Clinical-scale isolation of mesenchymal stromal cells from bone marrow with the CliniMACS® System and CD271 antibody–conjugated MicroBeads.

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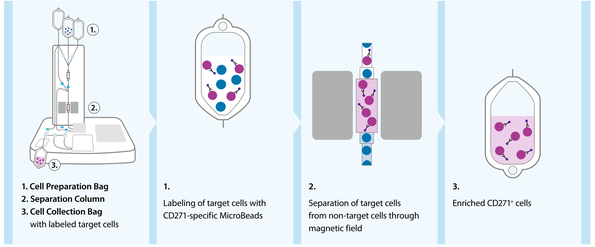
Introduction

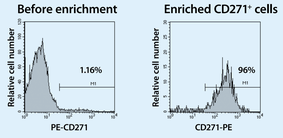
Mesenchymal stromal cells (MSCs) have shown their potential for cell therapy in various clinical trials, for example, for the treatment of graft-versus-host disease and tissue regeneration. Studies of several groups have indicated that the clonogenic potential of CD271$^+$ cells magnetically enriched from bone marrow mononuclear cells (BM-MNCs) was found to be about 100-fold higher compared to the potential found for cells isolated by plastic adherence (PA).[1] The expansion rate of enriched CD271$^+$ cells is one to three orders of magnitude higher than the rate of cells isolated by PA.[2] Expanded CD271$^+$ cells express MSC markers, such as CD73, CD90, and CD105 and retain their multilineage differentiation potential, giving rise to adipocytes, osteoblasts, and chondrocytes. In this study we show the isolation of CD271$^+$ cells from human bone marrow (BM) using the CliniMACS Instrument, an automated cell separation system based on MACS® Technology, which enables the operator to perform clinical-scale magnetic enrichment of target cells in a closed and sterile system.

Results

1 Enrichment of CD271$^+$ cells from BM with the CliniMACS System (n=4)

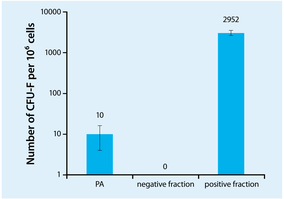
50 mL human BM was harvested and CD271$^+$ cells were purified by using CD271 antibody conjugated MicroBeads and the CliniMACS® Plus Instrument. BM samples before and after enrichment were labeled with CD271-PE (Miltenyi Biotec). PI immunofluorescence and light scatter signals were used to gate live cells. CD271$^+$ cells were enriched at purities of about 96% (median 93% ± 6.0%, n=4) and recoveries of about 94% (median 87%, n=2).





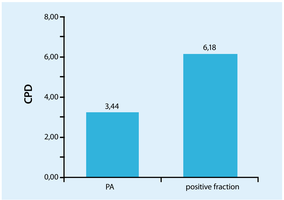
2 Colony-forming unit fibroblast (CFU-F) assay (n=3)

The clonogenic potential of the CD271$^+$ cell fraction was compared to CD271$^-$ cells and MSCs obtained by plastic adherence (PA). Cells were Giemsa-stained after 14 days of culture and CFU-F numbers were determined (median 2952 ± 431, n=3). The numbers of CFU-F increased by more than 200-fold when MSCs were isolated according to CD271 expression as compared to PA. No CFU-Fs were detected in the CD271$^-$ cell fraction.



3 Expansion of MSCs isolated by magnetic enrichment or plastic adherance

Cells isolated by plastic adherence (PA) and the CD271$^+$ cell fraction isolated by magnetic enrichment were cultivated with NH Expansion Medium (Miltenyi Biotec) in order to assess their proliferative capacity after 41 days of expansion. The population doubling (PD*) and cumulative population doubling (CPD**) was determined using the equation shown below. CD271$^+$ cells show a 50% increased CPD level in comparison to MSCs isolated by PA.



* PD for each subcultivation = $(\log_{10}(N_H) - \log_{10}(N_I))/\log_{10}(2)$
N_I = number of inoculated cells; N_H = number of harvested cells
** CPD for 41 days of cultivation: Σ(PD)

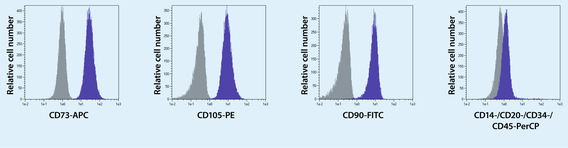
4 Phenotyping of *in vitro* expanded MSCs

After 41 days of culture in NH Expansion Medium, MSCs were trypsinized and stained by using the MSC Phenotyping Kit (Miltenyi Biotec) containing a staining cocktail (■) and an isotype cocktail (■). Expanded MSCs were CD73$^+$, CD90$^+$, CD105$^+$ and CD14$^-$, CD20$^-$, CD34$^-$, CD45$^-$ independent of whether MSCs were isolated by PA or according to CD271 expression (fig. 4A). The histograms (fig. 4B) exemplify marker expression of CD271$^+$ MSCs.

A

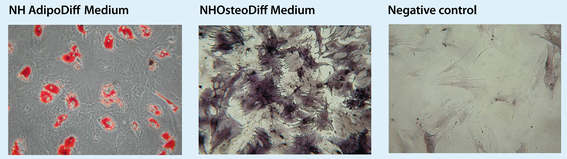
	CD73	CD90	CD105	CD14	CD20	CD34	CD45
CD271$^+$ MSCs	+	+	+	–	–	–	–
PA-MSCs	+	+	+	–	–	–	–

B



5 Differentiation potential of CD271$^+$ MSCs isolated by magnetic enrichment

Isolated CD271$^+$ cells were analyzed regarding their multipotent differentiation potential. After 10 days of cultivation in NH OsteoDiff Medium (Miltenyi Biotec) all MSCs showed a high alkaline phosphatase (AP) activity. Detection of AP activity was performed using SigmaFast BCIP/NBT (Sigma-Aldrich) as a substrate. After 18 days of cultivation in NH AdipoDiff Medium (Miltenyi Biotec), all MSCs showed an increased accumulation of intracellular lipid vacuoles, as revealed by Oil red O staining. Negative controls showed no differentiation.

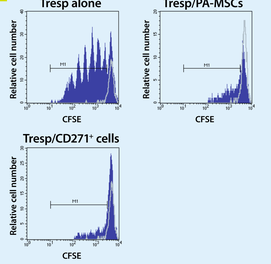


6 Immunosuppression of T cell proliferation by CD271$^+$ cells before and after cultivation

Human MSCs isolated by PA were expanded for 2 passages (P2) in NH Expansion Medium. These MSCs are further referred to as PA-MSCs. 1×10^5 CD4$^+$CD25$^-$ T responder (Tresp) cells were either co-cultured with 1×10^5 CD271$^+$ cells directly after magnetic isolation or with 1×10^5 PA-MSCs. Tresp cells were isolated from buffy coat by immunomagnetic isolation of CD4$^+$ cells and depletion of CD25$^+$ Treg cells. CD4$^+$CD25$^-$ Tresp cells were labeled with carboxyfluorescein succinimidyl ester (CFSE). Tresp cells were stimulated with Treg Suppression Inspector (Miltenyi Biotec), which contains Anti-Biotin MACSiBead™ Particles preloaded with biotinylated CD2, CD3, and CD28 antibodies. Cells were harvested on day 5 and the percentage of proliferating Tresp was measured as CFSE dye dilution (M1) analyzed by flow cytometry (fig. 6A). Data are presented as percentage of proliferating responder cells cultured in the presence of PA-MSCs or CD271$^+$ cells with respect to responder cells cultured alone (100%) (fig. 6B).

Suppression of T cell proliferation by isolated CD271$^+$ cells prior cultivation is as robust as by expanded MSCs.

A



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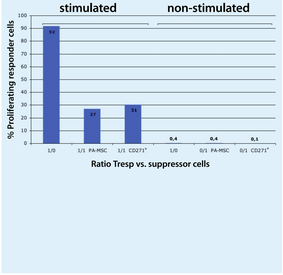

Conclusion

We developed a protocol for the isolation of CD271$^+$ cells in a closed and sterile system with high purity and recovery. With their increased expansion potential CD271$^+$ cells represent an optimal homogenous starting population for a time-effective clinical-scale MSC expansion.

References
1. Quirici *et al.* (2002) Exp. Hematol. 30: 783–791.
2. Poloni *et al.* (2009) Cytotherapy 11: 153–162.