Improved clinical-scale MSC isolation from primary tissue and expansion with a GMP matrix- and xeno-free medium

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Introduction

The need for xeno-free and GMP-compliant production of clinical-scale mesenchymal stem cells (MSCs) is rising. A number of clinical Phase II/III trials are registered. In this context, the use of fetal calf serum (FCS) is undesirable with regard to adverse events and lot-to-lot variations. StemMACS™ MSC Expansion Media Kit XF (StemMACS MSC XF) supports the isolation and expansion of MSCs from various tissues. The medium is xeno-free and can be used without additional coating of cell culture vessels. We transferred this formulation into a GMP version (MSC-Brew GMP), according to recommendations of USP<1043> on ancillary materials. In this study, we characterized MSCs isolated and further expanded from three human bone marrow (BM) samples. We compared a preliminary lot of MSC-Brew GMP Medium with StemMACS MSC XF and other commercially available xeno-free MSC media (medium A and B) as well as FCS- and platelet lysate (PL)-containing formulations.

Results

1 MSC-Brew GMP supports colony formation

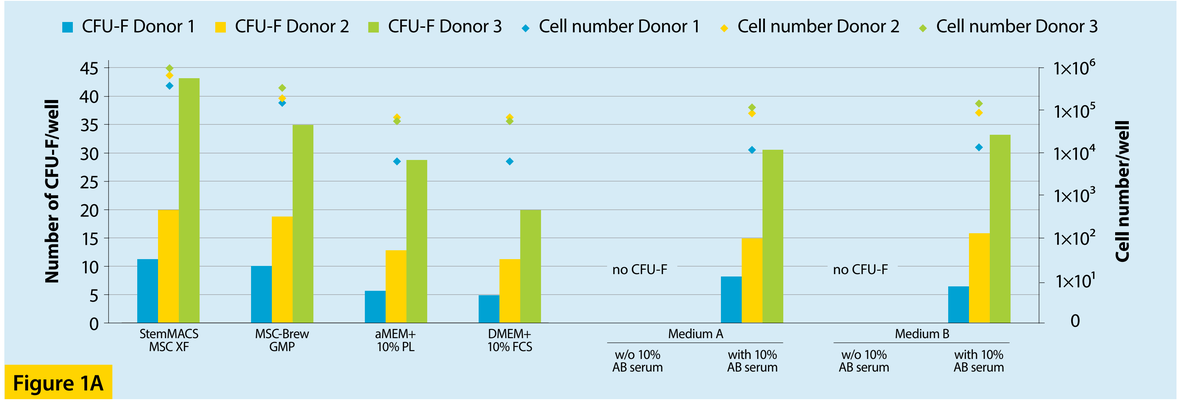

Figure 1A

First we analyzed the clonogenic potential of human bone marrow mononuclear cells (BM MNCs, three donors) using all six media. We assessed cell numbers of p0 cultures as well as CFU-F counts after 9 days (n = 3) (A). The number of CFU-F was higher for BM MNCs from all donors when cells were cultured in StemMACS MSC XF or MSC-Brew GMP compared to standard media containing PL or FCS. Medium A and B, without addition of 10% AB serum, neither supported attachment of MSCs nor growth of CFU-F (A). Initial expansion potential of MSCs per CFU-F was higher for StemMACS MSC XF or MSC-Brew GMP (B) than for all other media tested.

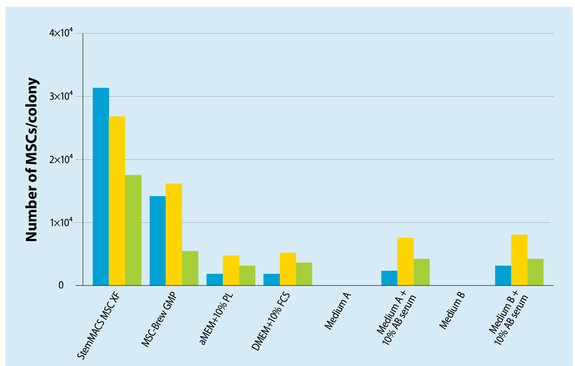

Figure 1B

2 Expansion of MSCs from BM using MSC-Brew GMP

To reach a clinical-scale number of MSCs, BM MNCs were seeded at 1.6×10^5 cells/cm^2 using the six different media. MSCs were passaged when reaching 80% confluence and reseeded at 3,000 – 5,000 MSCs/cm^2 depending on media specifications. Growth kinetics of MSCs were investigated for every medium (A) and morphology was examined microscopically. MSCs revealed a fibroblastoid morphology (B, shown is MSC-Brew GMP). A clinically relevant number of 2×10^8 cells could be harvested on average 12–13 days earlier in MSC-Brew GMP (17±0.9 days) compared to medium supplemented with PL (29±1.4 days) or FCS (30±1 days) (C). Therefore, MSC-Brew GMP expansion cultures required the lowest amount of medium (D). Samples from two donors completely failed to reach sufficient numbers of MSCs when expanded in FCS-containing media. Medium A and medium B never reached a sufficient number of MSCs (data not shown).

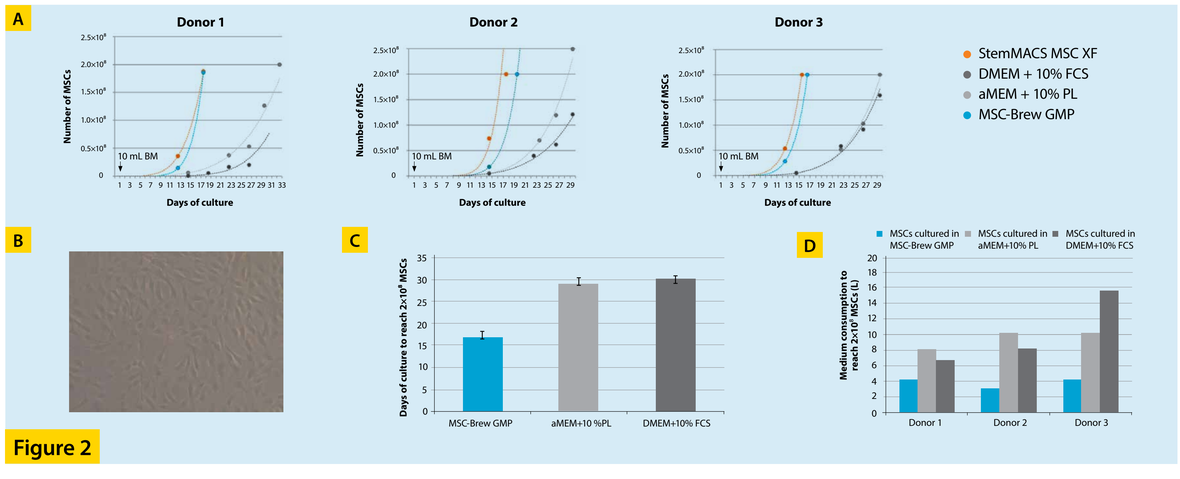

Figure 2

3 MSC-Brew GMP allows for stable expansion of MSCs

Growth kinetics of BM-MSCs were investigated for up to six passages (34–41 days) comparing all media (n = 3). One representative growth curve is depicted for donor 1. Average cell expansion of cultured MSCs was higher in MSC-Brew GMP (CPD 13.7±0.3) compared to standard media containing PL (*CPD 9.7±0.8) or FCS (CPD 7.4±1.9).

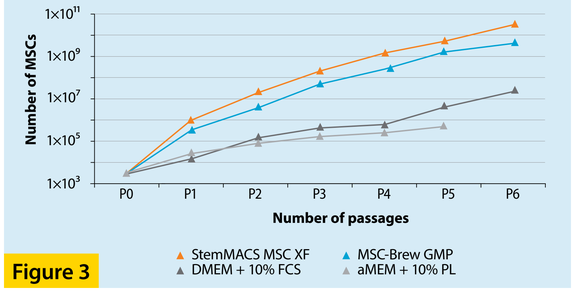

Figure 3

*CPD: Cumulative population doubling.

4 MSC-Brew GMP preserves standard MSC marker expression and multilineage differentiation potential

MSCs were analyzed by flow cytometry using the MSC Phenotyping Kit after culture expansion for six passages in different media (n = 3). Furthermore, the differentiation potential of MSCs was assessed for adipocytes, osteoblasts, and chondrocytes. To this end, MSCs were cultivated in StemMACS AdipoDiff Medium for 18 days. Adipocyte differentiation was analyzed using LipidTOX™ to demonstrate lipid accumulation. After 10 days of culture in StemMACS OsteoDiff Medium, osteoblast differentiation was analyzed by detection of alkaline phosphatase activity. After 26 days of culture in StemMACS ChondroDiff Medium, chondrocyte differentiation was analyzed using an anti-Aggrecan antibody for detection of proteoglycan. ISCT criteria were met for all conditions.

	StemMACS MSC XF	MSC-Brew GMP	DMEM + 10% FCS	aMEM + 10% PL
	% Positive			
Target	**Positive human MSC markers (ISCT Guidelines: >95%[1])**			
CD73	99.88±0.03	99.77±0.10	99.92±0.02	99.90±0.03
CD90	99.88±0.08	99.86±0.11	99.87±0.03	99.88±0.01
CD105	99.55±0.24	99.63±0.08	99.78±0.01	99.71±0.02
	Negative human MSC markers (ISCT Guidelines: <2%[1])			
Non-MSC markers: CD14, CD20, CD34, CD45	1.54±0.01	1.34±0.02	1.45±0.02	1.34±0.03
***In vitro* differentiation**	**Demonstrated by staining of *in vitro* cell culture according to ISCT Guidelines[1]**			
Adipocytes, osteoblasts, chondrocytes	✓	✓	✓	✓

Figure 4

5 MSC-Brew GMP preserves T cell–suppressive potential

T cell–suppressive potential of MSCs (p6) was analyzed using 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. T cells were cocultured with MSCs in different ratios. Flow analysis of MSCs expanded in MSC-Brew GMP is shown as an example (A). T cell–suppressive potential was observed for all conditions (B). Immunosuppressive potential was preserved even after a freeze/thaw cycle in MSC-Brew GMP Medium + 10% CryoMACS® DMSO or StemMACS Cryo-Brew.

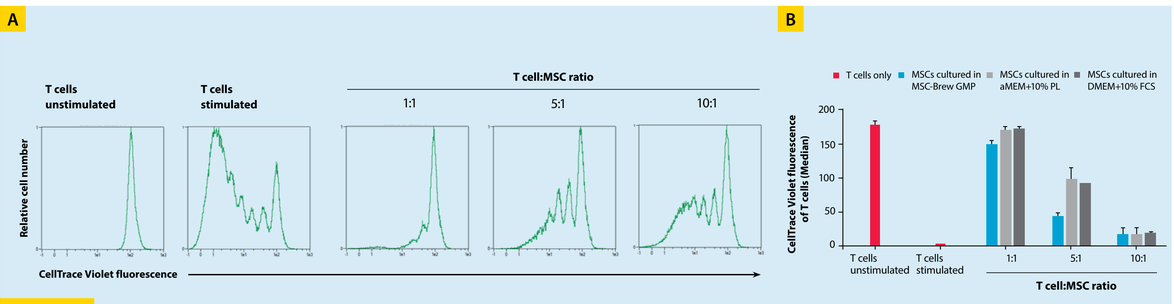

Figure 5

Conclusion

- MSC-Brew GMP Medium is a xeno-free, GMP-compliant medium and supports the attachment and expansion of MSCs from primary tissue without coating of cell culture vessels.
- MSC-Brew GMP Medium supports efficient expansion of bona fide MSCs at a clinically relevant scale suited for cell therapy and preserves an immunomodulatory phenotype.
- MSCs retain their immunosuppressive potential when using GMP-compliant freezing conditions.

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

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