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Cryopreservation of adipose-derived stromal/stem cells using 1–2% Me_2SO (DMSO) in combination with pentaisomaltose: An effective and less toxic alternative to comparable freezing media

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ABSTRACT

Mesenchymal stromal/stem cells (MSCs) derived from bone marrow, umbilical cord and especially adipose tissue are increasingly being explored for their therapeutic potential to treat a wide variety of diseases. A prerequisite for most allogeneic off-the-shelf and some autologous MSC therapies is the ability to safely and efficiently cryopreserve cells during production or for storage prior to treatment. Dimethyl sulfoxide (Me_2SO) is still the commonly used gold standard cryoprotectant (CPA). However, undesirable cellular impacts and side effects of Me_2SO have led to an increasing demand for the development of safe and effective alternatives.

This study investigated the effect of pentaisomaltose as a CPA for cryopreservation of adipose-derived stromal/stem cells (ASCs). We compared pentaisomaltose-based freezing media containing 1% Me_2SO (PIM1) or 2% Me_2SO (PIM2) to our in-house freezing media formulation containing 10% Me_2SO (STD10) and to CryoStor freezing media containing 2% or 10% Me_2SO (CS2 and CS10). We assessed the recovery of viable ASCs, their phenotype, differentiation potential, proliferation potential, and migratory potential. Further, their immunomodulatory potential was assessed by measuring their ability to suppress T cell proliferation and express immunomodulatory markers.

The results showed that the post-thaw viability of ASCs cryopreserved with STD10, CS10 and PIM2 was improved compared to that of CS2. The recovery of ASCs with PIM1 and PIM2 was also improved compared to that of CS2. Proliferation and migration were comparable among the tested freezing media. The results showed no difference in the induction of PDL1, PDL2 or IDO1 expression. Nevertheless, the potential of cryopreserved ASCs to suppress T cell proliferation was reduced when the Me_2SO concentration was reduced (CS10>STD10>CS2 and PIM2>PIM1).

Altogether, the migratory and immunomodulatory potential combined with improved recovery indicate that the addition of pentaisomaltose in the freezing media may allow for the reduction of the Me_2SO concentration to 2% while retaining a more potent cell product that what is recovered using comparable freezing media. With the desire to reduce the amount of Me_2SO, these results suggest that 2% and potentially even 1% Me_2SO in combination with 10% pentaisomaltose could be an effective and less toxic alternative to comparable freezing media.

1. Introduction

Cell therapy use has expanded extensively during the past decades and holds promise for being an important platform for future treatment modalities. Mesenchymal stromal/stem cells (MSCs) derived from bone marrow, umbilical cord and especially adipose tissue are increasingly being explored for their therapeutic potential to treat a wide variety of diseases, as researchers try to exploit their regenerative and immunomodulatory capability demonstrated in vitro [1,2].

A prerequisite for most allogeneic off-the-shelf and some autologous

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MSC therapies is the ability to safely and efficiently cryopreserve cells during production or for storage prior to treatment. However, cryopreservation of ASCs has been shown to reduce cell potency for the first 24–72 h post-thaw in terms of e.g. differentiation potential and immunosuppressive properties [3].

Successful cryopreservation that results in viable and functional cells post-thaw requires effective cryoprotectants (CPAs). Dimethyl sulfoxide (Me_2SO) is still the gold standard CPA used for cryopreservation of most cell types, including mesenchymal stem cells. However, side effects and undesirable cellular effects of Me_2SO [4–10,33] have led to an increasing demand from health care professionals, patients and authorities for a reduction in the concentration of CPAs such as Me_2SO and the development of safe and effective alternatives. It is therefore imperative to optimize and compare freeze media in order to best possibly preserve cell viability and function.

Although progress has been made in the development of optimized freezing media, mainly by combining existing CPAs and improving the carrier media, new alternative CPAs have not been introduced for clinical use [11]. The commercially available freezing media CryoStor (BioLife Solutions) combined Me_2SO and Dextran-40 to allow reduction of the Me_2SO content to as low as 2%. However, reducing the concentration of Me_2SO seems to come with a cost in regards to cell survival and recovery [12]. Other CPAs like polyampholyte poly-L-lysine, polyvinylpyrrolidone (PVP), sucrose and trehalose have also been investigated with the purpose of replacing or reducing Me_2SO for the cryopreservation of mesenchymal stem cells although with mixed success [13–16].

We have recently reported the first use of pentaisomaltose, a low-molecular-weight (1 kDa) linear subfraction of Dextran 1 which is approved for clinical use, as a potential new CPA, and demonstrated that its sole use as an CPA in a concentration of 16% was sufficient for successful cryopreservation of hematopoietic stem and progenitor cells (HSPC) from apheresis products [17,18].

The purpose of the current study was, based on our previous encouraging findings for HSPCs, to investigate whether pentaisomaltose could also be used as an effective CPA for cryopreservation of adipose-derived stromal/stem cells (ASCs). We therefore compared pentaisomaltose-based freezing media to our in-house freezing media formulation that contained 10% Me_2SO and to CryoStor freezing media, that contained 2% or 10% Me_2SO (CS2 and CS10). To compare the effectiveness of these different cryomedia, both quantitative and qualitative functional measures were used. The following was assessed:1) viability and recovery, 2) ASC defining surface molecules and differentiation potential, 3) IFN-licensing potential, 4) transwell migration and 5) suppression of T cell proliferation.

2. Materials and methods

2.1. Isolation and culture of ASCs

Adipose tissue was obtained from five healthy donors who underwent an abdominal liposuction. The isolation of the stromal vascular fraction (SVF) was performed as previously described [19]. Briefly, the lipoaspirate was washed and enzymatically digested with collagenase (NB 4 grade standard, KEM-EN-TEC Diagnostics) for 45–60 min at 37 °C with constant rotation. The enzymatic activity of the collagenase was neutralized by the addition of cell culture media (DMEM (Life Technologies), 1% penicillin-streptomycin (GIBCO-Invitrogen), 2 IU/mL preservative-free heparin (LEO Pharma) and 10% pooled human platelet lysate (pHPL, produced in house from buffycoat derived platelet pools using three freeze-thaw cycles [19]), and the suspension was filtered prior to centrifugation. The resulting SVFs were counted and resuspended in culture media, and seeded at a density of approximately 10.000 cells/cm^2. ASCs in the SVF were expanded in cell culture media in a CO_2 incubator (atmospheric gas supplemented with 5% CO_2, 37 °C), as previously described [19].

2.2. Cryopreservation of adipose-derived stromal cells

ASC cultures (passages 1–3) were washed once with PBS (w/o Ca^{2+} and Mg^{2+}) and then detached using TrypLE (Life Technologies). Following neutralization, centrifugation and washing, 2×10^6 ASCs were aliquoted into cryotubes (duplicate tubes) and were pelleted at 300×*g* for 5 min. The cell pellets were gently resuspended in the different cryopreservation media in duplicate tubes (STD10, CS2, CS10, PIM1 and PIM2; see Table 1 for formulation descriptions). The tubes containing cells and CPA solution were frozen to −100 °C in a rate-controlled freezer (−1 °C/min decrease to 0 °C, −2 °C/min decrease to −45 °C, −5 °C/min decrease to −100 °C; Kryo 560–16, Planer PLC), and then they were transferred to and stored in liquid nitrogen at −196 °C. ASCs from each of the five donors were cryopreserved with all of the investigated freezing media.

2.3. Viability immediately post-thaw and during the following 24 h

Recovery of viable cells was evaluated immediately after the cells had been thawed in a 37 °C water bath using Acridine Orange/DAPI (Via-1 cassettes,NC-3000, Chemometec). Both Acridine Orange and DAPI are DNA dyes. Whereas Acridine Orange is permeable to the cell membrane of both live and dead cells DAPI only enters dead or dying cells without an intact cell membrane.

Cytotoxicity was monitored for 24 h post-thaw. The thawed ASCs were seeded in 96-well plates (2000 cells/well) in culture expansion media containing Cytotox red DNA stain (Essen Biosciences). The number of dead ASCs post-thaw was monitored and quantified hourly using Incucyte live cell imaging and then were analyzed using Incucyte Zoom software (Essen Biosciences) according to the manufacturer's instructions.

2.4. Adipogenesis and chondrogenesis differentiation assay

The ASCs were differentiated into adipogenic or chondrogenic lineages using a STEMPRO Adipogenesis and Chondrogenesis Differentiation kit (Invitrogen) according to the manufacturer's instructions. Briefly, adipogenic differentiation was performed in 96-well tissue culture plates (NUNC, Thermo Fisher Scientific). The cells were seeded at a density of 1×10^4 viable cells/cm^2 in prewarmed adipogenesis differentiation medium and were then cultured for 14 days; the media was changed every 4 days. Chondrogenic differentiation was performed by seeding 3 droplets of 1.6×10^7 cells/mL in 24-well tissue culture plates (NUNC, Thermo Fisher Scientific). The cell droplets were incubated for 2 h to allow the cells to attach to the plastic surface before adding chondrogenesis differentiation medium. Cells were cultured for 14 days, and the medium was changed every 4 days. The staining analyses were performed with oil red O staining for adipocytes and Alcian blue for chondrocytes (all from Sigma Aldrich). Cells cultured in media without a differentiation substrate were stained as described and served as controls.

2.5. Proliferation assay

Thawed ASCs were seeded as 5 replicates in 96-well plates (NUNC, Thermo Fisher Scientific) in sterile filtered culture expansion media at a

Table 1
Tested freezing media formulations.

Formulation	Abbreviations
10% Me_2SO + 4% HA (in-house standard)	STD10
CryoStor 2% Me_2SO + Dextran-40	CS2
CryoStor 10% Me_2SO + Dextran-40	CS10
1% Me_2SO + 10% pentaisomaltose + 4% HA	PIM1
2% Me_2SO + 10% pentaisomaltose + 4% HA	PIM2

concentration of 3000 ASCs/well. The plate was monitored using Incucyte live cell imaging, and pictures were obtained every 2 h. Cell confluency was measured using the accompanying software and a pre-trained mask identifying cell morphology in culture. Cell proliferation was analyzed using Incucyte Zoom software, measuring proliferation as an increase in confluency. Confluency data from the Incucyte were used to calculate hours to confluency (e100%), length of lag-phase (hours) and Max PDT (hours). The PDT was estimated by using Shiny app (Rstudio) to determine the steepest point of the slope on the proliferation curve and then applying the following formula to calculate the population doubling time: T(h) = (log(2))/(log(1+(growth rate/100)).

2.6. Transwell migration assay

ASCs were seeded in Clearview 96-well chemotaxis plates at a concentration of 1500 cells/well insert. The insert (top well) contained 2% pHPL culture expansion media, and the reservoir (lower well) contained 5% pHPL culture expansion media. Controls contained growth with 2% pHPL in both the insert and reservoir. The ASCs were cultured for 24 h, after which images were captured, and the number of migrated cells was counted using an Incucyte live-cell imager; the data were analyzed by accompanying Incucyte Zoom software (Essen Biosciences).

2.7. Phenotypic surface marker analyses

Surface marker expression of the thawed ASCs was determined using a mix of antibodies against ASC-positive (anti-CD73 APC, anti-CD90 BV510 and anti-CD105 BV421) and ASC-negative markers (anti-45 FITC and anti-CD31 FITC) (all antibodies purchased from BD Biosciences). Fluorescence minus one (FMO) staining was used as a negative control. Briefly, cells were thawed, washed in PBS-EDTA with 0.1% human albumin and centrifuged for 5 min at 300×*g*. Cells were then resuspended in brilliant violet staining buffer (BD Biosciences) and were incubated for 5 min with an Fc-blocking antibody. The antibody mix described above was then added and incubated for 30 min. The samples were subsequently diluted with PBS-EDTA containing 0.1% HA and then were analyzed using a FACS Canto II flow cytometer (BD Biosciences). The flow data were analyzed using Kaluza analysis software 2.1 (Beckman Coulter).

2.8. IFN-ɣ licensing - induction of IDO, PDL1 and PDL2

ASCs were seeded in T25 flasks (Nunc, Thermo Scientific) at 20 × 10^3 cells/cm^2 in culture expansion media supplemented with 25 ng/mL IFN-ɣ. Cells were cultured for 24 h and harvested by trypsinization. Half of the ASCs were stained for the presence of intracellular IDO protein using a fixation/permeabilization kit (BD Biosciences) following the manufacturer's instructions and using anti-human IDO1 Alexa Fluor® 647 (Clone V50-1886, BD Biosciences) antibody. Nonstimulated cells were used as controls. The other half of the harvested ASCs were stained with anti-PDL1 BV421 and anti-PDL2 APC antibodies (Biolegend). FMOs were used as controls. Stained ASCs were analyzed on a Canto II flow cytometer (BD Sciences), and the data were analyzed using Kaluza analysis software 2.1 (Beckman Coulter).

2.9. ASC and T cell coculture

ASCs were seeded in 96-well plates at a concentration of 25 × 10^3/well or 12.5 × 10^3/well. PBMCs from healthy donors were isolated from buffy coats using preloaded Leucosep tubes (Greiner Bio-One) according to the manufacturer's instructions. Isolated and washed PBMCs were stained with CFSE for 10 min. Briefly, 25 × 10^6 PBMCs were centrifuged and resuspended in 1 mL of prewarmed RPMI (with 100 U/ml penicillin/streptomycin and 2 IE/mL heparin), mixed with 1 mL of prewarmed 2 μM CFSE solution and then were stained for 10 min at 37 °C. To each well, 50 × 10^3 PBMCs were added at a 1:2 or 1:4 ASC-to-PBMC ratio. Finally, the cells were stimulated with 500 ng/ml staphylococcal enterotoxin B (SEB; Sigma-Aldrich) and were cultured for 4 days. T cell proliferation was measured by harvesting the cells and staining them using 7AAD and an anti-CD3 PE antibody (Beckman Coulter). Quantification of proliferating T cells was performed using counting beads (Stem-Count, Stem-Kit, Beckman Coulter). Cells were analyzed on a Navios flow cytometer (Beckman Coulter), and data were analyzed using Kaluza analysis software 2.1 (Beckman Coulter).

2.10. Statistical analysis

A one-way Anova was used to analyze viability, recovery, proliferation, expression and T cell proliferation data, comparing means of each type of freezeing media. Tukey's multiple comparison test was used to when comparing mean values. All statistical analyses were performed with computer software (GraphPad Prism, Version 5.02, GraphPad Software Inc.).

3. Results

3.1. Screening of different pentaisomaltose concentrations

Initially, we screened and compared 16% pentaisomaltose alone or different concentrations of pentaisomaltose (5%, 10% and 16%) in combination with 2% Me_2SO. Based on viability, recovery and proliferation data, 10% and 16% pentaisomaltose were chosen for further screening (Supplemental Material Fig. 1).

In addition, 10% and 16% pentaisomaltose in combination with 1% or 2% Me_2SO were also compared. Both pentaisomaltose concentrations showed similar viability and recovery, whereas proliferation data was slightly improved when using 10% pentaisomaltose (Supplemental Fig. 2). Based on these data and a general effort to use the lowest efficient CPA concentration possible, 10% pentaisomaltose in combination with 1% or 2% Me_2SO was chosen for further investigations.

3.2. Improved viability and recovery when combining Me_2SO and pentaisomaltose

When comparing the post-thaw viability (mean ± SD) of cells cryopreserved with different freezing media, STD10 (95.2% ± 0.9), CS10 (95.8% ± 0.6) and PIM2 (95.7% ± 0.5) showed a significantly higher viability than CS2 (89%.8 ± 0.9) ($p = 0.003$, $p = 0.013$ and $p = 0.021$, respectively) (Fig. 1A). Subsequently, no further progression in cell death was observed using the 24-hour viability assay (Fig. 1C).

The calculated post-thaw recovery of ASCs ranged from 78.9 to 93.8%. The recovery (mean ± SD) of cells in both PIM1 (93.56% ± 5.2) and PIM2 (93.8% ± 6.0) was significantly higher than that of CS2 (78.9% ± 3.6) ($p = 0.045$ and $p = 0.0411$, respectively) (Fig. 1B).

3.3. The ASC phenotype is conserved post-thaw, and ASCs cryopreserved with pentaisomaltose are able to undergo adipogenesis and chondrogenesis

Flow cytometry analysis of thawed samples showed that ASCs cryopreserved with the different freezing media formulations were negative for CD45 and CD31 and positive for CD73, CD90 and CD105 (Supplemental Fig. 3A). Further, the ASC samples cryopreserved in freezing media containing pentaisomaltose were able undergo adipogenesis and chondrogenesis (Supplemental Fig. 3B).

3.4. Comparable proliferation of cryopreserved ASCs

Although comparable ASC proliferation curves were obtained when cells were cryopreserved with each CPA combination (Fig. 2A), the estimation of hours to 100% confluency (e100%) was significantly longer for cells in CS2 (90.5 h) and PIM1 (90.4 h) compared to that for cells in CS10 (81.3 h) ($p = 0.005$ and $p = 0.006$, respectively) (Fig. 2B).

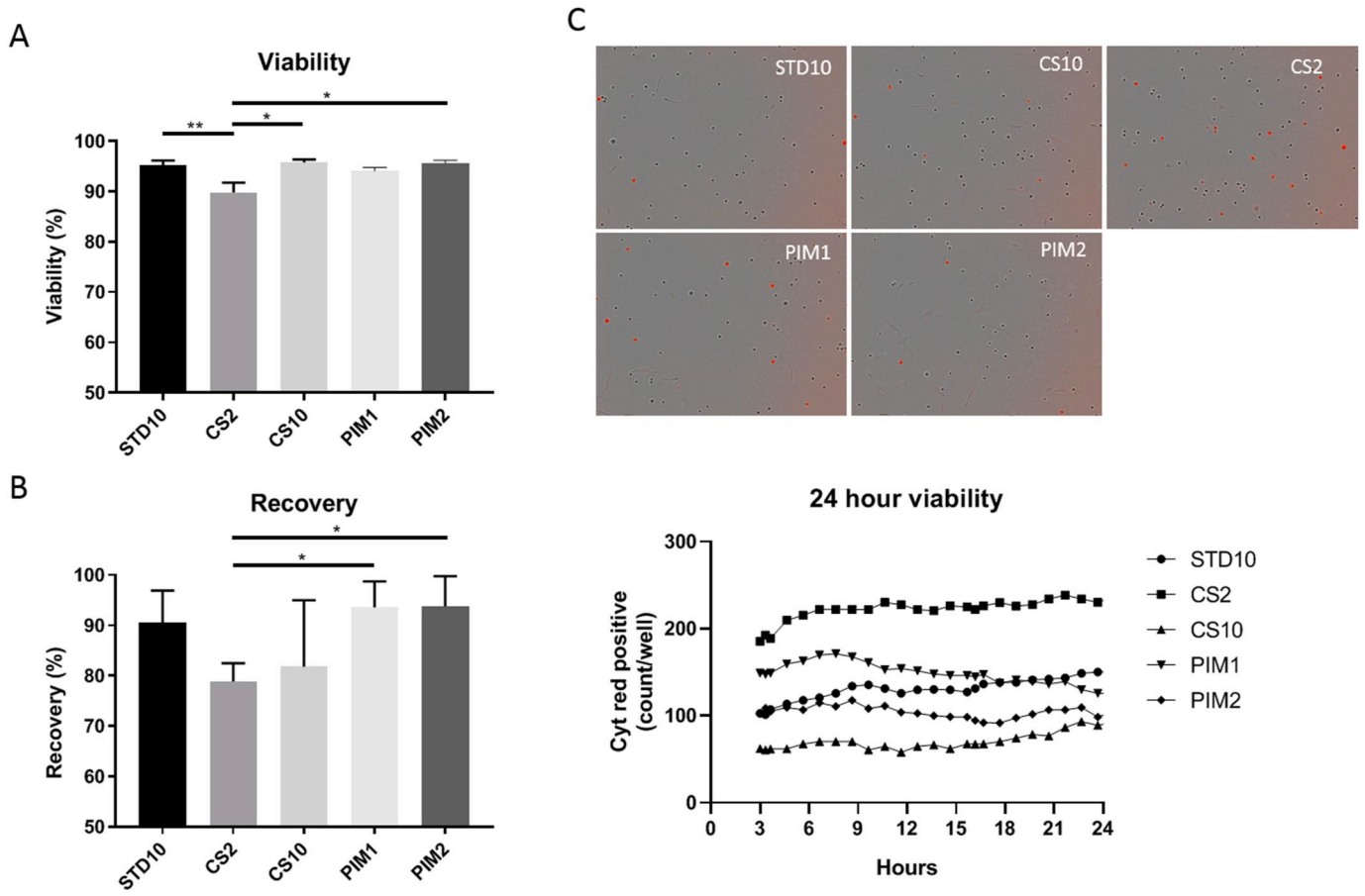

Fig. 1. Viability and recovery of cryopreserved ASCs.
Adipose-derived stromal stem cells (ASCs) were cryopreserved with STD10, CS2, CS10, PIM1 or PIM2; then, the cells were thawed and analyzed for (A) viability, (B) and recovery of live cells and (C) 24-hour increase in cell death. Significant differences between freezing media are presented as; * ($p < 0.05$) and ** ($p < 0.01$). Data represent 5 donors.

No significant difference was observed in the length of the lag phase or maximum population doubling time (max PDT) (Fig. 2C–D).

3.5. Comparable transwell migration potential

ASCs seeded in the transwell migration plate showed a comparable number of cells that migrated across the membrane. The mean ± SD number of migrating cells/well was 112 ± 18.9 (STD10), 116 ± 15.8 (CS2), 108 ± 16.6 (CS10), 113 ± 12.3 (PIM1) and 122 ± 18.2 (PIM2) (Fig. 2E). The number of migrated cells in the control wells was 79 ± 17.

3.6. Suppression of T cell proliferation and expression of immunosuppressive markers

The coculture of isolated PBMCs and cryopreserved ASCs showed that T cell proliferation was suppressed by all samples compared to PBMCs stimulated with SEB without ASCs (control). Suppression was decreased by increasing the ratio of ASCs to PBMCs from 1:4 to 1:2 (Fig. 3C). ASCs cryopreserved with STD10, CS10, PIM2 or CS2 showed significantly reduced T cell proliferation compared to PIM1 ($p < 0.0001$, $p < 0.0001$, $p = 0.0093$ and 0.0312, respectively). Furthermore, cells cryopreserved in CS10 showed significantly reduced T cell proliferation compared to that of PIM2 ($p = 0.0457$). However, only cells in CS10 showed significantly reduced T cell proliferation using both 1:2 and 1:4 ratios of ASCs to PBMCs when compared to that of PIM1.

When quantifying the number of proliferating T cells, ASCs cryopreserved with STD10, CS10, PIM2 or CS2 also showed significantly reduced T cell proliferation compared to that of PIM1 ($p = 0.0002$, $p < 0.0001$, $p = 0.0131$ and $p = 0.0293$, respectively). Using a ratio of 1:4 ASCs to PBMCs, CS10 showed significantly reduced T cell proliferation compared to that of PIM1 ($p = 0.011$) and PIM2 ($0 = 0.044$).

Flow cytometric analysis of the induction of PDL1 and PDL2 following IFN-ɣ licensing showed a comparable increase in PDL1 and PDL2 surface protein levels of approximately 4-fold and 2-fold, respectively, on ASCs cryopreserved by all the tested CPA combinations (Fig. 3A).

Intracellular staining of IDO1 also showed a comparable induction compared to non-IFN-ɣ licensed ASCs. All cryopreserved ASCs displayed an approximately 100-fold induction of IDO1 (Fig. 3B).

4. Discussion

In the current study, we compared different freezing media formulations for cryopreservation of adipose-derived stromal cells. We demonstrated that pentaisomaltose with 1–2% Me_2SO enabled successful cryopreservation when assessing recovery of viable ASCs, phenotype and differentiation, proliferative and migratory potential, and immunomodulatory effect (as shown by the ability to suppress T cell proliferation and express immunomodulatory markers).

ASCs cryopreserved with the different freezing media generally demonstrated comparable results with regards to i) proliferation, with the exception of CS10 showing a decrease in the estimated time to 100% confluency (e100%) compared to that of CS2 and PIM1, and ii) migration, indicating that they possess similar migratory potential towards an increasing gradient of human platelet lysate containing several chemoattractant factors such as PDGF-AB and TGF-β1 [19,20].

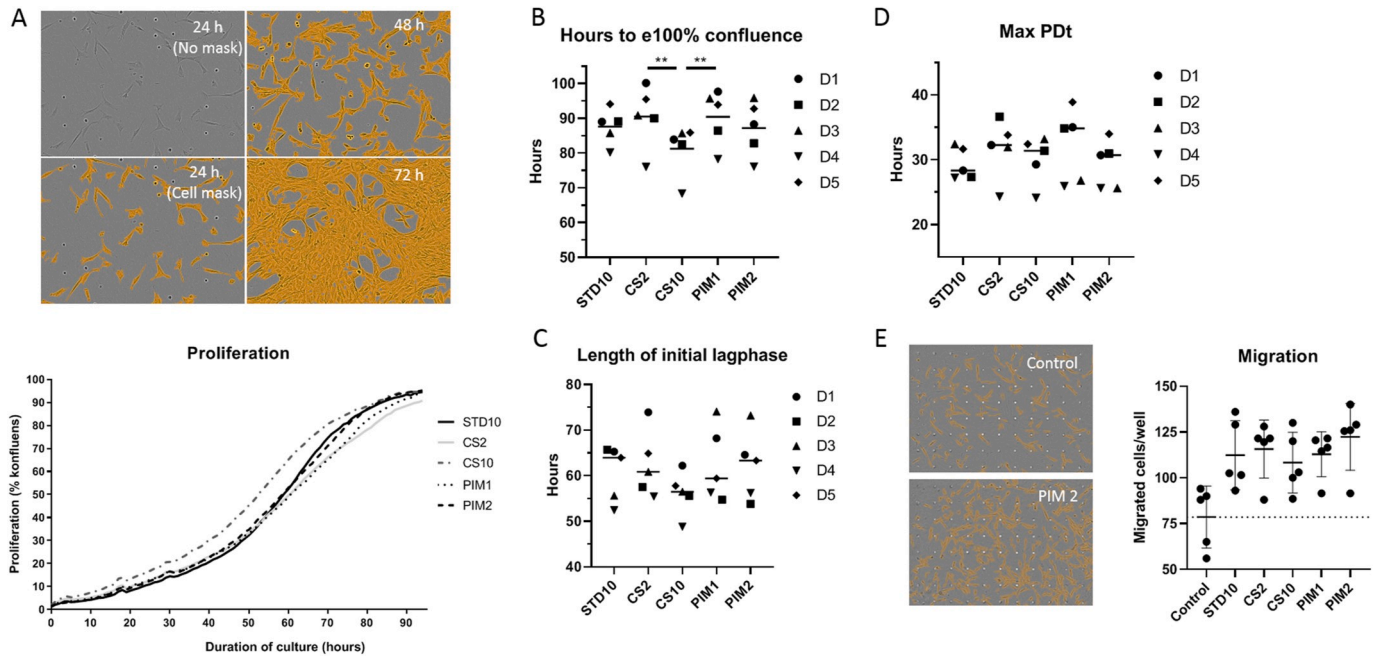

Fig. 2. Proliferation and migration potential of cryopreserved ASCs.
Adipose-derived stromal stem cells (ASCs) were cryopreserved with STD10, CS2, CS10, PIM1 or PIM2. Post-thaw assessment of the proliferative potential shown as (A) cumulative proliferation curves of ASCs shown as increase in confluency, (B) hours to estimated 100% confluency, (C) length of lag-phase and (D) maximum proliferation doubling time (fastest). (E) Migration towards a gradient of pooled human platelet lysate. Significant differences between freezing media are presented as; ** ($p < 0.01$). Data represent 5 donors.

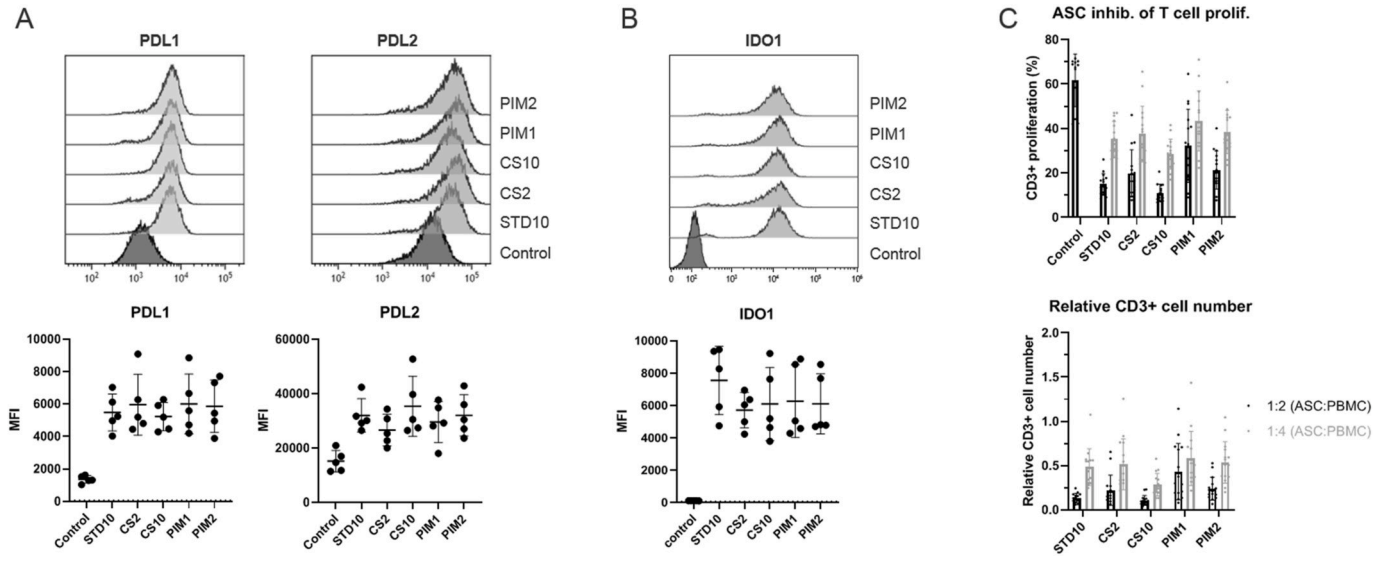

Fig. 3. Expression of immunosuppressive markers and suppression of T cell proliferation by cryopreserved ASCs.
ASCs were treated with IFN-ɣ for 24 h and then were investigated by flow cytometry for the expression of (A) PDL1 and PDL1 and (B) intracellular IDO1 protein. Non-IFN-ɣ treated ASCs served as controls. ASCs were cocultured with isolated PBMCs stained with CFSE in two different ratios (1:2 and 1:4). T cell proliferation was stimulated for 4 days with SEB, and (C) the percentage of proliferating T cells as well as the number of proliferating T cells assessed using beads were determined by flow cytometry. Data represent 5 donors.

The most significant difference between the tested cryomedia was the effect on the ability of ASCs to suppress the proliferation of T cells, indicating a Me_2SO concentration-dependent effect; ASCs cryopreserved with freeze-media containing 10% Me_2SO (CS10 and STD10) were more potent in inhibiting T cell proliferation in terms of the percentage of proliferating T cells and T cell numbers. ASCs may suppress T cell proliferation both by secreting soluble factors and by cell-to-cell contact [21–25]. Here, we investigated their ability to induce the expression of IDO1, which is a potent immunosuppressive molecule produced by ASCs [23,24], following post-thaw IFN-ɣ licensing. We found an approximately 100-fold increase in IDO1 protein levels after 24 h, regardless of the freezing media used. We also investigated IFN-ɣ-mediated induction of PDL1 and PDL2, which are two ligands known to be involved in the cell contact-dependent immunosuppressive function of ASCs [21,22]. Expression of both PDL1 and PDL2 was induced to comparable levels in a manner that was independent of the cryomedium. Thus, the observed

differences in their ability to suppress T cell proliferation do not seem to be related to the induction of IDO1, PDL1 or PDL2. However, ASCs are also able to suppress T cell proliferation by producing or secreting other soluble immunosuppressive factors, such as prostaglandin E2 (PGE2) or nitric oxide (NO), and by other direct cell contact-dependent mechanisms [24,25].

We have previously shown comparable viability, recovery, proliferative and differentiation potential of HSPCs cryopreserved with 10% Me_2SO (STD10) or 16% pentaisomaltose without the need for Me_2SO. However, ASCs cryopreserved with 16% pentaisomaltose alone showed a significantly reduced viability, recovery and proliferative potential compared to STD10 and 10% or 16% pentaisomaltose combined with either 1% or 2% Me_2SO, indicating the additive effect of combining a permeating CPA such as Me_2SO with a nonpermeating CPA such as pentaisomaltose. Furthermore, it shows that different CPAs have different cryoprotective efficiencies depending on the cell type. Differences can be caused by variation in parameters like cell surface area to volume, permeability of the cell membrane to water and the CPAs used and Arrhenius activation energy [26]. HSPCs and mesenchymal stromal stem cells are reported to differ in i) cell size (HSPC approximately 8–10 μm [27] and ASCs 16–20 μm in diameter (culture expanded, data not shown)) and thus in cell surface to volume ratio (approximately 0.6–0.75 and 0.3–0.375, respectively), and ii) permeability of the cell membrane to water (approximately 2.8–4.66 and 154 μm Pa^{-1} s^{-1}) and Arrhenius activation energy (approximately 26.8 and 56.6 kJ mol^{-1}), respectively [28–30]. Differences in these parameters could indicate why pentaisomaltose alone is more efficient for cryopreservation of HSPCs. Although HSPCs have a higher cell surface to volume ratio and lower activation energy, the significantly higher membrane permeability of ASCs to water could lead to excessive osmotic dehydration during freezing when using pentaisomaltose as the sole CPA. However, combining pentaisomaltose with 1–2% Me_2SO (permeating CPA) significantly improves the cryoprotective properties, which was especially observed in their proliferative ability.

A possible advantage of using pentaisomaltose as a nonpermeating CPA over e.g., Dextran-40 used in CryoStor formulations is that pentaisomaltose, a linear subfraction of dextran-1, is biocompatible and actually used to prevent dextran-induced anaphylactoid[ic] reactions (DIAR), which can be caused by even small amounts of high-molecular weight dextrans such as dextran-40 [31,32].

In conclusion, this study demonstrates that the addition of pentaisomaltose in the freezing media allows for the reduction in the concentration of Me_2SO needed for efficient cryopreservation of ASCs. The proliferative, migratory and immunomodulatory potential combined with the improved recovery indicate that it is possible to reduce the Me_2SO concentration to 2% (and potentially even to 1%) and retain a more potent cell product than that achieved using CS2. The results also show that lowering the concentration of Me_2SO to 1% or 2% reduced the ability of ASCs to suppress T cell proliferation compared to cryopreservation media using 10% Me_2SO (STD10 and CS10). Nevertheless, the increased number of recovered live cells might compensate for this effect. With the desire to reduce the amount of Me_2SO, these results suggest that a lower Me_2SO concentration of 2% and potentially even 1% in combination with 10% pentaisomaltose could be an effective and less toxic alternative to comparable freezing media.

Contribution

J.D.S. and L.M-F. designed the study, performed experiments, analyzed data, and wrote the manuscript. O.R.B performed experiments, analyzed data, and wrote the manuscript. P.T.B, F.R and P.V.V analyzed data and wrote the manuscript. E.K.H. and A. F–N. designed and the study, analyzed data, and wrote the manuscript.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cryobiol.2020.05.014.

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