Highly pure plasma cells isolated by a fully automated process based on MACS® Technology yield more conclusive results in downstream assays

Frank Ralf Morrissey-Wettey[1], Karin Hartmann[2], Thomas Nebe[2], Daniela Vorholt[1], and Andrzej Dzionek[1]
[1] Miltenyi Biotec GmbH, Bergisch Gladbach, Germany [2] Hämatologielabor Mannheim, Mannheim, Germany

Introduction

Multiple myeloma (MM) is a plasma cell (PC) neoplasm originating in the bone marrow with concomitant secretion of monoclonal immunoglobulins. MM accounts for 15% of all hematological malignancies and almost 1% of all cancers worldwide, predominantly affecting the elderly. Despite important advances in the treatment, most patients become refractory and relapse with a 5-year overall survival rate of 50%. Since early therapeutic intervention benefits the treatment outcome, sensitive diagnostics are required.

Isolation of PCs from MM samples facilitates subsequent cellular, cytogenetic, or molecular routine analysis, especially when cells are present at low numbers.
Here we present an optimized process for the isolation of PCs from MM samples with MACSprep™ Multiple Myeloma CD138 MicroBeads, human in a fully automated manner using the autoMACS® Pro Separator and compared it to a process employing column-free magnetic isolation.

Methods

1 Isolation and analysis of CD138⁺ PCs from multiple myeloma samples

PCs were isolated from 1 mL of MM bone marrow aspirates using MACSprep Multiple Myeloma CD138 MicroBeads, human combined with the autoMACS Pro Separator from Miltenyi Biotec. For comparison, we used a column-free magnetic isolation method, also based on CD138, from another supplier.
The starting material as well as the resulting enriched and depleted fractions were analyzed by flow cytometry with a MACSQuant® Analyzer 10 using a panel of fluorochrome-conjugated antibodies from Miltenyi Biotec (CD138-APC, CD38-sFITC, CD235a (Glycophorin A)-PE-Vio® 700, CD45-VioGreen™, CD19-VioBlue®, CD28-/CD56-APC-Vio® 770, and propidium iodide. The enriched fractions were further analyzed by microscopy and/or standard fluorescent *in situ* hybridization (FISH) using IGH or XL 1p32/1q21 probes with staining of antibody light chains.

Results

1 Fully automated process for fast and easy PC isolation

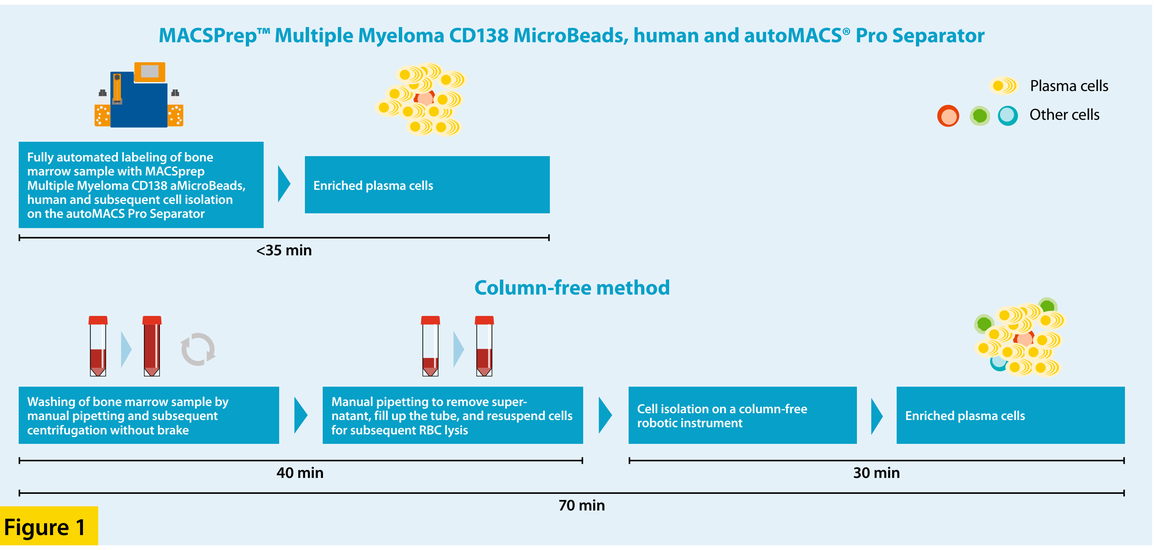

Figure 1

The autoMACS Pro Separator in combination with MACSprep Multiple Myeloma CD138 MicroBeads, human enables easy PC isolation with virtually no hands-on time. MM samples are simply placed in the autoMACS Pro Separator and the respective parameters are entered. In contrast, the automated column-free isolation process that we used for comparison requires two manual steps, namely i) preparation of the MM sample and ii) additional red blood cell (RBC) lysis, prior to loading of the robotic instrument. Depending on the number of samples, the autoMACS Pro Separator offers a processing time of <35 minutes compared to approximately 70 minutes with the column-free system. Figure 1 illustrates the workflow and the processing time required for one sample. The processing time of the autoMACS Pro Separator at full occupancy is comparable to the automated column-free process.

2 Gating strategy to define PCs by flow cytometry

After doublet exclusion (FSC-A/FSC-H), dead cell exclusion (PE/propidium iodide), and RBC definition (CD235a/FSC), cellular debris was excluded by scatter characteristics (FSC-A/SSC-A). Afterwards, erythroblasts were excluded in a CD45/CD138 dot plot. Finally, PCs were defined by coexpression of CD138 and CD38. Abnormal myeloma PCs were defined by down-regulation of CD19 and expression of CD56 (fig. 2; red population). The table shows the antibody panel designed for analysis of myeloma PCs.

Antigen/parameter	Fluorochrome
CD138	APC
CD38	sFITC
CD235a (Glycophorin A)	PE-Vio® 700
CD45	VioGreen™
CD19	VioBlue®
CD28/CD56	APC-Vio 770
Viability	Propidium iodide

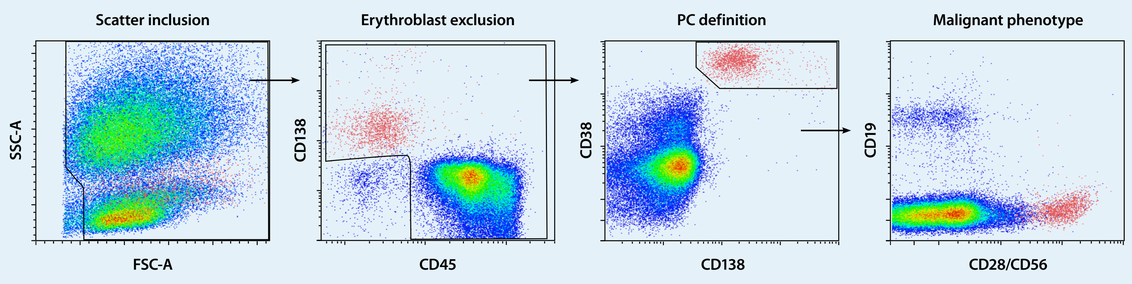

Figure 2

3 The autoMACS® Pro Separator yields higher PC purity and recovery compared to a column-free cell isolation method

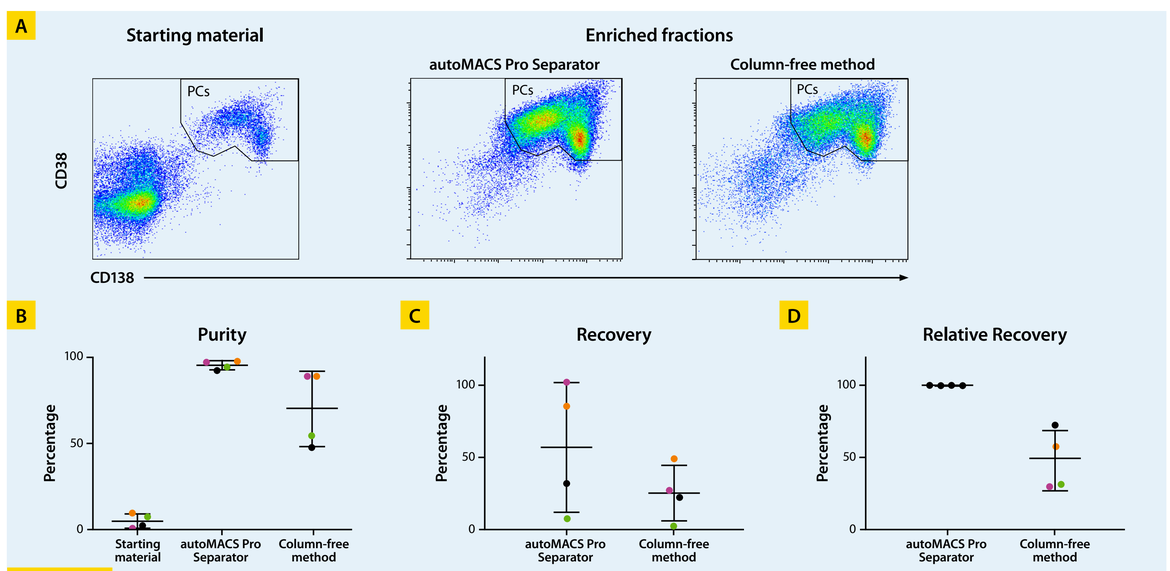

Figure 3

Analysis of separation performance was conducted by flow cytometry as depicted in figure 3A. PC isolation by the autoMACS® Pro Separator and MACSprep Multiple Myeloma CD138 MicroBeads, human resulted in a significantly higher purity of CD138⁺ PCs (fig. 3B) and a considerably better cell yield, i.e., recovery (fig. 3C), compared to the column-free method. In figure 3D recoveries are normalized to the results obtained with the autoMACS Pro Separator, which clearly shows that the autoMACS Pro Separator led to a higher cell yield in each experiment. Moreover, the autoMACS Pro Separator offers effective cell separation at both high and low CD138 expression levels as displayed in figure 3A, where two PC clones are visible (equal proportions of the fractions are shown for autoMACS Pro Separator and the column-free method).

4 PC enrichment by the autoMACS® Pro Separator results in higher PC:RBC ratios compared to a column-free method

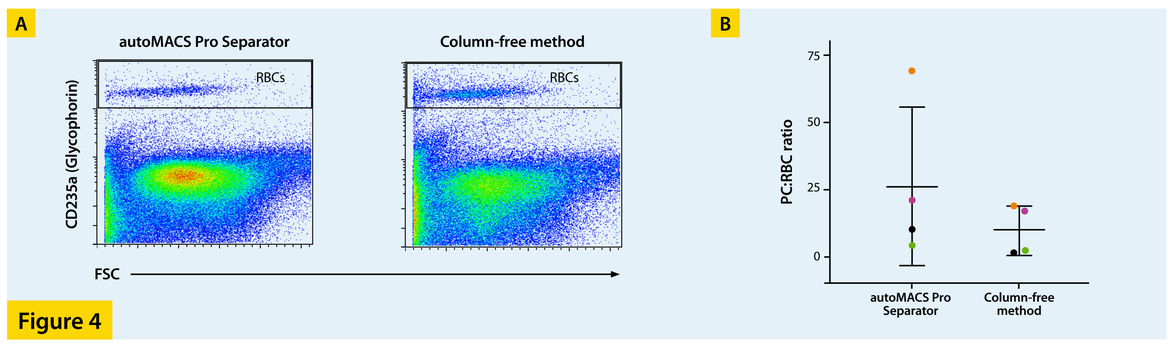

Figure 4

As depicted in figure 4A, the enriched PC fractions of both processes still contained erythrocytes. However, the ratio of PCs to RBCs was always higher in the PC fractions isolated by the autoMACS® Pro Separator compared to the column-free method (fig. 4B), despite the obligatory RBC lysis step included in the column-free process.

5 PCs isolated by the autoMACS® Pro Separator yield more conclusive results in downstream analyses

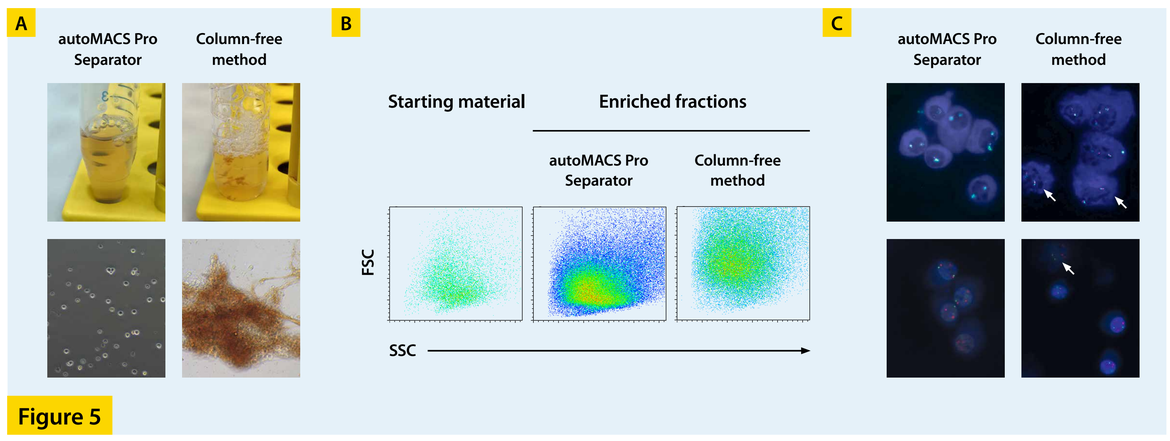

Figure 5

Macroscopic and microscopic inspection (fig. 5A) showed that the workflow based on the autoMACS® Pro Separator and MACSprep Multiple Myeloma CD138 MicroBeads, human yielded more single PCs and no aggregates compared to the column-free method. Scatter properties of PCs isolated with the autoMACS Pro Separator closely resembled the characteristics of PCs contained in the starting material. In contrast, cells isolated with the column-free method showed a different scatter pattern (fig. 5B; only PCs are shown as other cells were excluded from the analysis). The column-free process usually resulted in clearly visible aggregates and plasma cells with increased SSC values, possibly as a result of the obligatory RBC lysis. More importantly, the enriched fraction obtained with the autoMACS Pro Separator performed superior in downstream FISH analyses exhibiting a typical PC morphology and normal nuclear staining with clear probe signals (fig. 5C, left). In contrast, PCs from the column-free process often displayed abnormal nuclei (fig. 5C, upper right) and even apoptotic cells with fuzzy signals (fig. 5C, lower right). Additionally, staining artifacts, presumably due to considerable contamination with the large aggregates (fig. 5B), often complicated the analysis of samples obtained by the column-free method (not shown).

Conclusion

Miltenyi Biotec's automated PC isolation process is fast, with minimal hands-on time, and does not require any manual sample preparation. Without the need for RBC lysis it delivers pure PCs with high yield from myeloma samples. PC samples obtained by this workflow lead to more conclusive results in downstream assays like FISH. Thus the workflow facilitates the unambiguous analysis and study of myeloma PCs in research as well as in routine settings.