Efficient GMP-compliant expansion of mesenchymal stromal cells (MSCs) from umbilical cord, bone marrow, and adipose tissue using a closed cultivation system

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Introduction

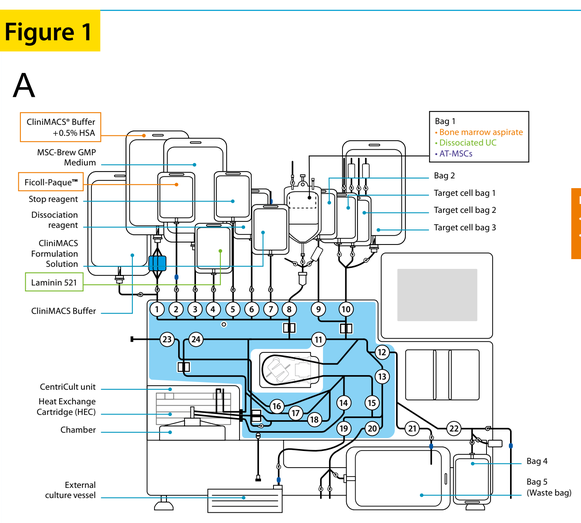
Human mesenchymal stromal cells (MSCs) hold great promise for clinical use and cell therapy applications and can be isolated from multiple tissue, e.g., bone marrow (BM), umbilical cord (UC), and adipose tissue (AT). To ensure highest quality and safety of the resulting cellular products, MSCs have to be maintained using standardized cultivation conditions and procedures. To this end, we have developed the 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 for use in clinical research.
To increase the level of process standardization and product safety we developed the CliniMACS Prodigy® Adherent Cell Culture System for automated and GMP-compliant isolation and cultivation of adherent cells using a closed single-use tubing set.

Methods

1 Manufacturing of MSCs using the CliniMACS Prodigy® Adherent Cell Culture System

The CliniMACS Prodigy® provides a range of ports for connecting bags containing buffer, media, reagents, and cellular material. Various tubing sets allow for a multitude of applications. For this cultivation process, we chose the tubing set CliniMACS Prodigy TS 730, which provides up to eight connections for bags. This setup also offers the option 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 vessels (ECVs) which are connected to the tubing set and are placed in an incubator next to the CliniMACS Prodigy (fig. 1A). Bags can be connected in a sterile manner prior to the installation procedure or later via sterile welding during the manufacturing process. Cellular starting material (BM aspirate, dissociated UC or adipose tissue-MSCs (AT-MSCs)) is provided in the application bag (bag 1) of the tubing set. Density gradient centrifugation (DGC) of BM aspirate is performed automatically by the system, while the preparation of tissues like UC and AT is performed outside of the system. After tissue preparation all following steps are performed semi-automatically by the CliniMACS Prodigy Adherent Cell Culture System, including the initial expansion step in the CCU or an ECV as well as all liquid handling steps, i.e., inoculation, washing of cells, medium exchange, and cell harvest (fig. 1B). Moreover, the system offers the possibility of taking samples to determine the cell number and analyze marker expression.

Figure 1



Results

Different human starting materials were used and processed to isolate and expand MSCs using the CliniMACS Prodigy® Adherent Cell Culture System. I) BM aspirate was processed directly within the closed system of the CliniMACS Prodigy by performing a DGC to isolate BM-mononuclear cells (BM-MNCs), which were then further cultivated to isolate MSCs by plastic adherence. II) UC was dissociated using the gentleMACS™ Dissociator together with the Umbilical Cord Dissociation Kit to obtain single cells. These cells were then transferred to the CliniMACS Prodigy and seeded for the isolation of MSCs. Here, the surface of the CCU was coated with Laminin 521. III) AT-MSCs, which can be obtained from the stromal vascular fraction (SVF) isolated from lipoaspirate by, e.g., enzymatic digestion, were pre-expanded prior to transfer to the CliniMACS Prodigy. Subsequently, all steps were performed within the closed system of the CliniMACS Prodigy for cultivation of cells.
For comparison, isolation and expansion were performed manually using standard tissue culture vessels. Proliferation data showed comparable results across all tissues (fig. 2A). Furthermore, cells showed a typical MSC morphology (fig. 2B).

1 Expansion of MSCs using the CliniMACS Prodigy® Adherent Cell Culture System

Figure 2

2 Characterization of MSCs

To confirm the quality of the cells processed with the CliniMACS Prodigy, MSC marker expression was analyzed using flow cytometry. MSCs met ISCT criteria, since the cells showed high expression levels of CD73, CD90, and CD105, while CD14, CD20, CD34, CD45 (Non-MSC), and HLA-DR expression levels were low[1] (tab. 1).

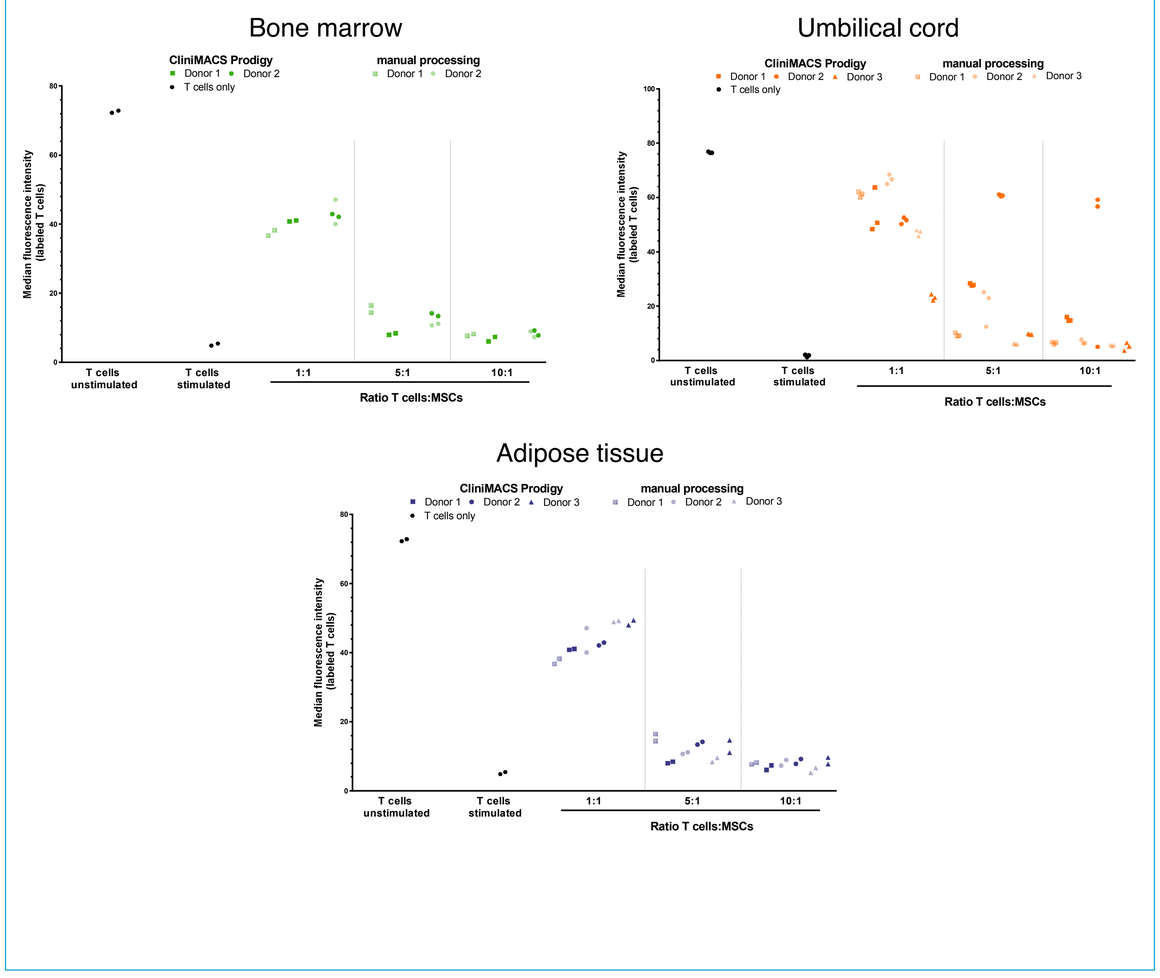
Table 1

	Bone marrow	Umbilical cord	Adipose tissue
Marker	**Positive markers (ISCT Guideline >95 %)**		
CD73	99.71±0.47	99.89±0.06	99.86±0.07
CD90	99.78±0.36	99.45±0.20	99.53±0.24
CD105	99.70±0.40	99.78±0.06	99.44±0.42
	Negative markers (ISCT Guideline <2 %)		
Non-MSC (CD14, CD20, CD34, CD45)	0.64±0.05	1.33±0.34	1.19±05
HLA-DR	0.22±0.01	0.42±0.17	0.34±0.20

3 Suppression of T cell proliferation

T cell–suppression potential of MSCs was analyzed using flow cytometry. To this end, $CD4^+CD25^-$T cells were isolated from whole blood by MACS® Cell Separation Technology and labeled with a cell tracking dye to monitor T cell division after stimulation with particles loaded with CD2, CD3, and CD28 antibodies. T cells were cocultured with MSCs in different ratios and showed a T cell–suppressive potential at all conditions (fig. 3).

Figure 3



Conclusion

- The novel CliniMACS Prodigy® Adherent Cell Culture System enables semi-automated cultivation of adherent cells in a closed system.
- MSCs from three different tissue types (bone marrow, umbilical cord, and adipose tissue) were expanded within this system. Automated expansion by the CliniMACS Prodigy Adherent Cell Culture System and manual expansion using standard tissue culture vessels led to comparable results.
- MSCs were expanded for at least two passages, which was sufficient to harvest clinically relevant numbers in up to 18 days. This time frame included the automated processing of bone marrow aspirate using a density gradient centrifugation within the system.
- The MSC-Brew GMP Medium used in this process is xeno-free and meets the recommendations of USP <1043> on ancillary materials.

References
[1]Dominici, M. *et al.*(2006) Cytotherapy 8: 315–317.

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