



# MSC-Brew GMP Medium supports human MSC isolation and clinical-scale expansion

## Introduction

Mesenchymal stem cells (MSCs) or stromal cells are a promising resource for cell-based therapies and are being tested in already registered phase II/III clinical trials. The production of clinical-grade cell products requires GMP-compliant instruments and consumables to assure high-quality and safe products. In addition, additives like fetal calf serum (FCS) can cause adverse effects and lot-to-lot variations.

The StemMACS™ MSC Expansion Media Kit XF is an optimized medium for the reproducible and reliable generation and expansion of MSCs from various tissues. The medium is serum- and xeno-free and can be used without pre-coating of cell culture vessels. We transferred this formulation into a GMP-compliant version (MSC-Brew GMP Medium) according to the recommendations of USP<1043> on ancillary materials. In this application note, we demonstrate the efficient use of the MSC-Brew GMP Medium for clinical-scale expansion of MSCs. MSCs were isolated from three human bone marrow (BM) samples and expanded in MSC-Brew GMP Medium. Growth kinetics and phenotypes of MSCs, cultivated in StemMACS MSC Expansion Media Kit XF, MSC-Brew GMP Medium, other commercially available xeno- and serum-free MSC media (medium A and B) as well as FCS- and platelet lysate (PL)-containing formulations were analyzed.

## Materials and methods

### Sample preparation

Human bone marrow or lipoaspirate samples were prepared according to our protocols "Isolation of mononuclear cells from human bone marrow aspirates by density gradient centrifugation"<sup>1</sup> or "Preparation of the stromal vascular fraction (SVF) from human lipoaspirate"<sup>2</sup>. Human umbilical cord samples were prepared using the Umbilical Cord Dissociation Kit, human.

### Isolation of human MSCs by plastic-adherent method

Cells were resuspended in pre-warmed MSC-Brew GMP Medium and transferred into a cell culture vessel at

appropriate cell density (tab. 1). Cells were cultured at 37 °C in an incubator with 5% CO<sub>2</sub> and >95% humidity. Medium was changed after 24–48 hours, and thereafter every 4–5 days. When MSCs had reached 80% confluency (presumably around day 10), MSCs were passaged (fig. 1).

Human tissue source	Seeding density (cell number/cm <sup>2</sup> )	Medium volume (mL/cm <sup>2</sup> )
Bone marrow mononuclear cells (BM MNC)	1.6×10 <sup>5</sup>	0.2
Stromal vascular fraction (SVF)	1×10 <sup>5</sup>	0.2
Umbilical cord	1.6×10 <sup>5</sup>	0.2

**Table 1:** Optimal seeding density and required cell medium volume per cm<sup>2</sup> for initial cultivation of MSCs starting with primary tissue using MSC-Brew GMP Medium.

### Expansion and passaging of human MSCs

MSC-Brew GMP Medium was removed and cells were washed with pre-warmed CliniMACS® PBS/EDTA Buffer to remove residual medium and dead cells. Pre-warmed trypsin-based detachment reagent was added to cover the cells and incubated for 5–10 minutes at 37°C. Detachment of MSCs was observed with a microscope. To ensure MSC detachment, the flask was gently tapped or incubation time was prolonged for another 5–10 minutes. Once MSCs were completely detached, trypsin inhibitor was added and cells were resuspended in pre-warmed MSC-Brew GMP Medium and transferred to an appropriate vessel. For optimal retrieval, the cell culture vessel was washed another time with MSC-Brew GMP Medium and the suspension was combined with the first one. Cells were centrifuged at 300×g for 5–10 minutes at room temperature and resuspended in MSC-Brew GMP Medium.

For further expansion, MSCs were seeded into new culture vessels (3×10<sup>3</sup> cells/cm<sup>2</sup> for MSC-Brew GMP Medium and StemMACS MSC Expansion Media Kit XF, and 4×10<sup>3</sup> cells/cm<sup>2</sup> for other media according to the manufacturers' recommendations) and cultured at 37 °C in an incubator with 5% CO<sub>2</sub> and >95% humidity. Medium was changed every 2–3 days. When MSCs had reached 80% confluency (approx. after 2–4 days), the passaging procedure was repeated.

### Calculation of population doubling and cumulative population doubling rates

Population doubling (PD) and cumulative population doubling (CPD) were determined using the following equations:

$$\text{PD for each passage} = (\log_{10} (N_H) - \log_{10} (N_I)) / \log_{10} (2)$$

$N_I$  = number of inoculated cells;  $N_H$  = number of harvested cells

$$\text{CPD} = \sum (\text{PD})$$

### MSC phenotyping and functional analysis

Cultured MSCs were analyzed by flow cytometry using the MSC Phenotyping Kit, human.

Differentiation potential of cultured MSCs was tested by culturing cells in StemMACS™ AdipoDiff Media, human for 18 days, StemMACS ChondroDiff Media, human for 26 days, or StemMACS OsteoDiff Media, human for 10 days according to our protocol "StemMACS™ Mesenchymal Stem Cell Media, human"<sup>3</sup>.

Immunosuppressive capacity of cultured MSCs was analyzed using the MSC Suppression Inspector, human.

### Cryopreservation of human MSCs

After centrifugation, MSC pellet was quickly resuspended with cold freezing solution (90% MSC-Brew GMP Medium + 10% CryoMACS® DMSO 10 (EP/USP) or StemMACS Cryo-Brew) and transferred to cryovials at a final concentration of  $0.5\text{--}1.0 \times 10^6$  cells/mL. Samples were transferred to an isopropanol freezing container and frozen immediately at  $-80^\circ\text{C}$ . After 24 hours, vials were transferred to a liquid nitrogen tank for long-term storage.

Product	Order no.
MSC-Brew GMP Medium	2L: 170-076-325 500mL: 170-076-326
StemMACS™ MSC Expansion Media Kit XF, human	130-104-182
MSC Phenotyping Kit, human	130-095-198
StemMACS AdipoDiff Media, human	130-091-677
StemMACS ChondroDiff Media, human	130-091-679
StemMACS OsteoDiff Media, human	130-091-678
MSC Suppression Inspector, human	130-096-207
CryoMACS DMSO 10 (EP, USP)	170-076-303
StemMACS Cryo-Brew	130-109-558
Umbilical Cord Dissociation Kit, human	130-105-737

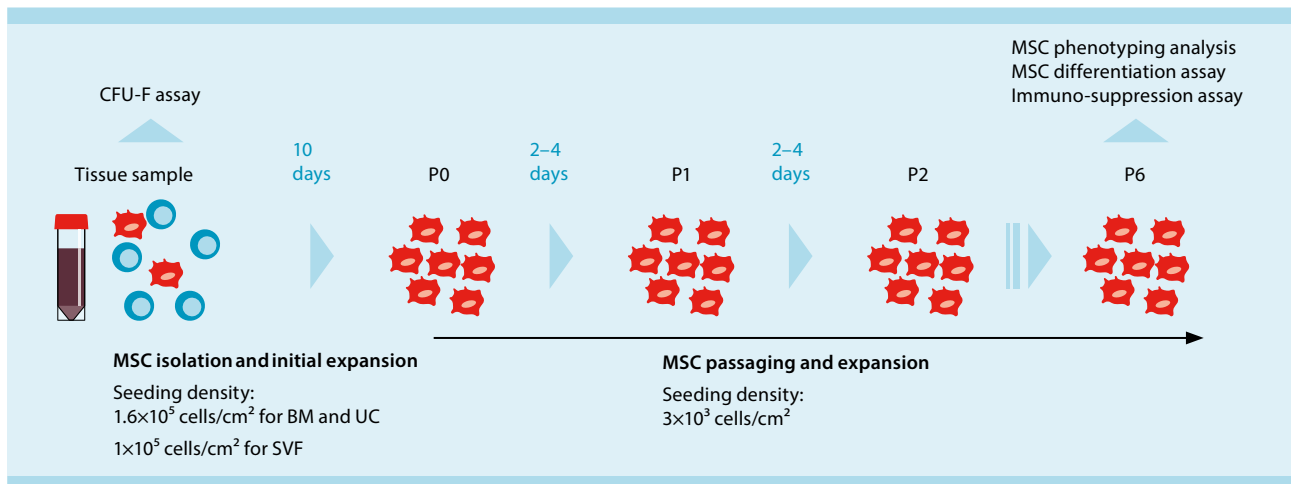


Figure 1: Overview of the MSC workflow.

## Results

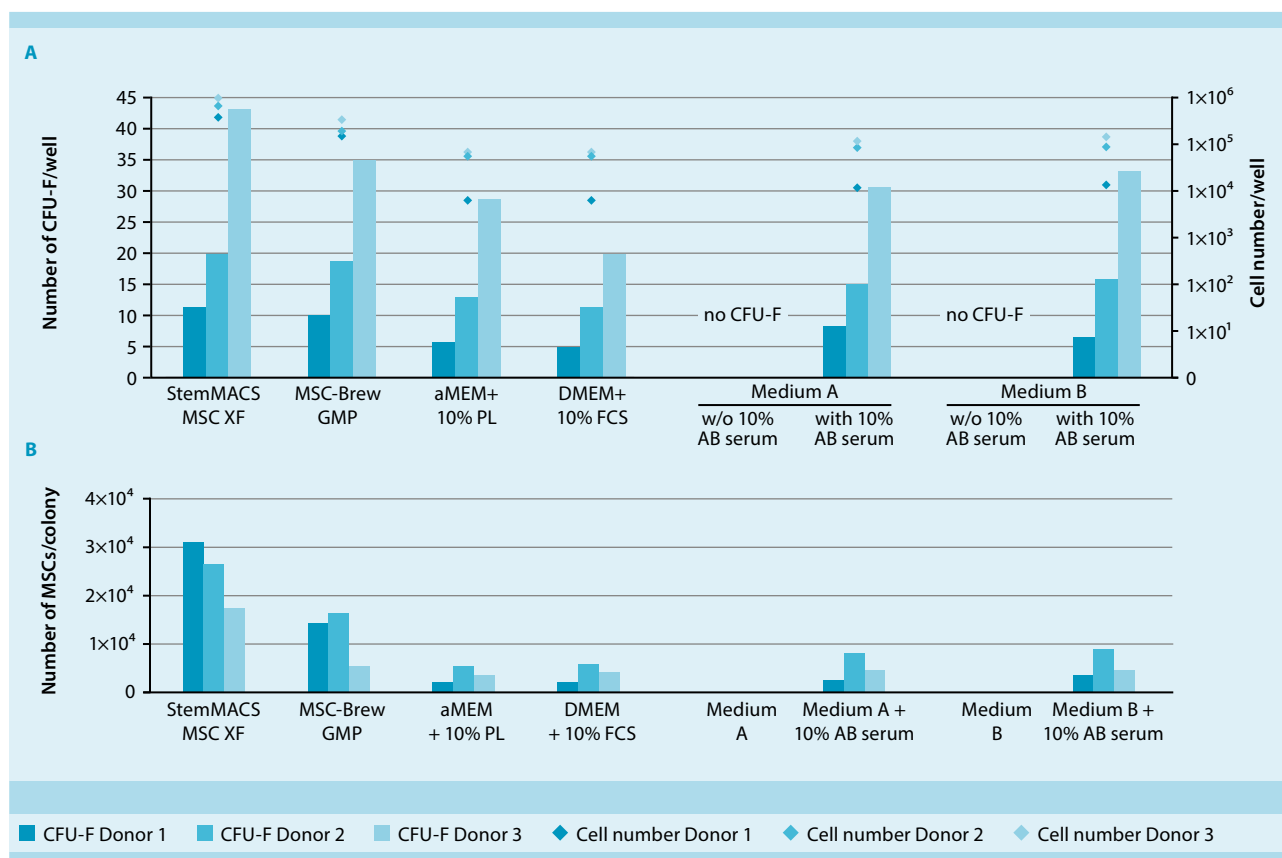
### MSC-Brew GMP Medium and StemMACS MSC Expansion Media Kit XF enhance initial cell attachment and growth

First, the clonogenic potential of human bone marrow mononuclear cells (BM MNCs) cultivated in different media was tested. We assessed total cell numbers as well as colony-forming unit–fibroblast (CFU-F) counts after nine days in culture (fig. 2A). The CFU-F count was highest when BM MNCs were cultured in StemMACS MSC Expansion Media Kit XF or MSC-Brew GMP Medium. No cell growth was observed when using Medium A and B without 10% AB serum. The initial expansion potential of MSCs per CFU-F was higher with StemMACS MSC Expansion Media Kit XF or MSC-Brew GMP Medium than with all other media tested (fig. 2B).

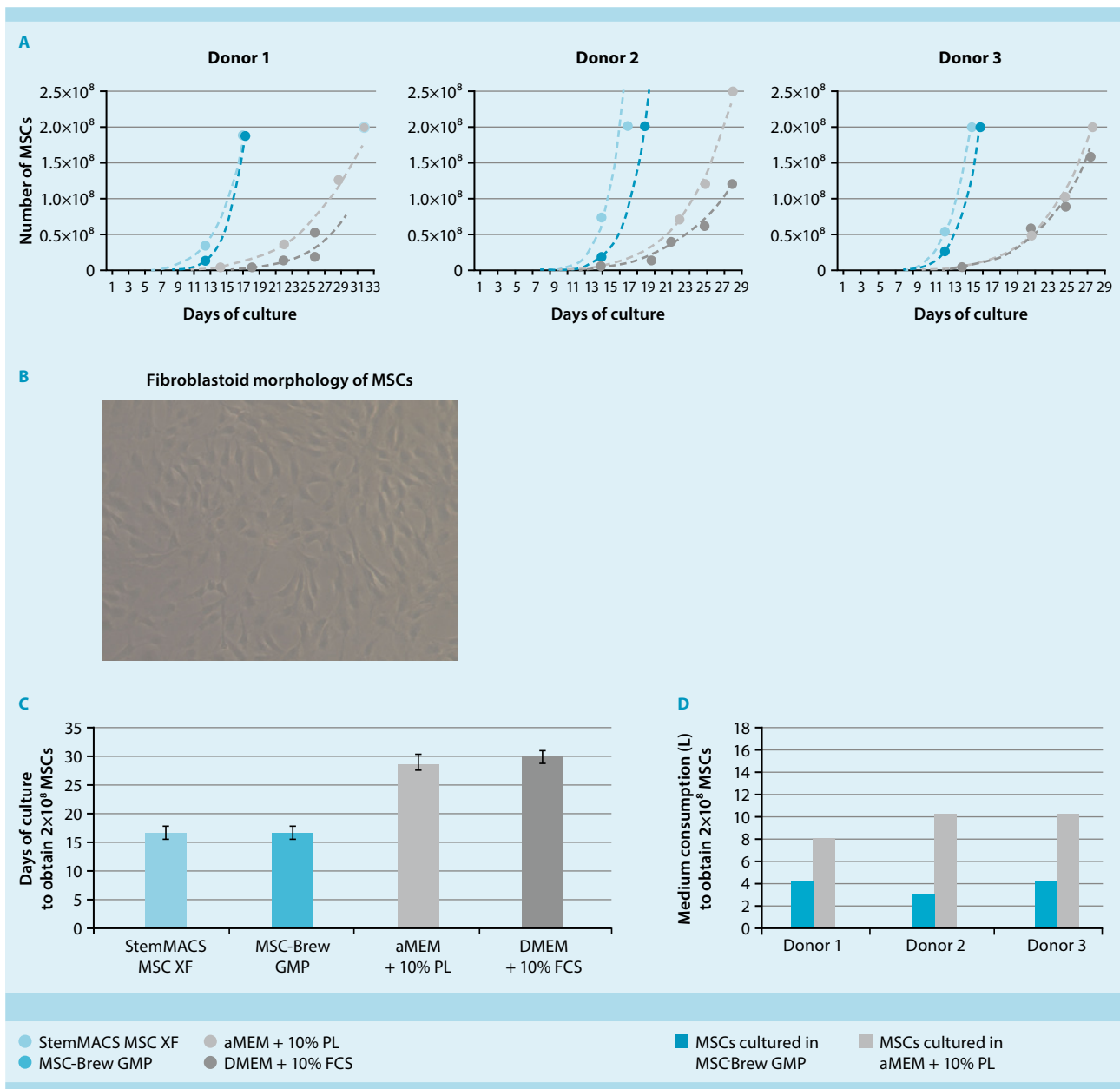
### MSC-Brew GMP Medium enables efficient MSC expansion

Growth of MSCs was monitored every other day from day one to day 33 (fig. 3A) and MSC morphology was examined microscopically. MSCs revealed a fibroblastoid morphology (fig. 3B).

A clinically relevant number of  $2 \times 10^8$  cells could be harvested on average 12–13 days earlier when using MSC-Brew GMP Medium (at day  $17 \pm 1.25$ ) or StemMACS MSC Expansion Media Kit XF (at day  $17 \pm 0.9$ ) compared to medium supplemented with PL (at day  $29 \pm 1.4$ ) or FCS (at day  $30 \pm 1$ ) (fig. 3C). Thus, to reach a clinical-scale number of MSCs, less MSC Brew GMP Medium is required (fig. 3D). Samples from donor 1 and 2 failed to reach sufficient numbers of MSCs when expanded in FCS-containing media (fig. 3A). MSCs cultured in medium A or B did not reach a sufficient number in the tested timeframe (data not shown).



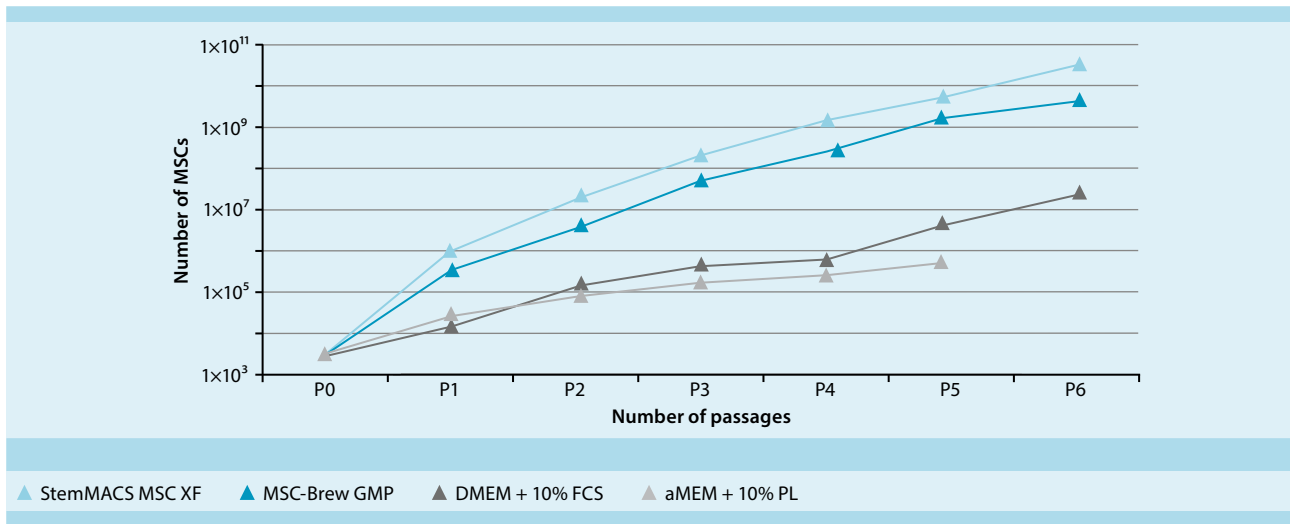
**Figure 2:** MSC-Brew GMP Medium and StemMACS™ MSC Expansion Media Kit XF support initial cell attachment and growth. (A) CFU-F counts and total cell numbers of cultures from three donors (passage 0, day 9. Performed in triplicate). (B) Initial expansion potential of MSCs per CFU-F.



**Figure 3: MSC-Brew GMP Medium enables efficient MSC expansion.** (A) Growth kinetics of MSCs cultured in different media. (B) Fibroblastoid morphology of MSCs cultured in MSC-Brew GMP Medium. (C) Time needed and (D) volume of medium needed to obtain a clinically relevant number of MSCs ( $2 \times 10^8$  cells) ( $n = 3$ , SD).

Growth kinetics of BM-MSCs cultivated in different media were investigated for up to six passages (34–41 days of culturing). One representative growth curve per medium is depicted for donor 1 (fig. 4). MSCs cultured in MSC-Brew

GMP Medium revealed higher numbers of cumulative population doublings (CPD  $13.7 \pm 0.3$ ) compared to MSCs cultured in medium containing PL (CPD  $9.7 \pm 0.8$ ) or FCS (CPD  $7.4 \pm 1.9$ ).



**Figure 4: MSC-Brew GMP Medium facilitates efficient and stable MSC proliferation.** MSCs cultured in MSC-Brew GMP Medium show higher expansion rates and stable proliferation in the tested timeframe (34–41 days of culturing).

### MSC-Brew GMP Medium preserves standard MSC marker expression, multilineage differentiation potential, and immunosuppressive potential

The expression of cell surface markers of cultured MSCs was analyzed by flow cytometry using the MSC Phenotyping Kit after expansion for six passages in different MSC media.

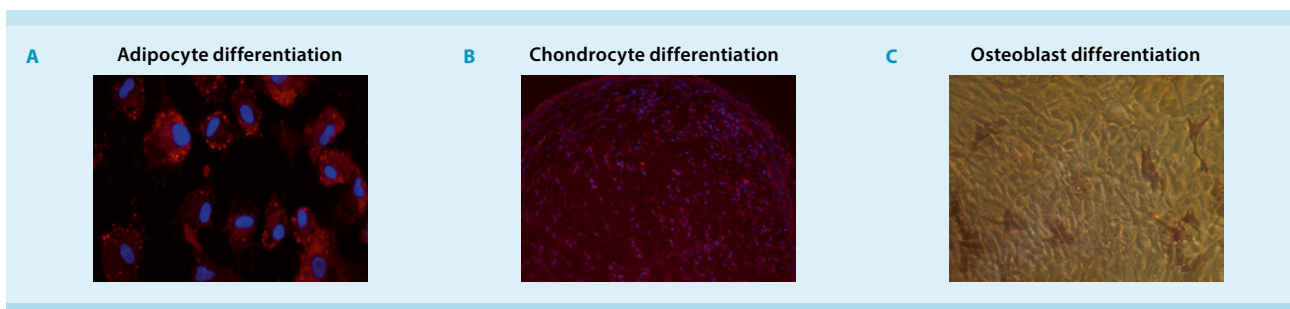
Irrespective of the medium, expanded MSCs showed the typical expression profiles of CD73, CD90, and CD105 (tab. 2) as described within ISCT Guidelines<sup>4</sup>.

	StemMACS MSC XF	MSC-Brew GMP	DMEM + 10% FCS	aMEM + 10% PL
<b>% Positive</b>				
<b>Target</b>	<b>Positive human MSC markers (ISCT Guidelines: &gt;95%<sup>4</sup>)</b>			
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
	<b>Negative human MSC markers (ISCT Guidelines: &lt;2%<sup>4</sup>)</b>			
Non-MSc markers: CD14, CD20, CD34, CD45	1.54±0.01	1.34±0.02	1.45±0.02	1.34±0.03

**Table 2: MSC-Brew GMP Medium preserves standard MSC marker expression.** ISCT Guidelines for positive and negative MSC markers<sup>4</sup> were met.

Next, we tested the differentiation potential of MSCs cultivated in MSC-Brew GMP Medium for six passages. MSCs were cultured in StemMACS™ AdipoDiff Medium, human, StemMACS ChondroDiff Medium, human, or StemMACS OsteoDiff Medium, human to induce their differentiation into

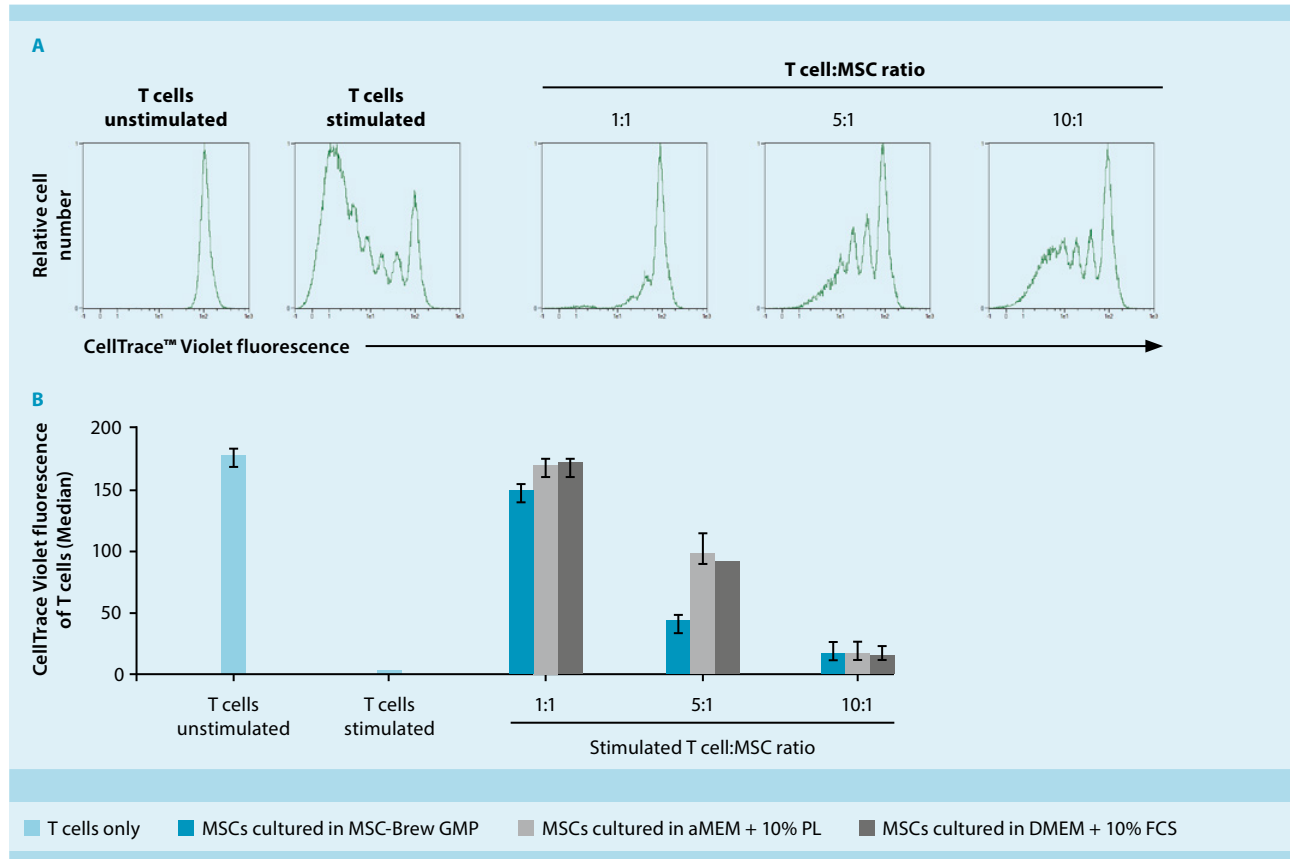
adipocytes, chondrocytes, and osteoblasts. Differentiated cells were stained according to ISCT Guidelines<sup>4</sup>, and successful differentiation into adipocytes, chondrocytes, and osteoblasts was observed (fig. 5).



**Figure 5: MSCs cultured in MSC-Brew GMP Medium maintain differentiation potential.** Isolated MSCs were cultivated in different media for 10–26 days. (A) Adipocyte differentiation was analyzed using a lipid-staining dye (red). (B) Chondrocyte differentiation was analyzed using an anti-aggregan antibody (red). (C) Osteoblast differentiation was analyzed by detection of alkaline phosphatase activity (blue-purple).

To investigate immune modulatory capacity of the expanded MSCs, cells from passage six were cocultured with CD4<sup>+</sup>CD25<sup>-</sup> responder T cells (Tresp) at different ratios. CD4<sup>+</sup>CD25<sup>-</sup> T cells were labeled with CellTrace™ Dye to monitor T cell proliferation after stimulation with CD2, CD3, and CD28 antibody-loaded particles included in the MSC Suppression Inspector, human. Flow cytometry analysis of proliferating T cells cocultured

with MSCs expanded in MSC-Brew GMP Medium is shown as an example (fig. 6A). T cell suppression was observed for all conditions (fig. 6B). The immunosuppressive capacity was preserved after a freeze-thaw cycle in MSC-Brew GMP Medium + 10% CryoMACS® DMSO or StemMACS™ Cryo-Brew (data not shown).

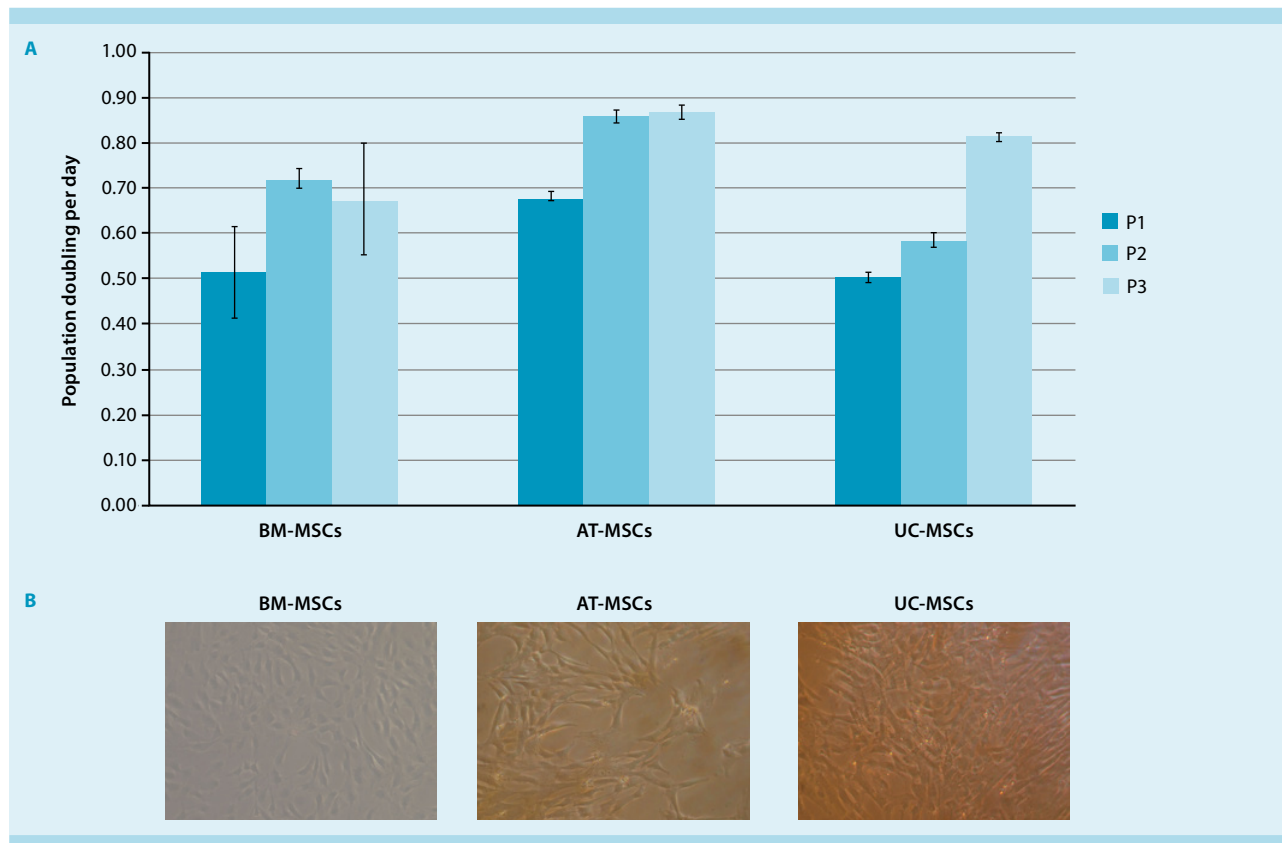


**Figure 6: MSC-Brew GMP Medium preserves immunosuppressive potential.** (A) Flow cytometry analysis of proliferating T cells cocultured with MSCs at different ratios. (B) T cell-suppressive potential was observed for all conditions, even with decreased MSC to T cell ratio (n = 3, SD).

### MSC-Brew GMP Medium supports efficient expansion of MSCs derived from various tissues

Next, we tested the growth capacity of MSCs generated from different tissues. Human adipose-derived (AT-), bone marrow-derived (BM-), and umbilical cord-derived (UC-) MSCs were cultured in MSC-Brew GMP Medium. MSCs were plated at  $3 \times 10^3$  cells/cm<sup>2</sup> and cell growth was monitored for

three passages to calculate the population doubling rate per day. Each passage was harvested at 80% confluency. MSCs generated from various primary tissues showed a stable proliferation for three passages when cultured in MSC-Brew GMP Medium (fig. 7A). Furthermore, cultured MSCs revealed a spindle-shaped morphology (fig. 7B).



**Figure 7: MSCs from various tissues can be expanded with MSC-Brew GMP Medium.** (A) Population doubling rates per day for MSCs from bone marrow (BM), adipose tissue (AT), and umbilical cord (UC), cultivated in MSC-Brew GMP Medium (passages 1–3). (B) Representative microscope images of the spindle-shaped morphology of MSCs (at passage three) from different tissues (n = 3, SD).

## Conclusion

- The serum- and xeno-free MSC-Brew GMP Medium is based on the formulation of StemMACS MSC Expansion Media Kit XF and shows comparable performance. It supports the efficient attachment of MSCs without requirement of AB serum and without requirement of pre-coating culture vessels.
- MSC-Brew GMP Medium supports efficient expansion of bona fide MSCs from different human tissues at a clinically relevant scale.
- MSC phenotypes are maintained and multilineage differentiation and immunomodulatory potentials of expanded MSCs are preserved.

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