An animal component-free, chemically defined media formulation for cryopreservation ensuring high cell recovery and viability

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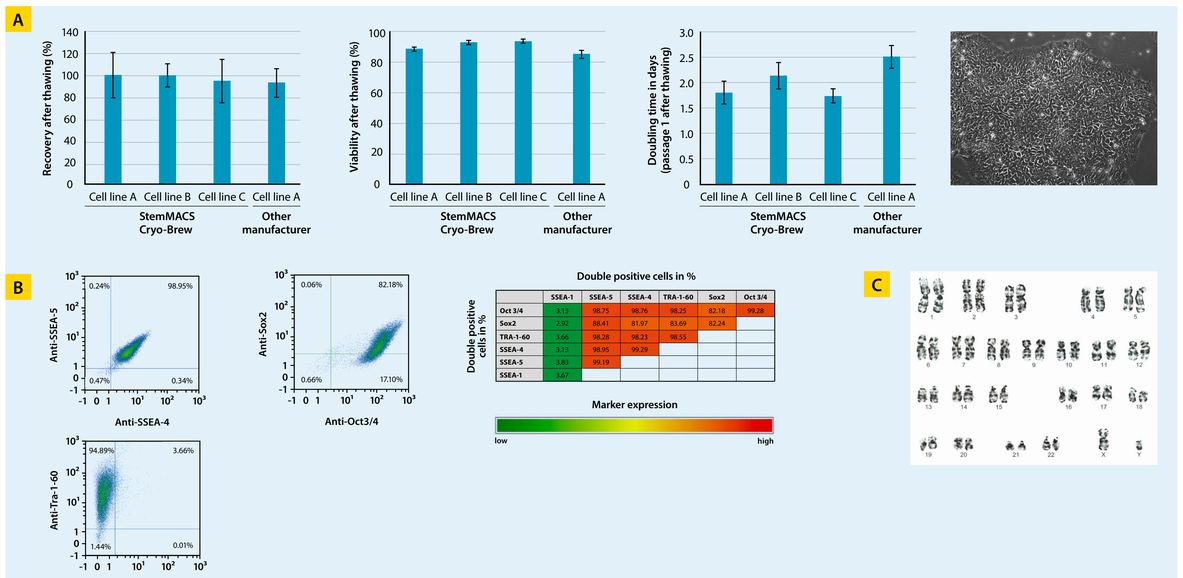
Introduction

Human pluripotent stem cells (PSCs) play an important role in disease modeling and drug discovery. Moreover, they hold great promise for future clinical applications, especially due to their potential usage in cell therapy. Therefore, optimal storage and cryopreservation of stem cells and progenies is of major importance. Our new animal component-free, chemically defined media formulation has been specifically designed for use with xeno- and serum-free culture systems. It ensures not only high recovery and viability but also functionality of different cell types right after thawing. The chemically defined nature of the formulation will allow rapid translation into a clinical-grade medium designed following the recommendations of USP <1043> on ancillary materials.

Results

1 StemMACS™ Cryo-Brew ensures high recovery and viability after cryopreservation of induced pluripotent stem cells (iPSCs)

Human iPSCs frozen in StemMACS™ Cryo-Brew showed a reliable and reproducible recovery of over 93% after thawing (mean +/– SD, n ≥ 3) as well as a viability of over 88% post-thaw (mean +/– SD, n ≥ 3) (A). Additionally, fast recovery after replating could be observed, with doubling times of less than 2.12 days (mean +/– SD, n ≥ 4). iPSCs showed typical stem cell morphology shortly after plating (example shown for cell line A on day 4 after thawing) (A). Marker expression was checked in passage 1 after thawing. The different cell lines displayed a high expression of pluripotency markers such as TRA-1-60, SSEA-4, SSEA-5, Oct3/4 and Sox2 while showing low expression of the differentiation marker SSEA-1 (example shown for cell line C). A heat map of the analyzed markers was used to visualize the percentage of double- and single-positive cells (B). To determine the genomic stability of iPSCs cryopreserved in our medium, we analyzed the karyotype in passage 5 post-thaw (n = 2, example shown for cell line B) (C).



	SSEA-1	SSEA-5	SSEA-4	TRA-1-60	Sox2	Oct 3/4
Oct 3/4	3.13	98.75	98.76	98.25	82.18	99.28
Sox2	2.92	88.41	81.97	83.69	82.24	
TRA-1-60	3.66	98.28	98.23	98.55		
SSEA-4	3.13	98.95	99.29			
SSEA-5	3.83	99.19				
SSEA-1	3.67					

Figure 1

2 Immunosuppressive potential of mesenchymal stem cells (MSCs) is preserved after cryopreservation in StemMACS™ Cryo-Brew

MSCs were isolated from human bone marrow samples and cryopreserved in StemMACS™ Cryo-Brew after expansion (passage 6). The cells showed a recovery of over 89% (mean +/– SD, n ≥ 2) and a viability of over 92% (mean +/– SD, n ≥ 2) after thawing as well as a low doubling time of 0.81 days (mean +/–SD, n = 3) (A). To assess their immunosuppressive potential, MSCs were co-cultured with activated T cells. Freshly thawed MSCs were compared to MSCs from the same donor that were maintained in culture (mean +/– SD, n ≥ 2) (B). Immunosuppressive properties were not negatively affected by the freezing step, thus ensuring full cellular functionality right after thawing.

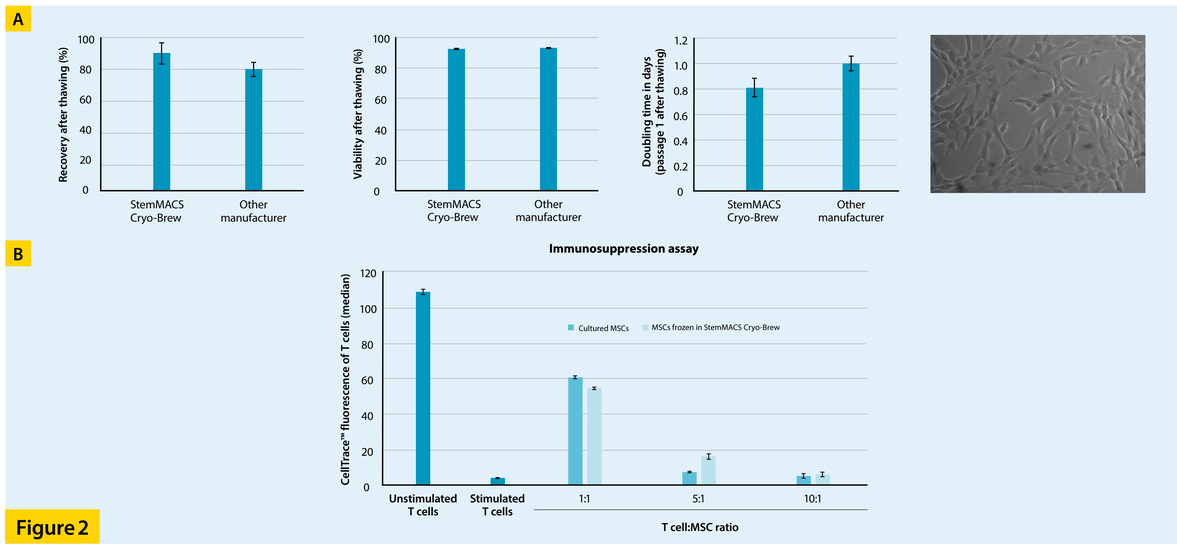
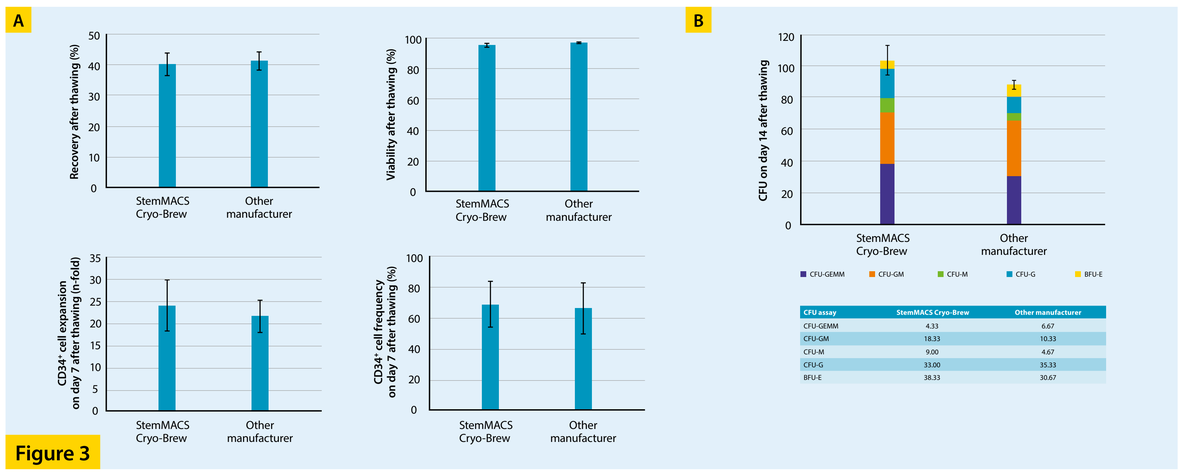


Figure 2

3 CD34⁺ hematopoietic stem cells (HSCs) show high recovery, viability, and functionality after cryopreservation in StemMACS™ Cryo-Brew

CD34⁺ HSCs, isolated from umbilical cord blood, were frozen in StemMACS™ Cryo-Brew. After thawing, the HSCs showed the expected recovery of 40% (mean +/– SD, n = 3) and a high viability of over 95% (mean +/– SD, n = 3). Furthermore, the HSCs could be expanded in culture 23.4-fold within seven days after thawing (mean +/– SD, n = 3) and showed a CD34⁺ cell frequency of nearly 70% (mean +/-SD, n = 3) (A). To assess the differentiation potential of the cells, colony-forming unit (CFU) assays were performed right after thawing. HSC stored in StemMACS Cryo-Brew showed normal CFU formation, including CFU-GEMMs, indicating full preservation of HSC differentiation capacity (mean +/– SD, n = 3) (B).



CFU assay	StemMACS Cryo-Brew	Other manufacturer
CFU-GEMM	4.33	6.67
CFU-GM	18.33	10.33
CFU-M	9.00	4.67
CFU-G	33.00	35.33
BFU-E	38.33	30.67

Figure 3

4 StemMACS™ Cryo-Brew enables cryopreservation of iPSC-derived cardiomyocytes with high recovery and viability

Human iPSCs were differentiated into cardiomyocytes and enriched using the PSC-derived Cardiomyocyte Isolation Kit. Cardiomyocytes cryopreserved in StemMACS™ Cryo-Brew showed good recovery of over 95% (mean +/– SD, n = 2) and high viability of over 70% after thawing (mean +/– SD, n = 2). Furthermore, the enriched cardiomyocytes initiated contractions as early as 24 hours after thawing indicating full functionality, independently of the freezing process.

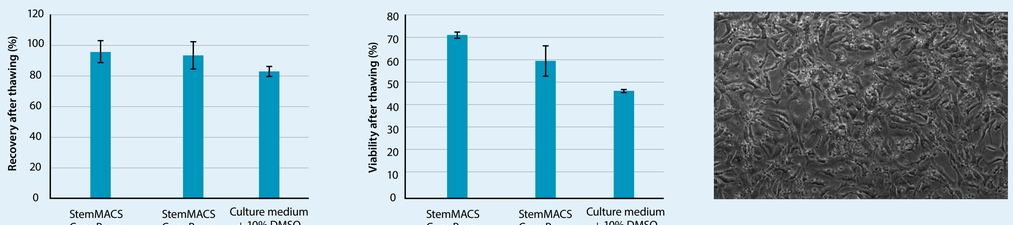


Figure 4

5 Human chimeric antigen-receptor (CAR T) cells show high recovery and viability after cryopreservation in StemMACS™ Cryo-Brew

Human CD4⁺/CD8⁺ T cells were isolated from leukapheresis, stimulated, and cultivated using the CliniMACS Prodigy®. Cells were further transduced with CD20-CAR via lentiviral transduction to generate gene-modified T cells with the TCT (T Cell Transduction) Process on the CliniMACS Prodigy. After cryopreservation in StemMACS™ Cryo-Brew the CAR T cells showed high recovery of nearly 85% (mean +/– SD, n = 2) and a viability of over 90% (mean +/– SD, n = 2).

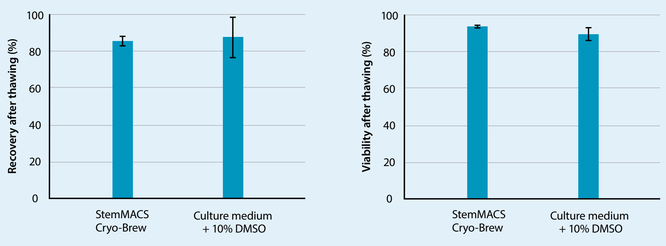


Figure 5

Conclusion

We have developed an animal-component free and chemically defined freezing medium that ensures high recovery, viability, and functionality after cryopreservation of the following cell types:

- Induced pluripotent stem cells
- Mesenchymal stem cells
- CD34⁺ hematopoietic stem cells
- iPSC-derived cardiomyocytes
- CD4⁺/CD8⁺ CAR T cells