Post-thaw stability of dental pulp derived stem cells after long-term cryopreservation

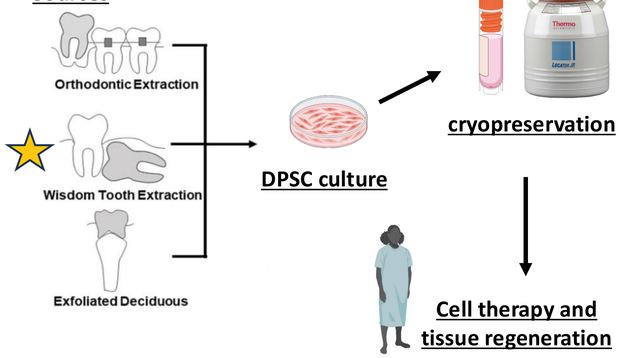
Malika A. Malik, Elizabeth Perkins, Alyssa Nedell, Hitesh Chopra, Jim Sugai, Darnell Kaigler*
University of Michigan School of Dentistry

BACKGROUND

- DPSCs are an ideal cell type for use in many regenerative stem cell therapies. Compared to other adult-derived stem cell sources, DPSCs can be readily obtained from extracted healthy third molars, premolars, which are mostly extracted during orthodontic treatment, human exfoliated deciduous teeth and supernumerary teeth.
- The previously described extractions are usually performed at a period of life when isolated dental pulp stem cells are not needed. To this end, cryopreservation represents an integral part of obtaining cell numbers for autologous or allogenic stem cell therapies and facilitating its off the shelf use.
- To enhance public accessibility, stem cells must be produced on a large scale and stored as off-the-shelf products for immediate use. A major challenge lies in ensuring that cellular properties and phenotypes remain unaffected during long-term storage and large-scale production. While cord blood banking services are widely available, there has been a recent increase in banking services for dental pulp-derived stem cells.
- DPSCs are able to maintain their regenerative properties after short-term cryopreservation. However, the functional phenotype of DPSCs cryopreserved after two years is not fully understood.
- **Therefore, an important question is how long do isolated DPSCs maintain their stemness features after long term storage in liquid nitrogen.**

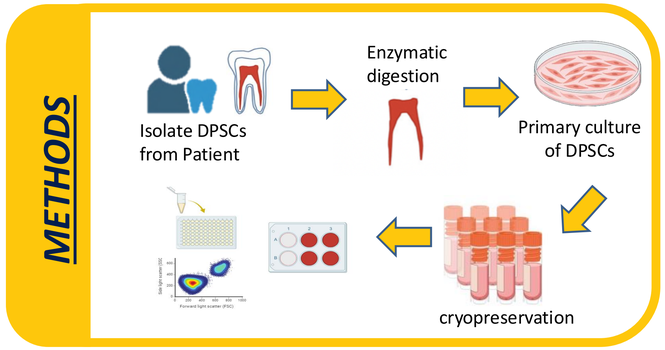
DPSCs for Regenerative Therapies

Dental Pulp Stem Cell Sources



AIM: The aim of the current study is to quantitatively investigate the impact of a standard cryopreservation procedure on cellular viability and functionality over a period of up to 13 years.

METHODS & RESULTS



Cell viability and proliferation potential

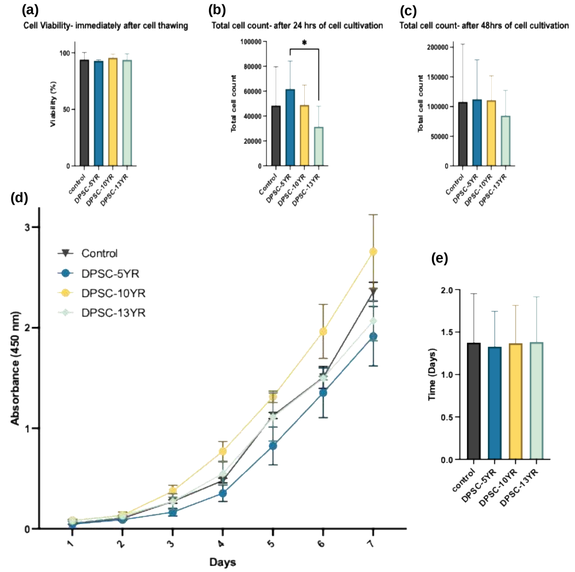


Figure 1: . Viability of DPSCs measured using trypan blue exclusion method (a) immediately after thawing (b) after 24 hrs of cell cultivation (c) after 48 hrs of cell cultivation. **p > 0.05* (d) The cellular growth curve after cryopreservation. (e) Cell proliferation, as measured by the PDT, between control versus cryopreserved groups. Data are presented as a mean and SD plotted as error bars

Senescence and Stemness

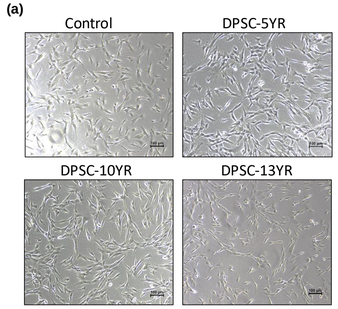
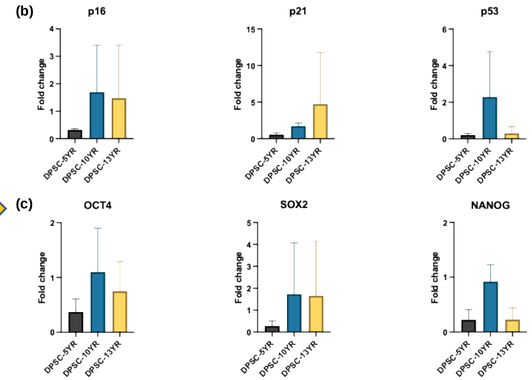


Figure 2: (a) Senescence-associated β-galactosidase activity of DPSCs at P6. Experiments were performed in triplicates, and representative images are shown. The images were captured at 4X magnification. Scale bar = 500 um. RT-PCR analysis of (b) senescence and (c) stemness markers on cryopreserved DPSCs. Data are presented as a mean, and SD is plotted as error bars.



Multipotency of DPSCs

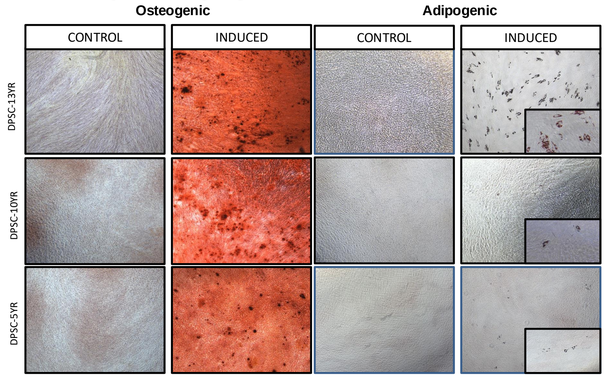


Figure 4: (a) Osteogenic differentiation assay. Representative images after alizarin red s staining with or without osteogenic induction. (b) adipogenic differentiation assay. Representative images of Oil Red O staining showing adipocyte-like cells with Oil Red O-positive droplets. Experiments were performed in triplicates and the representative images are shown. The images were captured at 4X magnification. Scale bar = 500 um

Phenotypic expression

Figure 3: presents flow cytometry analysis of surface marker expression in dental pulp stem cells (DPSCs) after cryopreservation compared to a fresh control group. The percentage of positive cells and their standard deviation (SD) are reported for each marker.

	CD34	CD45	CD73	CD90	CD105
control	0.6%±0.4	0.6%±0.4	99.9%±0.1	99.9%±0.1	96.4%±2.5
DPSC-5YR	3.8%±3.0	0%±0.1	99.9%±0.3	99.8%±0.2	99.3%±0.3
DPSC-10YR	1.4%±0.3	0%±0.1	99.6%±0.1	99.9%±0.5	90.9%±0.8
DPSC-13YR	1.3%±1.3	0%±0.1	99.9%±0.1	99.8%±0.1	99.9%±0.1

SUMMARY

- These findings suggest that cryopreservation has a minimal effect on DPSC viability within this experimental framework.
- Evaluation of proliferation demonstrated no statistically significant differences in PDT among the cryopreserved groups, with values of 1.32 ± 0.41 for DPSC-5YR, 1.36 ± 0.44 for DPSC-10YR, and 1.38 ± 0.53 for DPSC-13YR, comparable to DPSCs cryopreserved for less than one year (1.37 ± 0.57).
- Senescence-associated β-galactosidase staining revealed an absence of senescent cells at passage 6 and while analysis of gene expression showed variability between groups, no statistically significant differences were observed among the groups.
- Flow cytometry analysis revealed that DPSC-5YR, DPSC-10YR, and DPSC-13YR exhibited high expression (>90%) of CD73, CD90, and CD105, while CD34 and CD45 expression remained low (<4%).
- Multipotency analysis confirmed that osteogenic-induced cells formed mineralized nodules, whereas adipogenic-induced cells accumulated Oil Red O-positive lipid droplets, indicating the retention of multipotent differentiation potential post-thaw across all groups.

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