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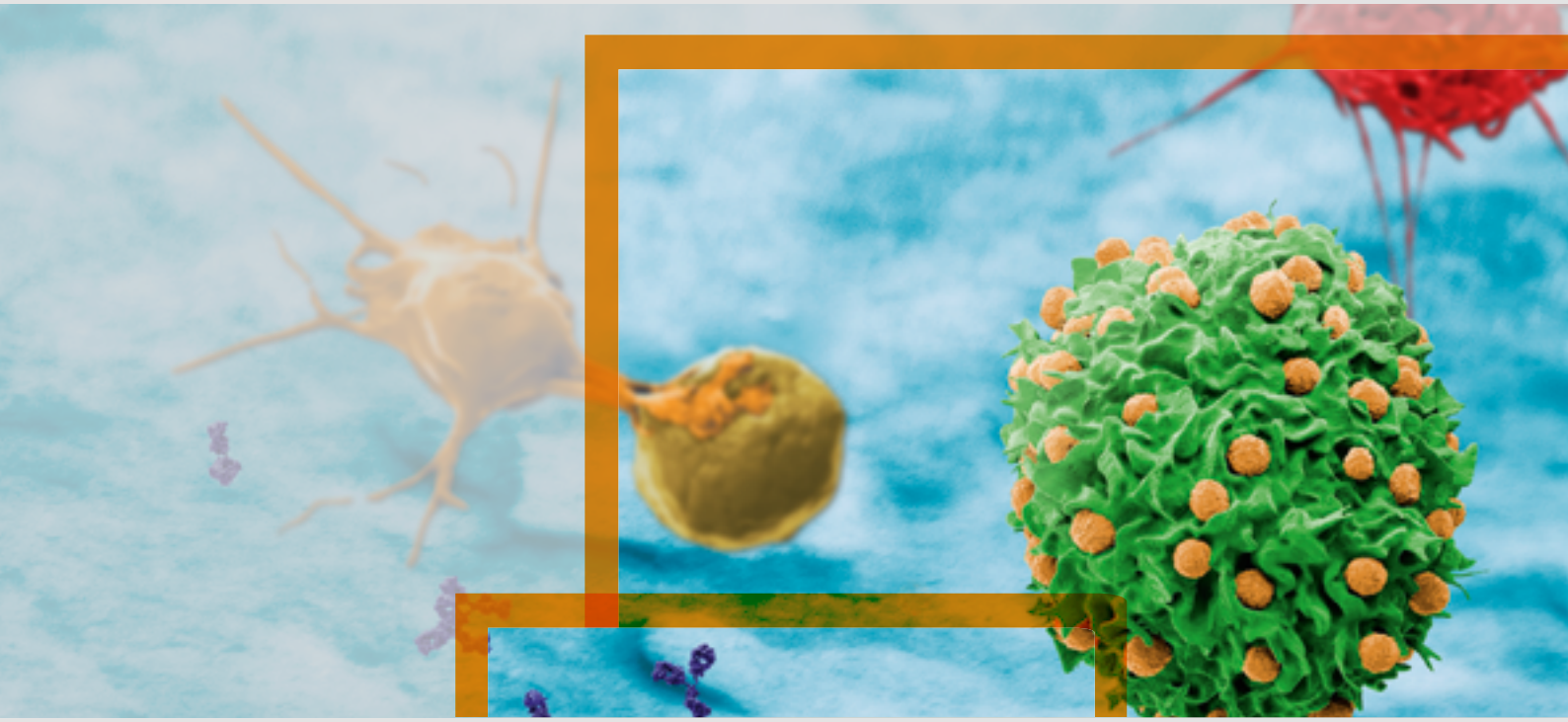

Miltenyi Biotec

An automated method for purification of CD138+ cells from whole bone marrow samples for multiple myeloma research

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Introduction

Multiple myeloma (MM), the second most common hematological malignancy, is a plasma cell (PC) disorder in the bone marrow (BM). MM is characterized by a large clinical heterogeneity despite the homogeneous morphological appearance of malignant PCs. Chromosomal aberrations are a hallmark of MM, and distinct genetic abnormalities characterize the major subtypes of the disease. As an addition to interphase fluorescence *in situ* hybridization (FISH), the more global assessment of the underlying cytogenetics by a genome-wide analysis of malignant PCs, using high-density, single-nucleotide polymorphism (SNP) arrays for molecular karyotyping, has significantly improved the detection and identification of genetic lesions.

FISH and/or molecular karyotyping experiments with unseparated BM samples have a 30 to 50% probability of showing false results or failure due to technical reasons. This is especially true for samples from BM aspirates that were affected by very low PC infiltration in the BM.

The CD138 antigen is a marker for PCs in BM. Purified CD138+ cells are a prerequisite to increasing the sensitivity of FISH analysis or SNP arrays. The quality of FISH and molecular karyotyping results depends on the degree of PC purity and DNA integrity after isolation.

Given the daily demands of laboratories there is a need for an automated platform for the preparation of pure CD138+ cell populations. Here we used StraightFrom® Whole Blood CD138 MicroBeads, human and the autoMACS® Pro Separator for the fast and reliable automated isolation of CD138+ PCs directly from whole BM samples for MM research, without density gradient centrifugation.

Materials and methods

Isolation of CD138+ cells

For the isolation of CD138+ cells directly from whole BM samples (1.5 to 3 mL), cells were magnetically labeled with StraightFrom Whole Blood CD138 MicroBeads (Miltenyi Biotec). Program "posselwb" in combination with the "Clean" process was used for separation of CD138+ cells on the autoMACS Pro Separator (Miltenyi Biotec) according to the manufacturer's protocol. Purities of isolated cell populations were determined by flow cytometry using CD138 and CD38 antibodies. The purity of DNA was analyzed using a NanoDrop™ Instrument (Thermo Fisher Scientific).

FISH analysis

After separation CD138+ PCs were hybridized with the LSI D13S25 (13q14.3) Single Color and/or the LSI IGH/FGFR3 Dual Color, Dual Fusion Translocation Probe (t(4;14)(p16;q32)), and/or the LSI P53 (17p13.1) Single Color Probe and analyzed by fluorescence microscopy.

Results

Isolation of CD138+ cells directly from BM samples

In a study of 100 MM samples from whole BM, we evaluated the efficiency and performance of separation, cell purity, and the quality of DNA after purification of CD138+ cells. Purities of CD138+ PCs after isolation with StraightFrom Whole Blood CD138 MicroBeads and the autoMACS Pro Separator amounted to 95% in average (table 1). For the vast majority of MM samples (95%) we obtained enough cells for the performance of the recommended panel of FISH analyses and genome-wide analysis.

Sample	Detectable PCs in bone marrow samples (%)	CD138+ PCs following isolation (%)
1	2	86.3
2	1	88.9
3	0	91.8
4	5	93.0
5	5	93.5
6	16	93.7
7	11	95.5
8	24	95.5
9	25	97.3
10	30	97.5

Table 1 Frequency of CD138+ PCs prior to and after enrichment from BM samples using StraightFrom Whole Blood CD138 MicroBeads and the autoMACS Pro Separator. PC purity was determined by flow cytometry using CD138 and CD38 antibodies.

FISH analysis of CD138$^+$ cells

CD138$^+$ cell enrichment prior to FISH analysis more than doubled the detection rate of abnormalities (83% vs. 40% in unseparated cells), and frequencies of abnormalities reached significant levels. Figure 1 shows examples of FISH analyses of isolated CD138$^+$ cells, comparing normal cells (left) with malignant cells (right). The normal cell in figure 1A shows two orange signals representing the two alleles of the D13S319 locus. The malignant cell shows only one orange signal, due to a deletion affecting locus D13S319 or monosomy of chromosome 13.

The normal cell in figure 1B shows two orange (FGFR3) and two green (IgH) signals. The malignant cell (fig. 1B, right) shows an abnormal signal pattern with one orange (FGFR), one green (IGH), and three fusion signals, resulting from the chromosomal translocation (t(4;14) FGFR3/IgH).

NanoDrop™ Analysis of DNA extracted from isolated CD138$^+$ PCs revealed high purity (data not shown).

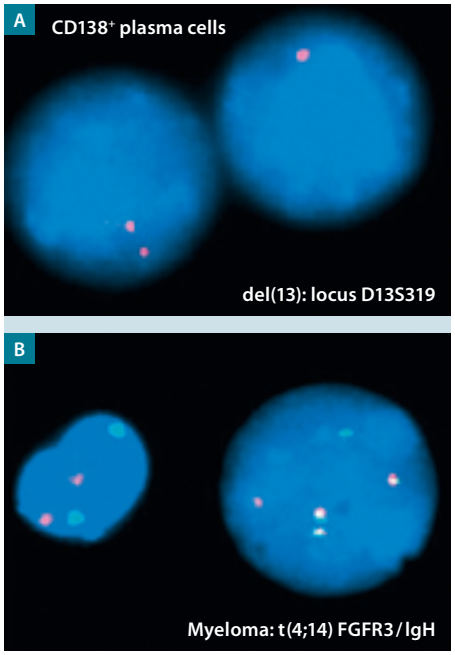

Figure 1 FISH analysis of CD138$^+$ cells. PCs were isolated from whole BM using StraightFrom Whole Blood CD138 MicroBeads. Isolated cells were subjected to FISH analysis with (A) LSI D13S25 Single Color Probe, or (B) LSI IGH/FGFR3 Dual Color, Dual Fusion Translocation Probe. In both pictures a normal cell is shown on the left and a malignant, mutated cell on the right.

Conclusion

As a routine MM research laboratory, we receive numerous BM samples every day. Isolation of CD138$^+$ cells from these samples is necessary to increase the sensitivity of downstream assays, such as FISH analysis or molecular karyotyping by SNP arrays. Since 2007, we have performed more than 6,000 PC isolations. This large number necessitates a reliable, rapid, and standardized method, allowing us to isolate CD138$^+$ cells from multiple samples in a convenient way, while maintaining sample integrity. StraightFrom® Whole Blood CD138 MicroBeads in combination with the autoMACS Pro Separator meet all of our lab's requirements. The autoMACS Pro Separator allows the standardization of cell separation processes and ensures a rapid handling of MM samples.

- StraightFrom Whole Blood CD138 MicroBeads enable fast isolation of CD138$^+$ cells directly from BM samples, thus minimizing hands-on time and maximizing the yield of target cells.
- No sample preparation is required, such as density gradient centrifugation or red blood cell lysis.
- Purified CD138$^+$ cells can be immediately subjected to FISH or molecular analyses.
- The detection rate of chromosomal abnormalities per sample in MM and PC dyscrasia significantly improves when analysis is performed on purified populations of CD138$^+$ PCs.
- The platform allows the generation of reproducible and consistent results – even in multi-user settings.

MACS Product*	Order no.
autoMACS Pro Separator – Starter Kit	130-092-545
StraightFrom Whole Blood CD138 MicroBeads	130-093-062
CD138-PE	130-081-301
CD38-APC	130-092-261
CD19-FITC	130-091-328

* Products are for research use only.

Dr. Mossafa, Laboratoire Cerba:

"As a routine multiple myeloma research laboratory, we receive numerous bone marrow samples every day. We must isolate CD138$^+$ cells from these samples to increase the sensitivity of downstream assays such as FISH analysis and whole genome arrays. For this reason, I really required a fast, reliable, standardized method, which allows me to process multiple samples in a convenient way, while maintaining sample integrity.

After testing different technologies, we decided to use Whole Blood CD138 MicroBeads in combination with the autoMACS Pro Separator. From October 2007 to July 2011 we have separated more than five thousand specimens with the autoMACS Pro Separator, as this technology meets all of the requirements of our lab."