GMP-compliant expansion of mesenchymal stromal cells (MSCs) using the CliniMACS Prodigy® and xeno-free MSC-Brew GMP Medium

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Introduction

Human mesenchymal stem cells (MSCs) hold great promise for clinical use and cell therapy applications. To ensure highest quality and safety of the resulting cellular products, MSCs have to be maintained using standardized cultivation conditions and procedures. To this end, we have developed our xeno-free MSC-Brew GMP Medium following the recommendations of USP <1043> on ancillary materials, thus enabling isolation and expansion of MSCs from various tissue sources (e.g. human bone marrow, adipose tissue, and umbilical cord). To increase the level of process standardization and product safety we developed a procedure using the integrated cell processing platform CliniMACS Prodigy® for i) density gradient centrifugation (DGC) of human bone marrow, ii) cultivation of MSCs starting from bone marrow mononuclear cells (BM-MNCs), and iii) passaging of MSCs.

MSCs from human bone marrow were isolated by plastic adherence using MSC-Brew GMP Medium and automated feeding and harvesting procedures in a closed, single-use tubing set. Subsequently, these cells could be replated and expanded within the closed system, illustrating the feasibility of an automated cell production.

Methods

1 Concept for clinical-scale MSC manufacturing using the CliniMACS Prodigy®

The CliniMACS Prodigy® provides a range of ports for connecting bags containing buffer, media, and reagents. Various tubing sets allow for a multitude of applications. For the cultivation process, we chose the tubing set CliniMACS Prodigy TS 730, which provides up to eight connections for buffer and media and the possibility to pre-warm solutions during transfer from an external 4 °C storage compartment to the cultivation and centrifugation unit (CCU) as well as external tissue culture (TC) vessels which are connected to the tubing set (fig. 1A). Bags can be connected in a sterile manner prior to the installation procedure or later via sterile welding. The MSC-Brew GMP Medium is provided in a bag with a PVC tube for reliable, sterile welding to the tubing set.

Bone marrow aspirate is transferred into the application bag (Bag 1) of the tubing set in a sterile manner. The following steps are performed automatically by the CliniMACS Prodigy using the Adherent Cell Culture Process_Beta_0.4. These steps comprise the density gradient centrifugation (DGC) of human bone marrow aspirate with the possibility of taking samples to determine the cell number. Furthermore, an initial expansion step was performed using external tissue culture (TC) vessels, which are connected in a sterile manner to the tubing set and are placed in an incubator next to the CliniMACS Prodigy. All liquid handling steps, including washing the CCU, inoculation, medium exchange, and cell harvest can be performed automatically, except potential handling of the external culture vessel (fig. 1B).

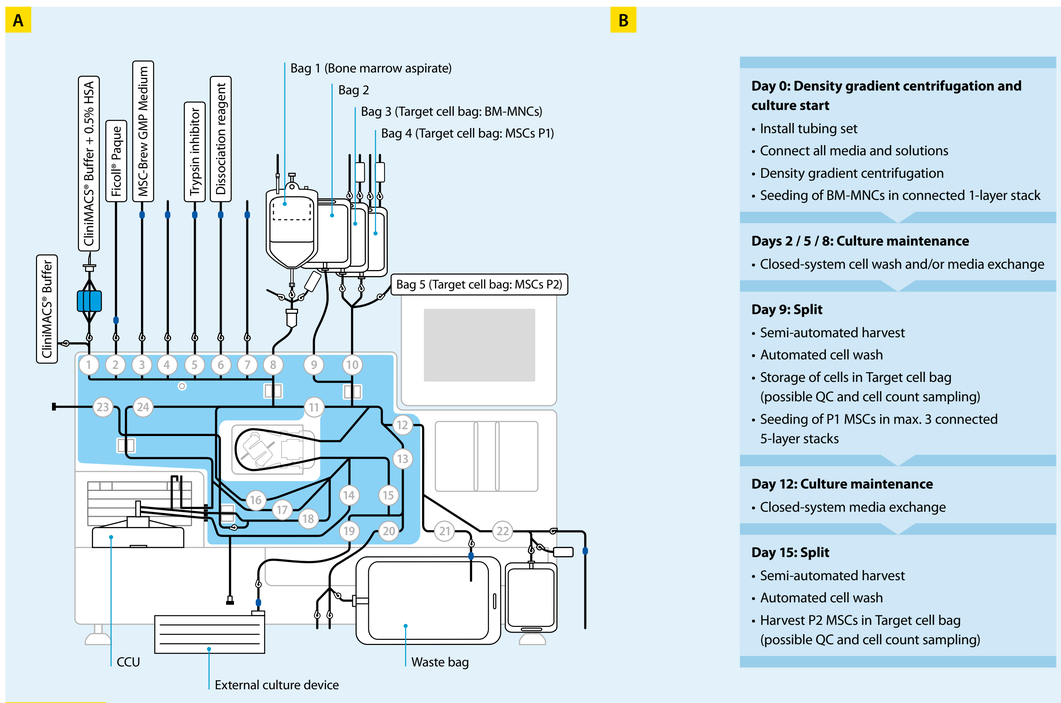

Figure 1

Results

1 Density gradient centrifugation using the CliniMACS Prodigy®

Density gradient centrifugation (DGC) was performed with four human bone marrow samples (30–100 mL) using the CliniMACS Prodigy® Adherent Cell Culture Process. The whole process including installation and priming of the tubing set TS 730 took ~2 hours. Lymphocytes and monocytes were quantified via flow cytometry using CD45, CD14, and side scatter as well as propidium iodide detection for analysis of cell viability. BM-MNCs revealed a viability of 98.2% (±1.9%) (results not shown). MSCs were quantified using a flow-based quantification kit (MSC Enumeration Kit, Miltenyi Biotec) based on a $CD271^+MSCA\text{-}1^+CD45^-$ gating strategy (figure 2A). A median of 84.5% (±23%) MSCs were recovered after DGC (figure 2B). Just 0.3% (±0.15%) of all erythrocytes and 2% (±1.4%) of all platelets reside within the BM-MNC fraction (figure 2C).

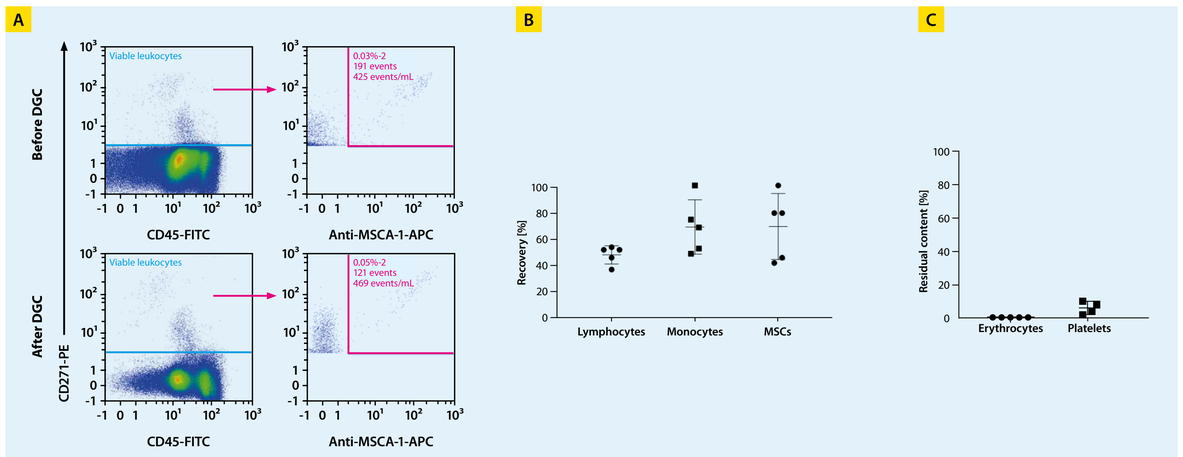

Figure 2

2 Expansion of MSCs using the CliniMACS Prodigy®

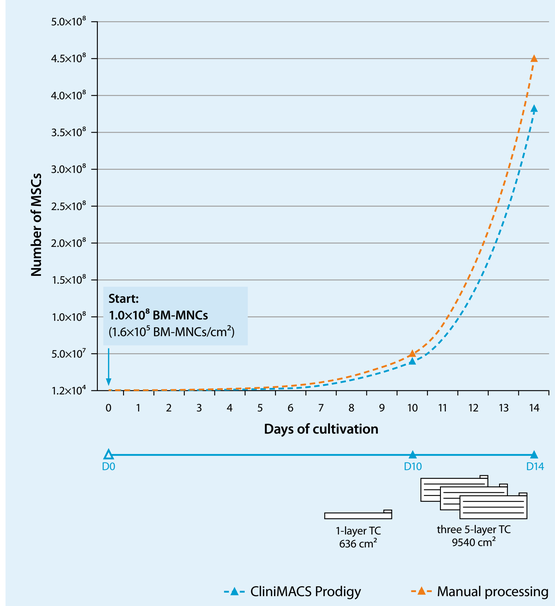

Figure 3

Human MSCs were isolated by plastic adherence from BM-MNCs using the CliniMACS Prodigy® and compared to the manual laboratory standard (T175 flask). A clinically relevant number of MSCs was harvested after 14 days in the CliniMACS Prodigy using MSC-Brew GMP Medium (fig. 3). In this experiment we used 30 mL of bone marrow aspirate. We performed an automated DGC as described in result 1 and continued the Adherent Cell Culture Process_Beta_0.4 with the cultivation of BM-MNCs for isolation of MSCs. Subsequently, all steps were performed within the closed system of the CliniMACS Prodigy. These steps comprised: i) an initial expansion step starting with a density of 1.6×10^5 BM-MNCs/cm² using a 1-layer stack, ii) media exchange and washing of cells, iii) semi-automated harvesting of P1 MSCs from the 1-layer stack and transfer to three 5-layer stacks connected to the tubing set in a sterile manner, and iv) semi-automated harvesting of P2 MSCs from three 5-layer stacks. In comparison, all steps were performed manually using standard T175 cell culture vessels.

3 Characterization of MSCs

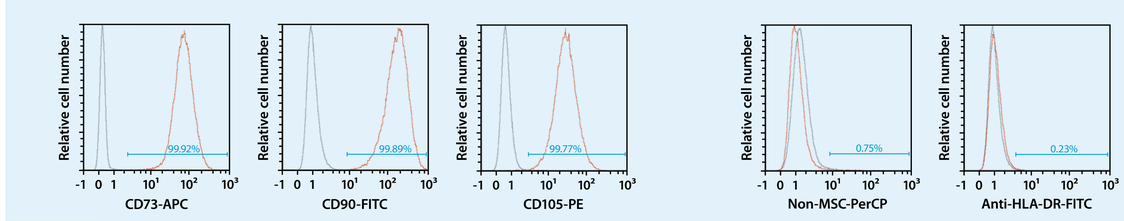

Figure 4

To confirm the quality of the cells processed with the CliniMACS Prodigy®, MSC marker expression was analyzed using flow cytometry. MSCs met ISCT criteria showing high expression levels of CD73, CD90, and CD105 while CD14, CD20, CD34, CD45 (Non-MSCs) as well as HLA-DR expression was low (fig. 4). Morphology during MSC expansion was as expected (not shown).

Conclusion and outlook

We have developed a method for DGC preparation of bone marrow and adherent, closed-system cultivation of MSCs using the CliniMACS Prodigy® Adherent Cell Culture Process and the CliniMACS Prodigy. Clinically relevant numbers of MSCs were expanded, starting from a human bone marrow sample. MSC-Brew GMP Medium is xeno-free and meets the recommendations of USP <1043> on ancillary materials.

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