Sorting of MSCs with CD271 and MSCA-1 antibodies in a closed-cartridge system using the MACSQuant® Tyto® Sorter

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Introduction

Mesenchymal stem cells (MSCs) have raised great expectations in a number of clinical trials studying the regeneration of bone, cartilage, and heart or autoimmune diseases including GvHD, Crohn's disease, and diabetes. MSCs are an extremely rare cell type in cord blood, adipose tissue, and bone marrow (BM, 0.01%). Several groups worked on the identification of MSCs, using markers such as CD271, Anti-MSCA-1 (TNAP), CD73, and STRO-1.
Recent technological advances like the MACSQuant® Tyto®, a benchtop microfluidic flow sorter, enable the purification of cells in a fully closed, sterile cartridge, which completely eliminates the risk of external contaminations and sample-to-sample carryover. Here, we demonstrate the possibility to sort CD271$^+$MSCA-1 (TNAP)$^+$ MSCs from human BM samples using the MACSQuant Tyto. The clonogenic potential was compared between the sorted MSC fraction, CD271$^-$ cells, and MSCs obtained by plastic adherence (PA-MSCs). Furthermore, we analyzed the expansion potential with and without sorting. Sorted and PA-MSCs were analyzed regarding their potential to suppress T cell proliferation. The gentle cell sorting process on the MACSQuant Tyto resulted in a highly pure population of viable and functional CD271$^+$MSCA-1$^+$ MSCs.

Results

1 Sorting of CD271$^+$MSCA-1$^+$ MSCs

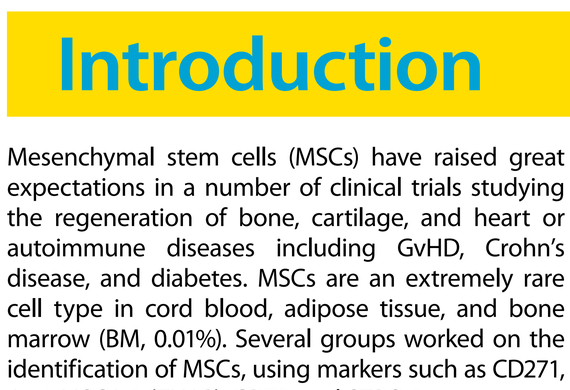

Figure 1A

Density gradient centrifugation (DGC) was performed with 50–100 mL human BM in a closed system using the automated CliniMACS Prodigy®. Subsequently, bone marrow mononuclear cells (BM-MNCs) were stained with CD271-PE and Anti-MSCA-1-APC and sorted for MSCs on the MACSQuant® Tyto® (fig. 1A) using a single-use cartridge in a two-step sorting approach. In total, a maximum of 5×10^8 BM-MNCs including $1–8\times10^4$ MSCs were isolated. Depending on the volume, the sort took 3–3.5 h. Samples before and after sorting were analyzed via flow cytometry using the MACSQuant® Analyzer 10 (fig. 1B). The purity of target cells increased from 0.03% (±0.02%) before sorting to 91% (±3%) after sorting (fig. 1C).

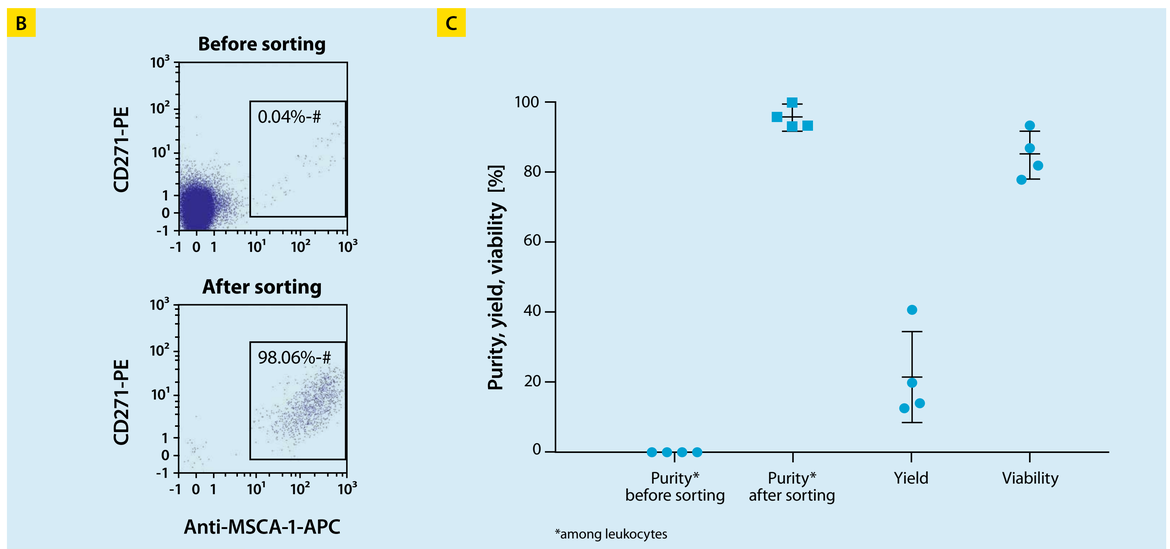

Figure 1 (continued)

2 Colony-forming unit fibroblast (CFU-F) assay

The clonogenic potential of the sorted CD271$^+$ MSCA-1$^+$ cell fraction (Sorted MSCs) was compared to CD271$^-$ cells and MSCs obtained by plastic adherence (PA; PA-MSCs). Cells were Giemsa-stained after 10 days of culture in MSC-Brew GMP Medium (fig. 2B) and colony-forming unit fibroblast (CFU-F) numbers were determined (n=3). The CFU-F numbers increased by more than three orders of magnitude when CD271$^+$MSCA-1$^+$ MSCs were sorted as compared to PA (fig. 2A). A significant depletion of clonogenic potential was detected in the CD271$^-$ cell fraction (fig. 2A).

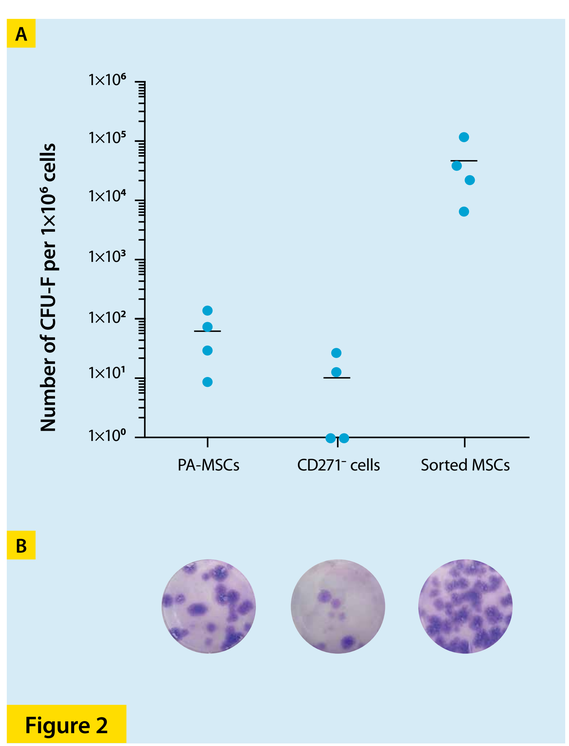

Figure 2

3 Expansion of MSCs isolated by flow sorting or plastic adherence

Sorted MSCs and PA-MSCs were cultivated in MSC-Brew GMP Medium in order to assess their growth curves over 33 days of expansion (fig. 3A). Cell quality after expansion was investigated by flow cytometry MSC marker expression analysis using the MSC Phenotyping Kit (Miltenyi Biotec). Sorted MSCs as well as PA-MSCs met ISCT criteria showing high expression levels of CD105, CD90, and CD73, while CD14, CD20, CD34, and CD45 (Non-MSCs) was low (fig. 3B, example for sorted MSCs). Morphology during MSC expansion was as expected (not shown).

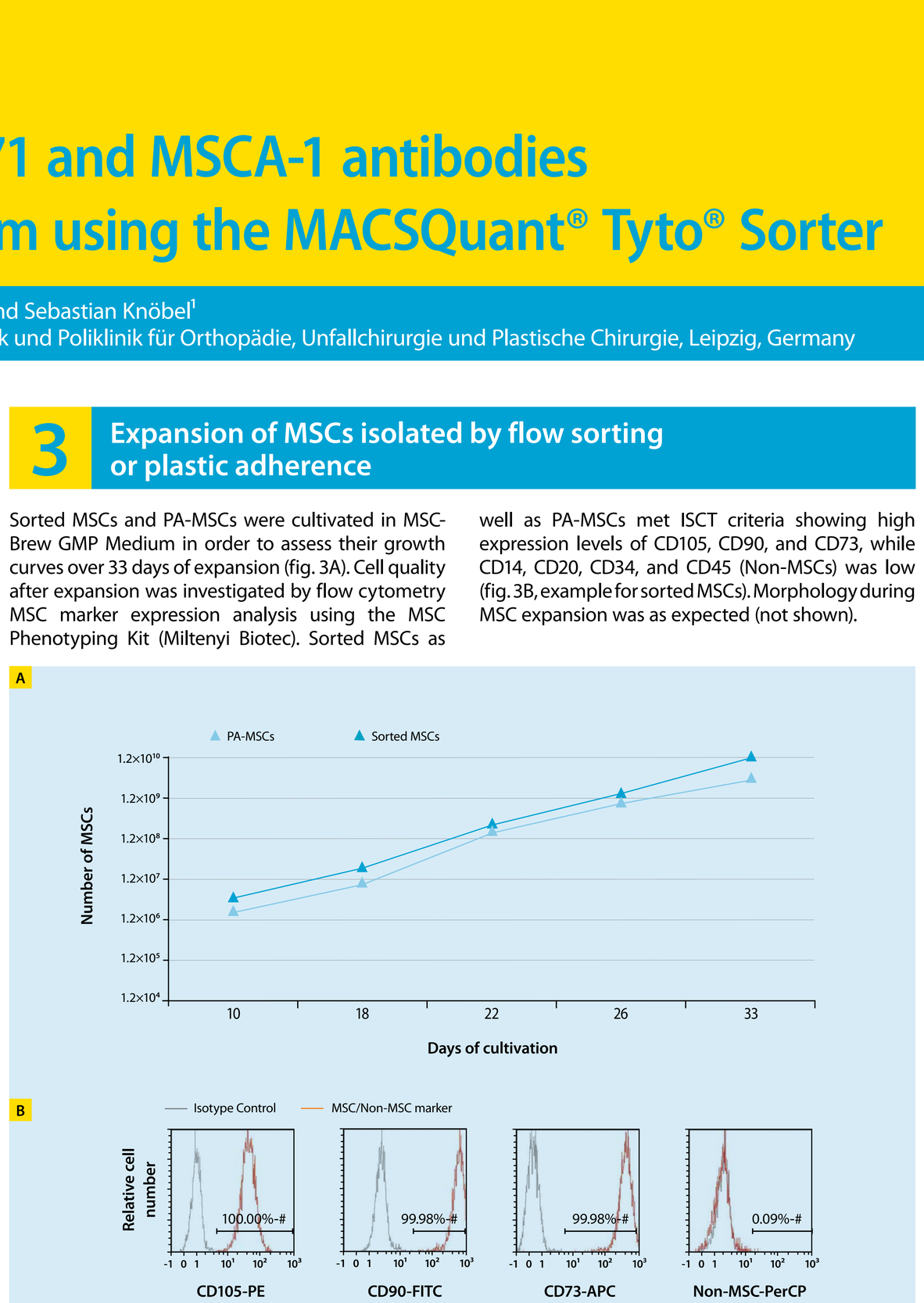

Figure 3

4 T cell suppression assay

T cell-suppressive potential of sorted MSCs and PA-MSCs after expansion (P3) was analyzed by flow cytometry. CD4$^+$CD25$^-$ T cells were labeled with CellTrace™ Dye to monitor T cell division after stimulation with CD2, CD3, and CD28 antibody–loaded particles (MSC Suppression Inspector, Miltenyi Biotec). T cells were co-cultured with MSCs in different ratios. T cell-suppressive potential was observed for all conditions (fig. 4).

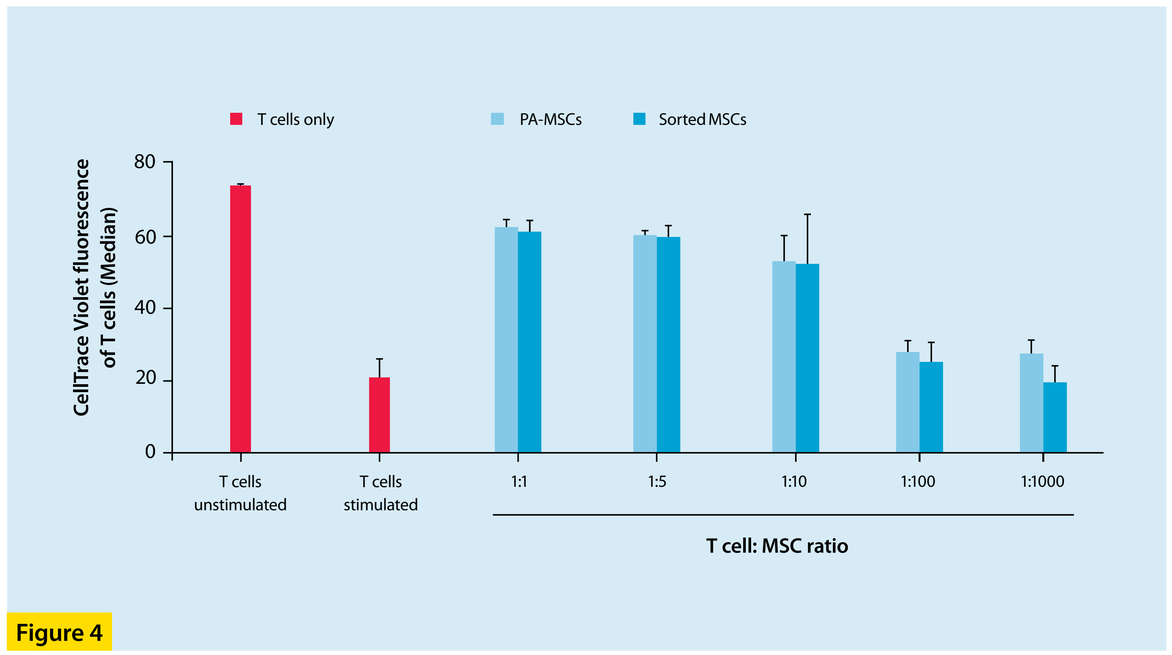

Figure 4

Conclusion

We have shown the potential of the MACSQuant® Tyto® Sorter for the isolation of the rare CD271$^+$ MSCA-1$^+$ MSC population from human BM-MNCs. The gentle sorting conditions and the sterile, fully closed system of the MACSQuant Tyto Cartridge provides an optimal basis for subsequent expansion of the isolated cells. Sorted and expanded CD271$^+$MSCA-1$^+$ MSCs are viable and functional, as shown by their high clonogenic and T cell–suppressive potential. Furthermore, multi-parameter cell sorting with the MACSQuant Tyto might also enable the sorting of MSC subpopulations.