GMP compliant generation and expansion of iPS derived MSCs and their extracellular vesicles in a closed cultivation system using xeno-free MSC-Brew GMP Medium

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Introduction

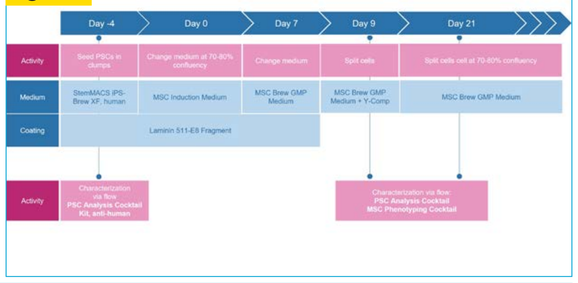
Human mesenchymal stem cells (MSCs) hold great promise for clinical use and cell therapy applications and can be isolated from multiple tissue, e.g. bone marrow (BM), umbilical cord (UC) or adipose tissue (AT). To overcome limitations in proliferation and the risk of senescence induced pluripotent stem cells (iPSC) have become a promising source of MSCs. Recent studies of extracellular vesicles (EVs) derived from MSCs have indicated high potential in clinical applications for many diseases. This brings also iPS-derived MSC (iMSC) EVs into the focus of preclinical research. To increase the level of process standardization and product safety we developed the CliniMACS Prodigy® Adherent Cell Culture System for GMP compliant cultivation of adherent cells using a closed single used tubing set. Here we show that iMSCs can be generated and expanded using the named CliniMACS Prodigy System. Stable iMSC expansion was achieved by using the xeno-free MSC-Brew GMP Medium. After iMSCs reached 80-90% confluency cell culture medium was changed to produce iMSC-EVs. Resulting iMSCs displayed MSC specific characteristic as defined by the ISCT consortium. iMSC-EVs were characterized using the MACSPlex EV MSC Kit, human.

Method

1 Generation and expansion of PSC derived MSCs

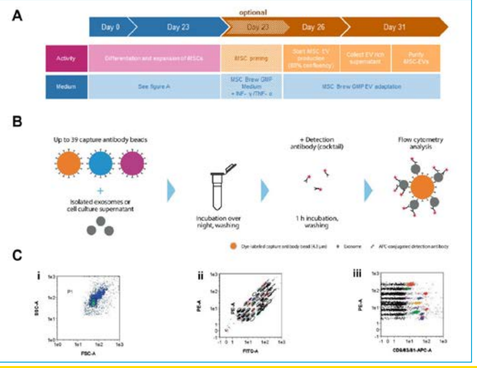
MSCs were generated as described in figure 1. In detail PSCs were cultivated in StemMACS iPS-Brew XF medium. When reaching 70-80% confluency, medium was exchanged to MSC Induction Medium (Prototype) modified from Grosh *et al*[1]. After seven days medium was changed to MSC-Brew GMP Medium for further two days. Cells were harvested at day nine and reseeded using MSC-Brew GMP Medium without coating. iMSCs were subsequently passaged when reaching 70-80% confluency in MSC-Brew GMP medium without Rock Inhibitor. Pluripotency of the starting PSCs was analysed using an antibody panel based flow assay. Characterization of iMSCs was performed according to ISCT guidelines[2] using the MSC Phenotyping Kit.

Figure 1



2 Characterization of iMSC-EVs using multiplex bead–based assay

Figure 2



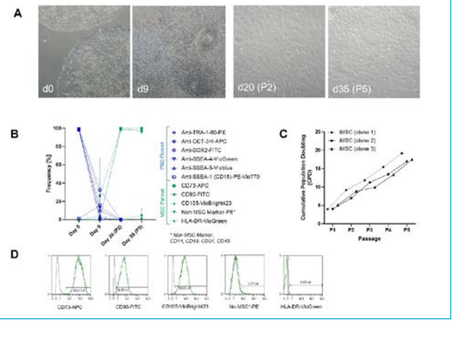
iMSCs were generated as described in figure 1. For EV production iMSCs at passage 3 reaching 80% confluency were washed with CliniMACS® PBS/EDTA Buffer and EV free, adapted MSC Brew GMP medium, including MSC-Brew GMP Basal Medium + MSC-Brew Supplement I was added. MSCs were further cultivated at 37°C and 5% CO_2 for up to five days. EV rich supernatant was harvested. For production of primed iMSC-EVs IFN-γ 1200 IU/mL and TNF-α 1000 IU/m were added to the medium for three days (fig 2A). Non-primed and primed iMSC-EVs were purified via ultracentrifugation. Purified iMSC-EVs were analyzed for diameter and quantity using a myriad videodrop analysis system. To characterize surface protein composition, the MACSPlex EV Kit MSC was used. (fig. 2B and C).

Result

1 PSC derived MSCs show long term proliferation

Four iPSC lines, originated from human fibroblasts using the StemMACS™ iPSC mRNA Reprogramming Kit, were differentiated into MSCs. Pluripotent state was validated via embryonic stem cell (ESC) morphology (fig 3A, day0) as well as detecting the expression of key pluripotency markers (fig 3B, day0). Induction of MSC differentiation until day 9 resulted in morphological changes as well as downregulation of pluripotency markers. After splitting cells into MSC-Brew GMP Medium without coating a stable MSC like phenotype was established via revealing a fibroblastoid morphology. Furthermore upregulation of MSC specific markers like CD73, CD90, CD105 as well as lacking hematopoietic markers was detected via flow cytometry from passage two on (Fig. 3B). iMSC expansion for all four lines was continued until passage five revealing a stable proliferation rate (CPD) (fig 3C).

Figure 3



2 Comparison of iMSC-EVs after priming with IFN-γ/TNF-α using multiplex bead-based assay

The capacity of iMSCs for priming using IFN-γ/TNF-α culture conditions was revealed by a strong intracellular expression of IDO (Indolamine 3,5-dioxygenase) (fig. 4A i), a very important enhancer for the immunosuppressive effect of MSCs. iMSC-EVs were collected with and without previous priming conditions and EV products revealed a very comparable diameter. The yield of EVs was slightly increased for primed iMSC-EVs (fig 4A ii). Characterization of iMSC-EVs via multiplex bead-based assay using the MACSPlex EV Kit MSC enables fast and reliable screening of up to 39 markers within just one sample (fig 4B). Primed and unprimed iMSC EVs showed expression of EV specific markers CD9, CD63 and CD81. As expected, the MSC specific markers CD73, CD90 and CD105 were detected while CD14, CD19, CD34, CD45 are not, in agreement with ISCT guidelines. Noteworthy, the CD90 signal was quite low on EVs, while being much higher expressed on corresponding iMSCs (fig. 3D). CD54 as well as HLA-ABC were higher expressed after priming and were already known to be upregulated after MSC stimulation with IFN-γ/TNF-α. CD54 is involved in interaction with pro-inflammatory macrophages and increases the immunosuppressive function of MSC[3]. This might also hold true for primed iMSC-EVs.

Figure 4A

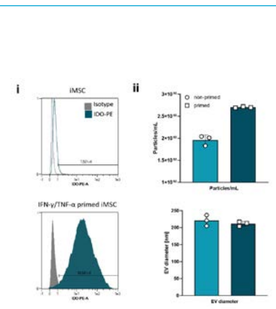
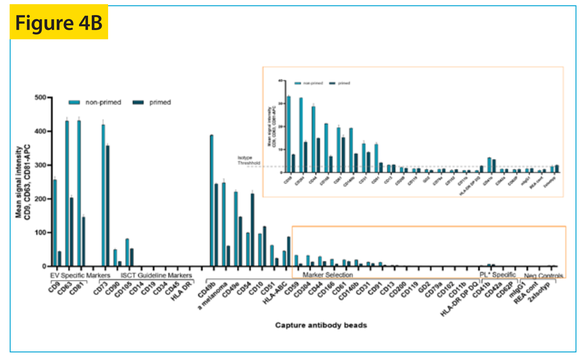


Figure 4B



3 Large scale differentiation and expansion of iMSCs using the CliniMACS Prodigy Adherent Cell Culture System

Figure 5A

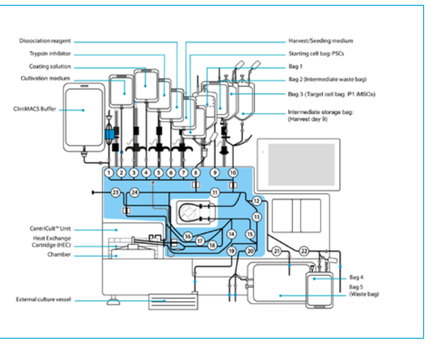
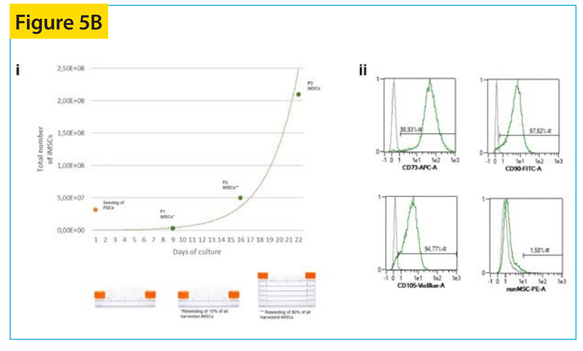


Figure 5B



Clone 1 was differentiated and expanded in the closed and semi-automated CliniMACS Prodigy® Adherent Cell Culture System connecting Cell Stacks (external cell culture vessel = ECV) via sterile connection to the CliniMACS Prodigy tubing set TS 730. All reagents used for the iMSC differentiation and expansion process (fig. 1) were sterile connected to the CliniMACS Prodigy tubing set as depicted in figure 5A. iMSCs were harvest at passage three and cytometry-based quality control was applied. Immunophenotyping revealed that iMSCs expressed MSC specific markers (fig. 5B i). Starting with about 3xE07 iPSCs resulted in about 2xE08 iMSCs after three passages, whereas only a small amount of iMSCs was reseeded per passage (fig. 5B ii).

Conclusion

- iPSC derived MSCs were successfully differentiated as well as further expanded using MSC-Brew GMP Medium.
- iMSCs were primed under IFN-γ/TNF-α conditions and revealed high production of IDO, an immunosuppressive factor.
- EVs resulting from primed and un-primed iMSCs were characterized using the flow based MACSPlex EV MSC Kit.
- The MACSPlex EV MSC Kit is a fast and reliable way to analyze up to 39 markers to characterize the surface protein composition of MSC specific EVs using flow cytometry.
- Production of iMSCs was successfully performed in large scale using the closed CliniMACS Prodigy® Adherent Cell Culture System.

Reference: 1. Grosh, BMC Biology 2020 2. Dominici, Cytotherapy 2006 3. Espagnolle, StemCell Reports, 2017