Recovery of Viable MSCs Isolated From Fresh Umbilical Cord Tissue, Measured After Cryopreservation, Is on Average 8-Fold Higher when Compared to Recovery of Viable MSCs Isolated From Previously Cryopreserved Umbilical Cord Tissue

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Background.: The human umbilical cord (hUC) is comprised of a gelatinous connective tissue that contains mesenchymal-like stem cells (TC-MSCs), proteoglycans and collagen. Isolation and cryopreservation of TC-MSCs for potential therapeutic use is an active area of research in the stem cell banking industry. We have compared two methods of cryopreservation to determine the optimal method for storing TC-MSCs: (1) cryopreservation of isolated TC-MSCs from fresh hUC tissue; and, (2) cryopreservation of intact hUC tissue followed by TC-MSC isolation.

Methods.: hUCs were aseptically obtained, and parallel hUC segments from the same hUC were processed by one of the two methods. Method 1: TC-MSCs were isolated from partially dissected fresh hUC segments by overnight collagenase digestion (2.5mg/mL-g), filtration and centrifugation. Isolated TC-MSCs from this process were cryopreserved in a cryopreservation solution (11% DMSO, 1% Dextran40, 4% HSA in 0.9% NaCl), frozen and stored at –80°C for < 30 days. Cells were rapidly thawed at 37°C, washed and resuspended in D-PBS for flow cytometric analysis. Method 2: hUC tissue was cryopreserved in the cryopreservation solution as in Method 1, frozen and stored at –80°C for < 30 days. Frozen hUC tissue was rapidly thawed at 37°C and processed as described above to isolate the TC-MSCs. TC-MSCs recovered from each procedure were enumerated and phenotyped by flow cytometry.

Results.: The recovery of viable CD45–/CD90+ TC-MSCs (after thawing) from fresh hUC tissue (Method 1), on average resulted in 5.04x10⁵ cells/gram of tissue (n=15), whereas the yield of CD45–/CD90+ TC-MSCs from cryopreserved/thawed hUC tissue (Method 2) resulted in 8.20x10⁴ cells/gram of tissue (n=15). Cell viabilities from both processes were comparable.

Conclusions.: Significantly higher (p<0.001) viable cell recoveries (8.4-fold average, 6.8-fold median, range 2.1–18.1) were obtained when TC-MSCs were harvested prior to cryopreservation.


Footnotes
* Asterisk with author names denotes non-ASH members.

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