of CD34+ cells increased from 19% to 66%. Moreover, CD34 MicroBeads UltraPure led to a pronounced reduction of non-specific events, as shown in the side scatter/forward scatter dot plots (fig. 2). Similar results were obtained for the enrichment of CD133+ stem cells (data not shown).

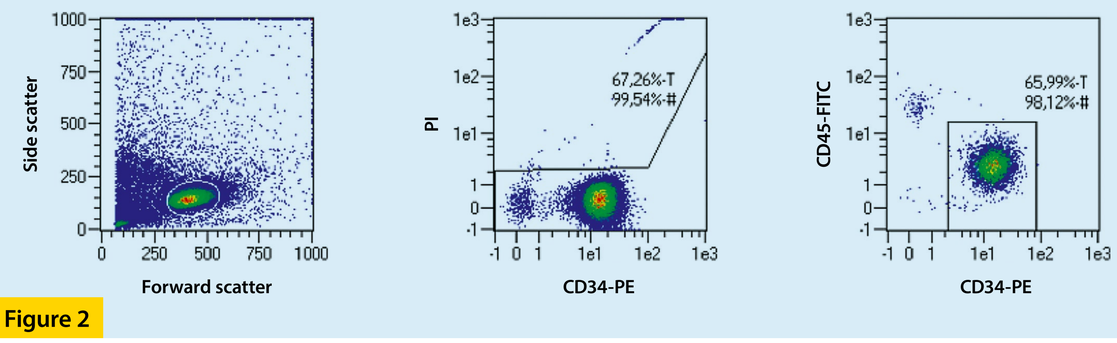

Figure 2

3 Isolation of HSC from PBMC in an automated system

We also tested CD34 MicroBeads UltraPure in an automated procedure, using the autoMACS® Pro Separator (fig. 3) for the isolation of CD34+ cells with results similar to the manual procedure (table 1). The magnetic cell separation was done using 1×10^8 PBMC and the autoMACS Pro Separator program Possel D.

Donor	Total purity (%)	Viability (%)	Gated purity (%)	Recovery (cell no.)
A	68.4	95.6	89.9	4.3×10^4
B	42.5	94.4	90.4	2.6×10^4
C	75.1	99.7	97.1	6.5×10^4
D	45.6	99.3	95.1	1.5×10^4

Table 1: Automated cell separation using CD34 MicroBeads UltraPure.

Figure 3

4 Isolation of HSC from cord blood– or bone marrow–derived MNC using the CD34 MicroBead Kit UltraPure

Next, we extended the study to the isolation of HSC from cord blood (CB)–derived mononuclear cells (MNC). The starting frequency of HSC in MNC amounted to 0.1–0.5%. Again, the total purity of CD34+ cells increased from 48% (using the CD34 MicroBead Kit) to 75% (using the CD34 MicroBead Kit UltraPure) for positive selection of CD34+ (fig. 4A). In further experiments, we found that there is an even greater benefit of using the CD34 MicroBead Kit UltraPure with MNC from frozen CB. This material generally contains significantly more cell debris than fresh CB. Similar results were obtained when using MNC from bone marrow as starting material (fig. 4B).

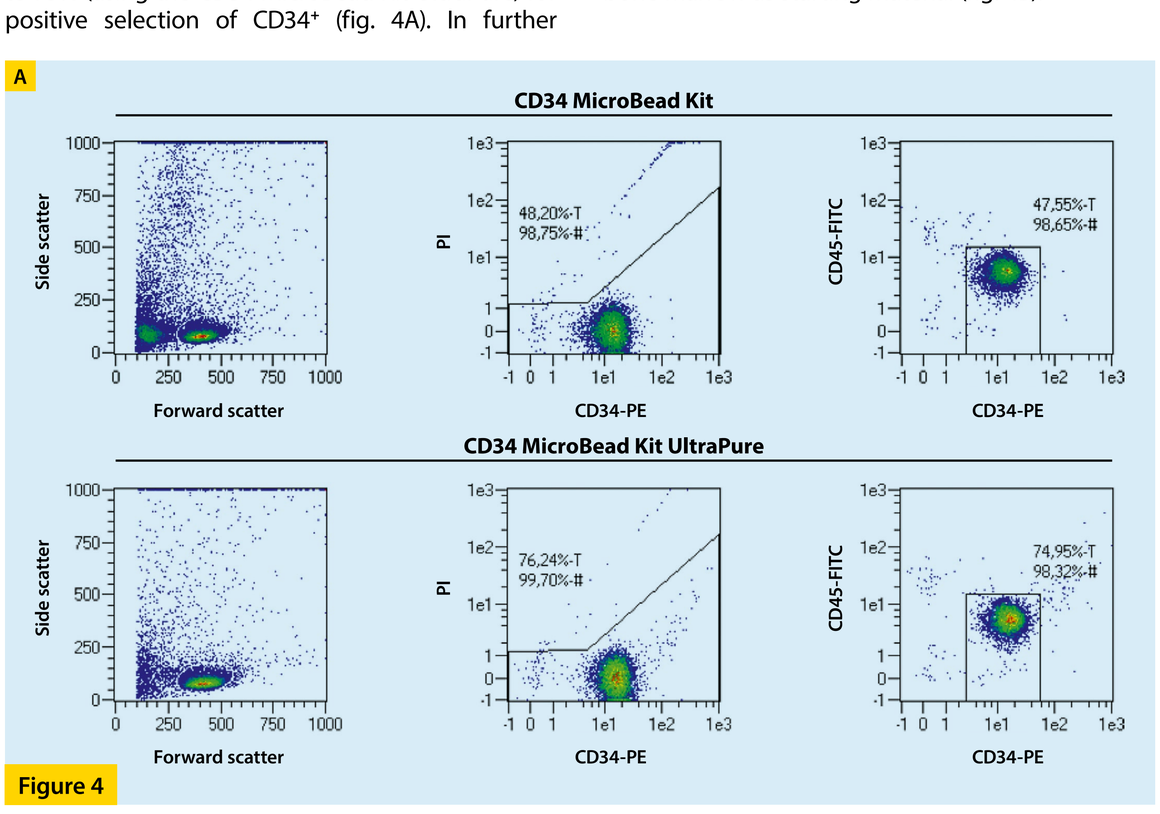

Figure 4

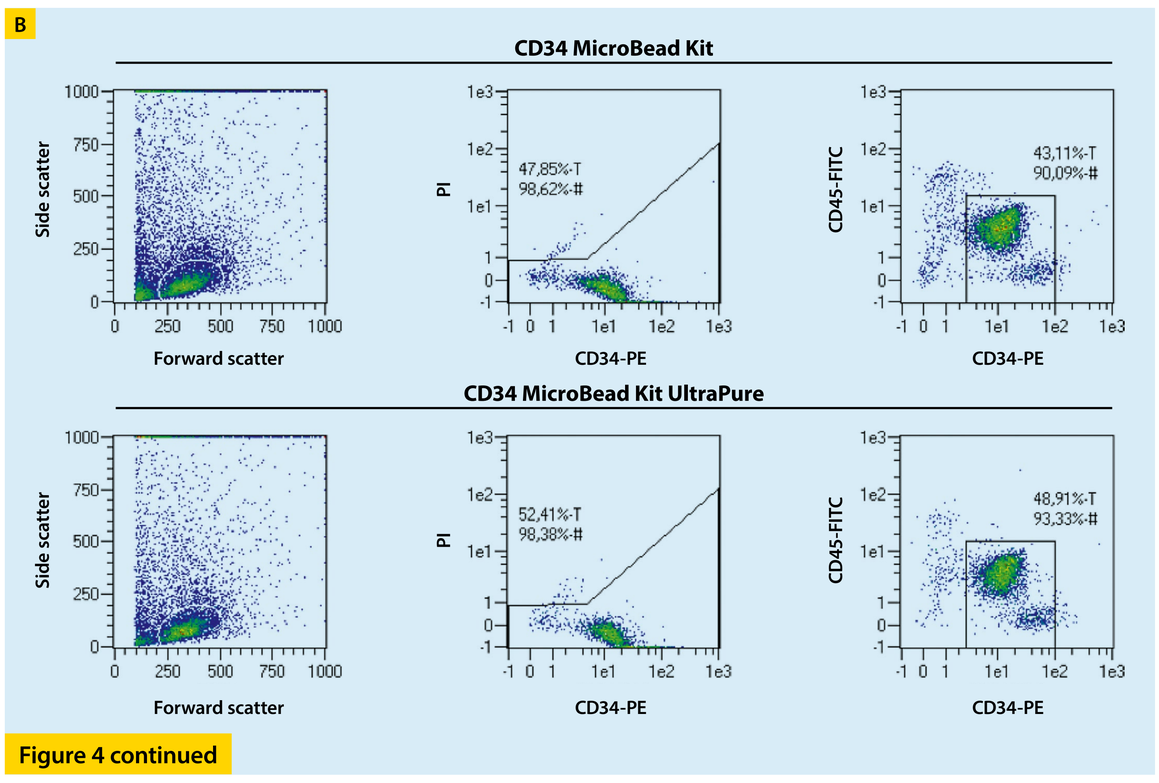

Figure 4 continued

5 Performance of the CD34 MicroBead Kit UltraPure compared to another commercially available immunomagnetic method

A direct comparison of the CD34 MicroBead Kit UltraPure vs. a commercially available immunomagnetic method involving a column-free positive selection system was done with PBMC. On average, the CD34 MicroBead Kit UltraPure led to a 50% increase in total purity compared to the other method. Both methods yielded similar results with regard to viability, gated purity, and recovery (table 2).
However, the column-free technology from another manufacturer compromised the quality of the enriched cells (fig. 5) and thus affected downstream applications such as NGS (fig. 6). Figure 5 shows that the column-free technology led to the co-enrichment of significant amounts of debris, in contrast to the CD34 MicroBead Kit UltraPure.
NGS analysis revealed improved results (higher read numbers) for cells isolated with the CD34 MicroBead Kit UltraPure (fig. 6). The enrichment of debris during column-free cell isolation negatively affected NGS results. Shown are the results of paired-end sequencing. The total number of reads is the sum of read counts of mate 1 and mate 2 (n = 3).

	Total purity (%)	Viability (%)	Gated purity (%)	Recovery (cell no.)
CD34 MicroBead Kit UltraPure	45.6	81.7	87.9	6.6×10^4
Other commercially available method	29.1	66.1	75.7	7.8×10^4

Table 2: Comparison data for the CD34 MicroBead Kit UltraPure. Data are means of four experiments.

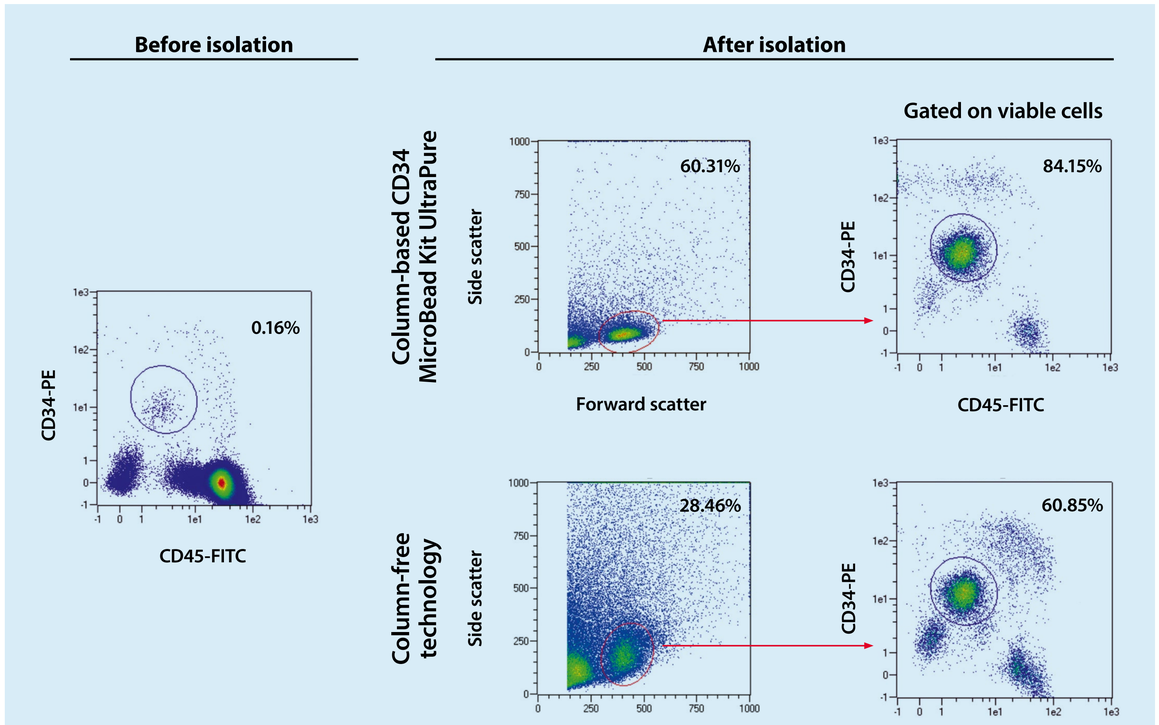

Figure 5

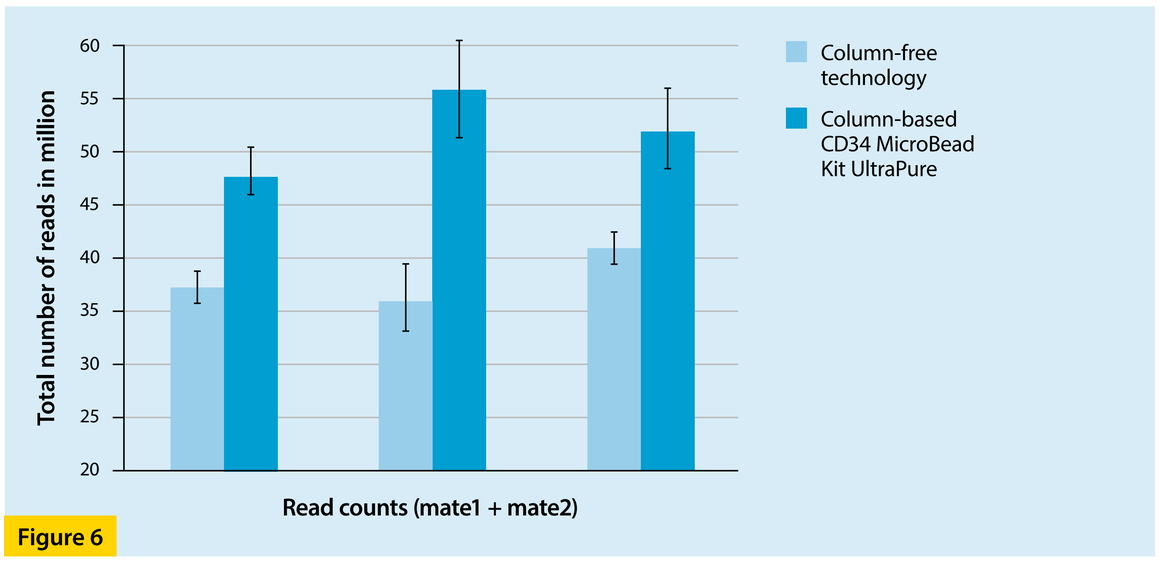

Figure 6

Conclusion

- Use of the CD34 MicroBead Kit UltraPure results in a strong reduction of the dead cell content in the enriched cell population.
- The CD34 MicroBead Kit UltraPure allows for the effective enrichment of HSC from various starting materials, including peripheral blood, CB (fresh and frozen), and bone marrow.
- The CD34 MicroBead Kit UltraPure also allows for automated magnetic cell separation at high purity and viability.
- The automated workflow allows a high level of standardization and reduces human errors, which is of particular importance when working with valuable and limited sample materials.