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Cold Storage And Cryopreservation Animal-Component Free Solutions For Short And Long-Term Storage Of Cells

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Introduction

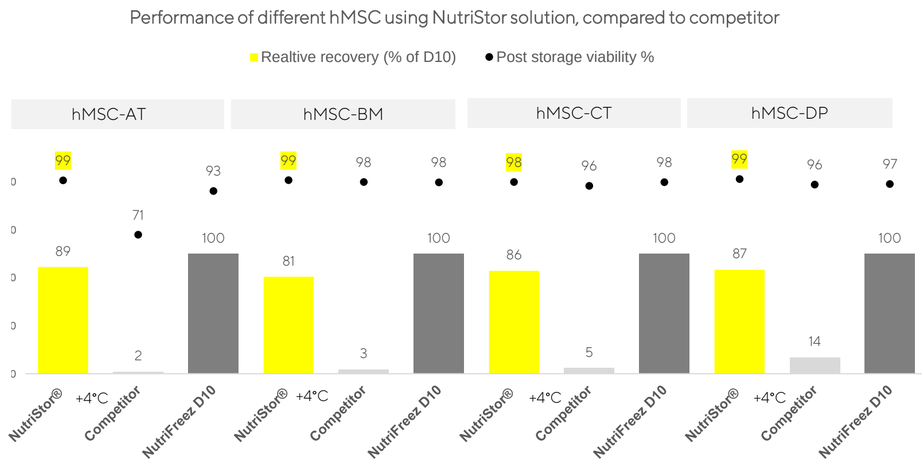
Storage of cells is a crucial step for cell-based products and for "off-the-shelf" cell therapy approaches. To date, the common practice is to cryopreserve the cells for long- term (years) using cryopreservation solutions composed of 10% DMSO or other toxic permeable cryoprotective agents (CPAs), such as Ethylene Glycol. Exposure of cells to these CPAs can impact the quality, safety, and efficacy of the cellular therapy product and clinical outcome. However, for applications that a short-term storage of cells (days) is required (e.g., therapeutic processes and shipment), cold storage alternative to the freezing step, is an advantage. Cold storage may reduce post-freezing necrosis and apoptosis, eliminates the risk of the exposure to undesired toxic CPAs and enables shipping stability. Facing strict regulatory requirements, the development of a defined solution for short- term storage of cells without any CPA as well as a cryopreservation product with a reduced concentration of DMSO, is required and holds a unique opportunity to advance the widespread implementation of cellular therapies. The current study presents the performance of NutriFreez® D5, a defined Animal Component Free (ACF), salt base cryopreservation solution with a reduced concentration of DMSO (5%) for cryopreservation of the cells and NutriStor®, a novel defined ACF, salt base solution, that enables short term cold storage of cells. Post storage cells viability, growth recovery and cell characterizations of various therapeutic cells (e.g., hMSC, pluripotent stem cells and immune cells), were successfully evaluated after being stored in NutriStor® and/or frozen in NutriFreez® D5. Continued work is invested in the development of the next generation, DMSO-free cryopreservation solution with non-toxic CPA alternatives.

NutriStor®

1. NutriStor suitability to various hMSC

NutriStor was designed for variety sources of hMSC (AT, BM, CT, DP). hMSC are increasingly being used in cell therapy-based applications and clinical trials. Figure 1 represents the impact of different bio-preservation solutions on variety sources of hMSCs - cells viability directly post 4 days of cold storage (2-8°C) and after being cultured for 3 days in XF culture system (relative proliferation - % of frozen cells using NutriFreez D10). hMSC were stored at conc. of 500 x10^3 cells/vial for 4 days in NutriStor and in competitor solution (at 2-8°C), simultaneously to cryopreservation of vials in NutriFreez D10 (as positive control). The growth recovery of cells were then evaluated. The results show that NutriStor promotes high viability and recovery of hMSC with similar performance as frozen cells using NutriFreez D10 and with significant advantage over commercially available bio-preservation solution.

(A)



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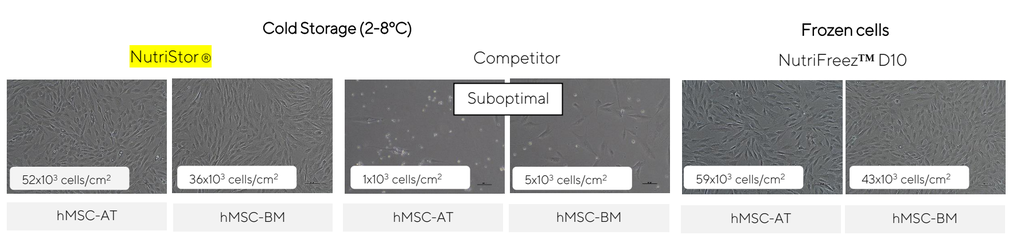
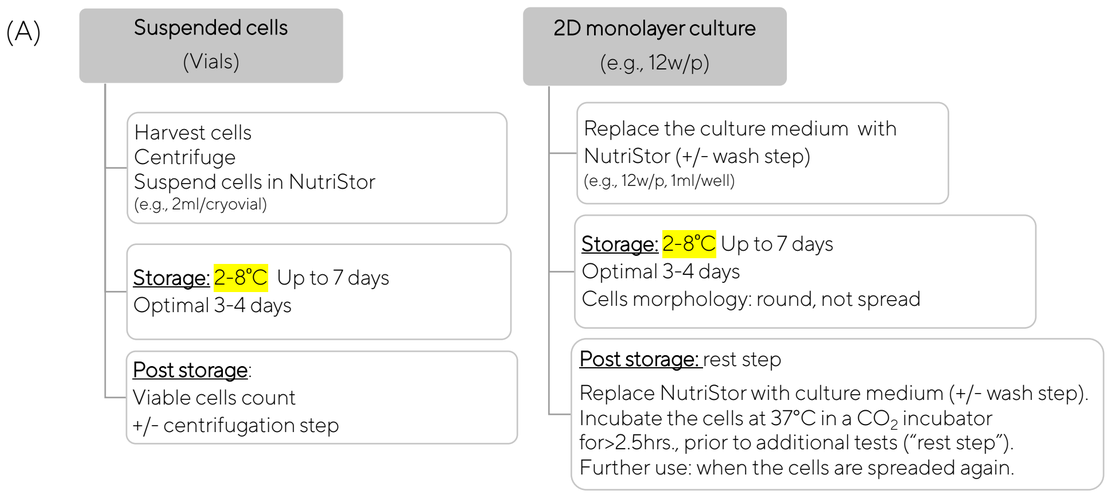


Figure 1: Summary performance results of various sources of hMSC post storage and recovery using NutriStor vs. other commercial bio-preservation solution (competitor) and cryopreservation solution (NutriFreez D10). (A) Post 4 days storage viability (%), and relative proliferation (% of frozen cells) post 3 days of culture in MSC NutriStem XF medium. (B) Representative images (x100) taken 3 days post seeding after storge (cells growth recovery). The numbers represent the viable cells count at day 3 (proliferation).

2. NutriStor available applications

NutriStor enables cell storage in both vials (suspension) and plates (2D monolayer culture). Figure 2 represents a schematic workflow for using NutriStor for short-term storage and the comparison of the two applications: cells viability directly post 4 days of cold storage (2-8°C) and after being cultured for 3 days in XF culture system (viable cells count).

(A)



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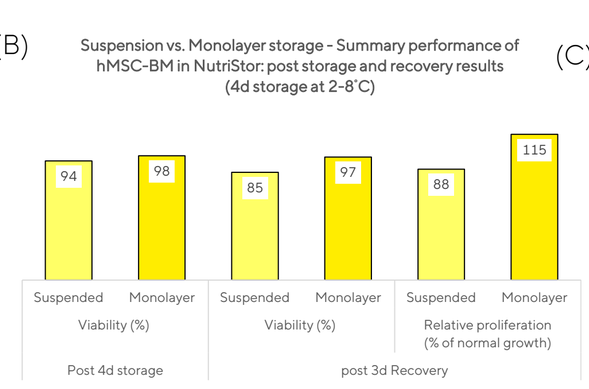

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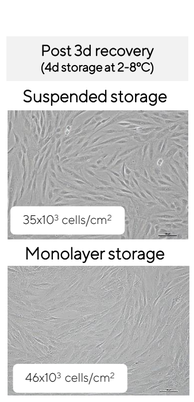

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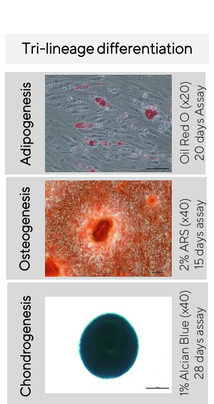


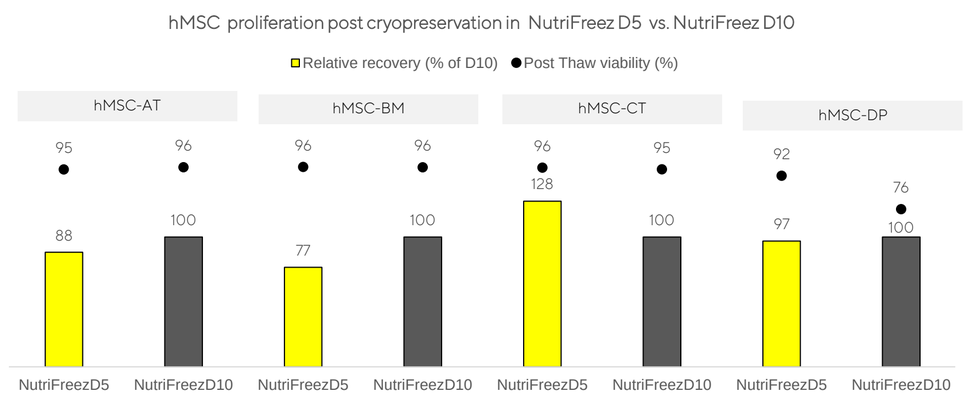
Figure 2: (A) Schematic work diagrams for practical applications using NutriStor. (B) hMSC performance post 4 days storage: Post storage cells viability (%), recovery viability (%) and relative growth after cells reseeding (% of the average of cells proliferation after 3 days in MSC NutriStem XF medium , PDL 3, 40,000 cells/ cm²). (C) Representative images (x100) taken 3 days post seeding (cells growth recovery). The numbers bellow represent the viable cells count (proliferation). (D) Representative images of hMSC' Tri-lineage differentiation ability after being stored for 4 days at 2-8°C in NutriStor suspension storage).

NutriFreez® D5

3. NutriFreez D5 is optimal for hMSC

Cryopreservation is a crucial step for a scalable manufacturing process for hMSC therapies. NutriFreez D5 was developed for optimal cryopreservation of hMSC. Figure 3 represents the performance of NutriFreez D5 on different sources of hMSC and the impact of different cryopreservation solutions (represented on hMSC-BM), directly post thawing (cells viability) and after being cultured for 3 days in XF culture system (cells viability and proliferation). Cells were cryopreserved at conc. of 200-500 x10^3 cells /ml for 7 days in cryopreservation solutions followed by growth recovery evaluation. Results show that the novel salt based freezing solution, NutriFreez D5 (composed of 5% DMSO) promotes high viability and recovery of hMSCs of various sources with similar performance as NutriFreez D10 (composed of 10% DMSO) and with advantage over commercially available freezing solution contains of 5 % DMSO.

A)



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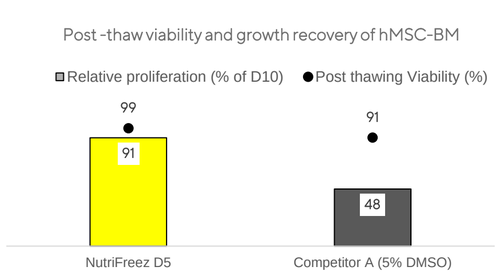

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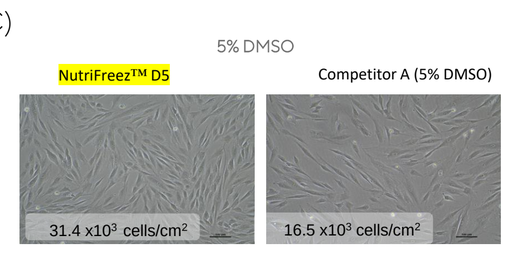


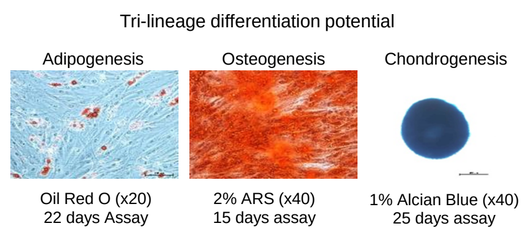
Figure 3: hMSC evaluation post 7 days of cryopreservation in NutriFreez D5 vs. NutriFreez D10 and commercial freezing solution composed of 5% DMSO. (A) Performance e of different hMSC sources after freezing, including Post thaw cell viability (%) and proliferation (viable cells count). (B) NutriFreezD5 vs. 5% DMSO competitor freezing solution. Post thaw cell viability (%) and proliferation (viable cells count). (C) Representative images (x100) taken 3 days post seeding (cells recovery). The numbers represent the proliferation rate (viable cells count).

4. Maintaining of hMSC features

hMSC-BM were frozen for 7 days in NutriFreez D5 followed by thawing and seeding in XF culture system (MSC NutriStem XF) for evaluation of hMSC features (tri-lineage differentiation potential, self- renewal potential, marker expression and genomic stability). The differentiation assays were done using MSCgo differentiation media.

The results indicate that hMSC after being frozen in NutriFreez D5 maintain their unique features.

A)



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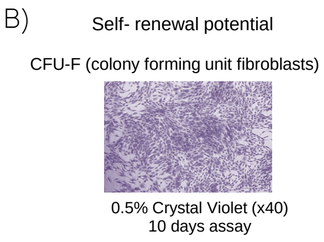

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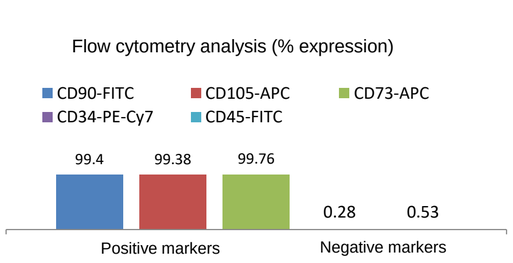

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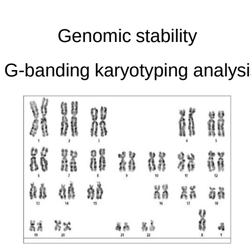
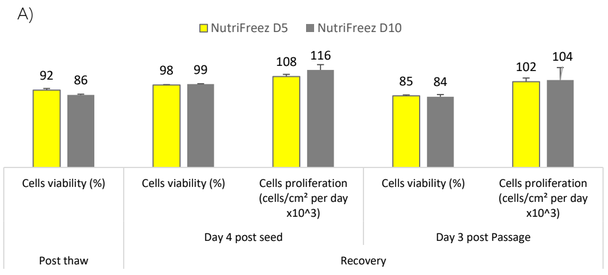


Figure 4: (A) Representative images of stained adipocytes (x20) after 22 days assay, stained osteocytes (2% Alizarin Red, x40) after 15 days assay, and stained chondrocytes (Alcian Blue, x40) after 25 days assay. (B) Representative image of mature colony (>50 cells /colony) stained with 0.5% Crystal violet after 10 days of CFU-F assay. (C) Immunophenotyping results of hMSC-BM using flow cytometry analysis. (D) G-banding karyotype analysis of hMSC-BM.

5. Human embryonic stem cells cryopreservation

The suitability of NutriFreez D5 to cryopreserve human embryonic stem cells was tested utilizing hESC cultured as single cells in XF culture medium (NutriStem hPSC XF) on laminin matrix (LN521). The assay was done in comparison to NutriFreez D10. hESC were cryopreserved (in triplicates) at conc. of 200 x10^3 cells /ml for 11 days in each cryopreservation solution followed by evaluation of growth recovery and hESC characteristics. NutriFreez D5 found applicable for hESC similar to NutriFreez D10.

A)



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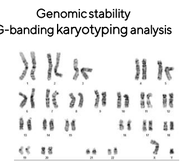

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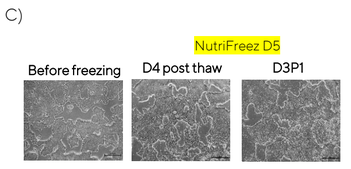

Figure 5: Growth recovery results of hESC post cryopreservation with NutriFreez D5 and NutriFreez D10. (A) Post thaw cell viability (%), recovery cell viability (%) and proliferation rate (viable cells count during 2P). (B) G-banding karyotype analysis of hESC (D3P1). (C) Representative images (x40) taken before and after freezing in NutriFreez D5, during 2 passages (cells recovery).

6. Conclusion

Storage of cells is a crucial step in cells'-based therapy that may impact the clinical outcome. The data presented demonstrates two applicable options for cells storage . A novel bio-preservation solution with flexible applications, NutriStor, for short term storage at 2-8ºC (without a freezing step), and upgraded cryopreservation solution with 5% DMSO, NutriFreez D5, enables long term cells storage (-196°C) suitable for hMSC as well as other cell types e.g., hESC.

Both solutions hold the following features :

- High grade salt-based solutions, ready-to-use, chemically defined, animal component free, protein-free, serum-free.
- Enable optimal storage of various of hMSC sources, providing high quality assured cells.
- Show advantage over commercially available solutions.