Cryopreservation of human amniotic fluid-derived stem cells for clinical and research purpose

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Objective

Cryopreservation of stem cells for future use is becoming more important, including for perinatal applications. Herein we compared different freezing protocols for the cryopreservation of human Amniotic Fluid-derived Stem Cells (hAFSC) harvested at the time of clinically indicated amniocentesis.

Methods

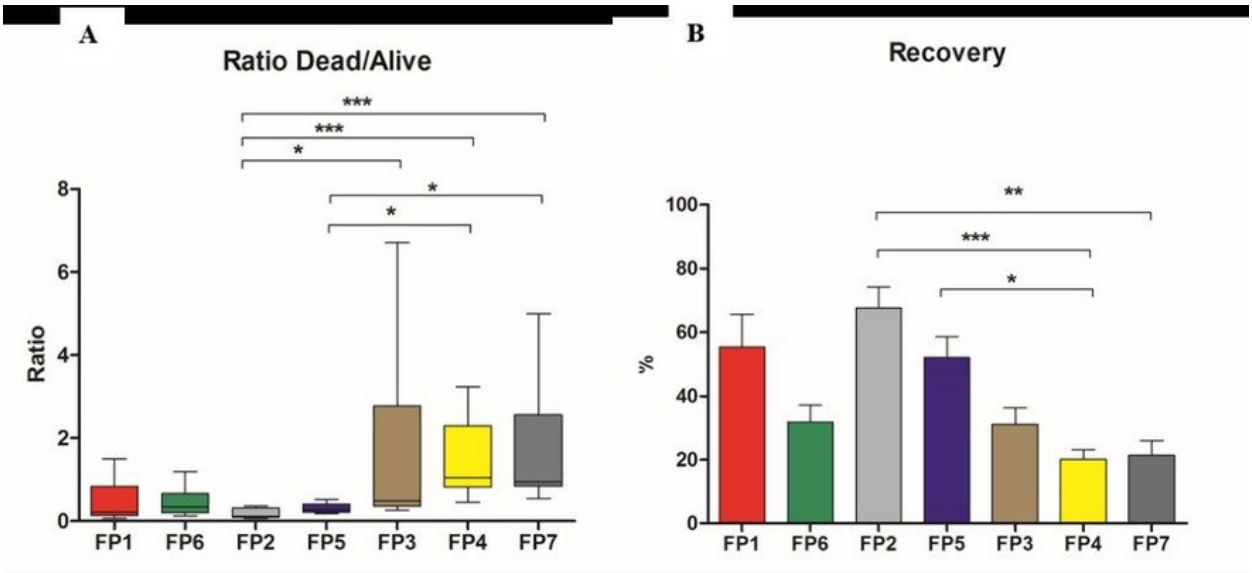
7 different freezing protocols were initially tested, i. e. protocol 1 (10% DMSO), protocol 2 (2, 5% DMSO, caspase inhibitor, bio-antioxidants and catalase), protocol 3 (5% glycerol, caspase inhibitor, bio-antioxidants and catalase), protocol 4 (Sperm Freezing medium), protocol 5 (Slow Freezing solution), protocol 6 (ethylene glycol, sucrose and ficoll70) and protocol 7 (Vitrification solution). Initially testing was done on 2 well-established hAFSC lines (6 independent experiments), with cell viability, recovery, doubling time and mesenchymal stem cell markers following thawing as outcome measures. Best performing freezing protocols (1, 5 and 6) were subsequently tested on freshly harvested amniotic fluid (n=29).

Results

Initial comparison demonstrated that protocols 1, 2, 5 and 6 resulted in successful recovery of hAFSCs using cell viability and recovery ratio (no statistical difference between these groups). Differences in CD marker expression resulted in exclusion of protocol 2. Protocols 3, 4 and 7 proved unsuccessful as suboptimal recovery of cells could be obtained. Protocols 1, 5 and 6 were further examined on fresh amniocentesis samples (n=11) and the reproducibility of these protocols was confirmed in another facility on 18 consecutive samples to exclude inter-researcher variability (p=0. 5497).

Conclusion

We have identified 3 cryopreservation protocols suitable for hAFSC. All of these protocols resulted in a high cell recovery and did not change the stem cell characteristics. Given one of these (Slow Freezing solution) is compatible with current GMP-legislation, this may be clinically used.



AFSCs cell viability and recovery.

Cell viability and recovery of AFSC-C1 and AFSC-C2 immediately after thawing was quantified with the Trypan Blue exclusion dye.

A. Viability is represented as the ratio of dead / alive cells for each freezing protocol.

B. Recovery is represented as the percentage of surviving cells after thawing.

** p < 0.05; ** p < 0.01; *** p < 0.001; FP: freezing protocol; FP1-FP6: n=9; FP7: n=8*