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DEVELOPMENT OF A QUANTITATIVE MEASUREMENT OF MESENCHYMAL STEM CELLS EXPLANT OUTGROWTH FROM UMBILICAL CORD TISSUE

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Background

Mesenchymal stem cells (MSCs) hold great promise in regenerative medicine and are actively being researched for a number of potential therapeutic benefits. The umbilical cord (UC) tissue can be easily collected in a non-invasive procedure following delivery and is a rich source of MSCs and other progenitor cell populations with potential therapeutic or research value. Cryopreserving UC as a composite material, in its raw state, allows for future isolation of any desired cellular component. MSCs can be harvested from fresh or cryopreserved UC in a robust and reliable manner by explant outgrowth with no apparent impact on functionality.[1,2] Characterization of explant outgrowth results can provide information about the quality of the tissue from which the MSCs were isolated. We have, therefore, developed a novel scoring assay for the purpose of quantifying explant outgrowth results which allows for statistical analysis of cell recovery and can be used with both fresh and cryopreserved umbilical cord tissue.

Study Design

Donated umbilical cords (n=10) were collected from consenting mothers and transported to a processing facility. Upon receipt, the cords were subjected to a series of rinses consisting of buffered saline and an antiseptic wash. The cords were then segmented into small sections. A portion of the UC was plated fresh while the remaining pieces were prepared for cryopreservation and stored at -196°C in a DMSO-based clinical grade cryopreservation freeze media (CryoStor®, BioLife Solutions). After at least 1 month in the vapor phase of liquid nitrogen (average 62.3 days), cryopreserved tissue was rapidly thawed, taken through a series of buffered saline rinses, and a portion of the material (<1 gram total) plated.

A tissue explanting procedure was designed to facilitate reliable and consistent quantification of growth results between samples, rather than to optimize cell yield. Fresh or thawed tissue segments were cut into smaller, uniform pieces and placed at regular intervals in a 10 cm culture dish. Tissue pieces were arranged in a 5 x 5 grid pattern (Figure 1) within the dish and incubated dry for 10 minutes to allow for secure attachment prior to media addition. Plating was performed in duplicate for fresh or frozen tissue from each cord. Media was exchanged after 7 days of incubation at 37°C, at which time the tissue pieces were discarded. After a total of 14 days in culture, the plates were evaluated and scored.

To evaluate outgrowth results, plates were observed under a microscope at 100X magnification. For each plate, each grid location was observed and assigned a score on a scale of 0 to 4 based on degree of cell attachment and cell proliferation (Figure 2). Cells were then detached from the plate with trypsin and enumerated. Percent viability was determined by trypan blue dye exclusion.

Findings

All fresh and frozen UC units yielded proliferating, plastic adherent cells with fibroblastic morphology. Flow cytometric analysis had previously confirmed that the majority of cells isolated through this explant method have a phenotype consistent with MSCs (data not shown). Average plated tissue weight, % viability, plate score, and cell yield per gram of tissue for fresh and frozen samples are given in Table 1. There was no difference in average plated tissue weight between fresh and frozen samples ($p>0.05$). Mean viability of harvested cells was 98.3% with no difference observed between fresh and frozen samples ($p>0.05$). Mean plating score was 71.8 and the average frozen sample score did not differ significantly from the average fresh sample score ($p>0.05$). Likewise, cell yield per gram did not differ significantly between fresh and frozen units ($6.2x10^5$ and $6.9x10^5$ cells/gram respectively; $p>0.05$), with an average yield across all samples of $6.5x10^5$ cells/gram. Biological variability between cords was observed as qualitative differences in explant outgrowth results between units and was confirmed by the quantitative scoring measurements (Figure 3). Number of confluent colonies (number of locations per plate receiving a score of 4) was a good predictor of cell yield (R^2=0.84; data not shown).

Figure 1.
Example explant plating layout.

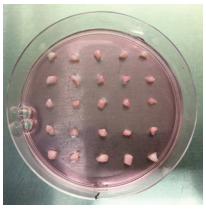
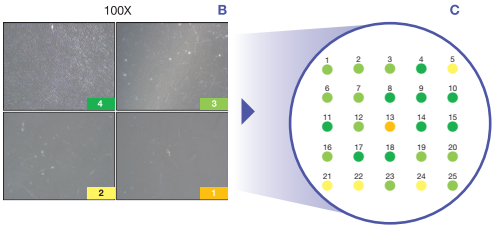


Figure 2.
Explant scoring matrix.

Score	Attachment	Growth
4	YES	CONFLUENT
3	YES	YES
2	YES	MINIMAL
1	MINIMAL	NO
0	NO	NO



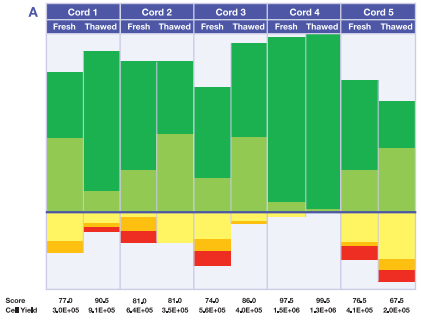
Each grid location was assigned a score on a scale of 0 to 4 based on degree of cell attachment and cell proliferation (A and B). An example of one plate from Cord 1 of the study is depicted (C).

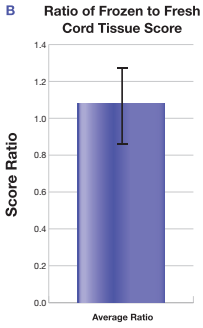
Table 1.
Average explant metrics for biologically identical fresh and frozen cord tissue units.

	Number of Cords with Successful Isolation of Proliferative Cells (%)	Plated Weight (gram)	% Viability	Score	Cells/Gram
Fresh (n=10)	10 (100%)	0.75	98.2	69.3	$6.2x10^5$
Frozen (n=10)	10 (100%)	0.69	98.3	74.3	$6.9x10^5$
Total Average	NA	0.72	98.3	71.8	$6.5x10^5$
p-value Fresh:Frozen	NA	>0.5	>0.5	>0.5	>0.5

Figure 3.
Explant scoring results comparing fresh and frozen tissue.

Heat map comparing outgrowth from explants of representative fresh and frozen umbilical cord tissue units (A). Fresh platings indicate biological variability between cords at the time of receipt, prior to freezing. Frozen cord tissue units consistently yield adherent, proliferating cells. Based on the average ratio of frozen to fresh scores from 10 cord tissue units, explants from biologically identical fresh and frozen cord tissue are comparable (B).





Conclusion

In a stem cell bank, manufacturing must be optimized for robustness to minimize the impact of non-controllable factors, such as biological variability. Cryopreserving UC as a composite material allows for isolation of the desired cellular component at the point of care when the specified clinical application is known. We have developed an explant plating and scoring system that provides quantifiable information about UC quality.

Previous studies have reported that cells harvested and expanded from UC explants express a surface marker profile consistent with MSC phenotype, and that no obvious differences are apparent between cells isolated from fresh and previously cryopreserved UC.[1] Here we expand on those findings by also demonstrating reliable and consistent isolation of cells by explant outgrowth from UC units collected and cryopreserved as a composite material at -196°C.

The present plating procedure and scoring matrix may be useful to determine the quality of umbilical cord tissue cryopreserved as a composite material for future isolation of MSCs. Additionally, the correlation of confluent colonies suggests it can be used to rapidly estimate cell yield which may be beneficial for process monitoring in a large volume biobank setting and can guide product development. Explant outgrowth is a robust isolation method and cryopreservation of UC as a composite material did not compromise cell recovery from explants with fresh and frozen tissues yielding similar numbers of cells when normalized by weight of tissue plated. Scoring metrics supported this observation with no significant difference in average score between fresh and frozen tissues. Uniform plating of tissue combined with a normalized scoring matrix provides a quantitative measurement of explant outgrowth, useful for statistical analysis of biological variability and process monitoring without product sampling or the successive stresses of enzymatic digestion and freezing.

Citations

1. Choudhery, M.S., Brown, K.S., Harris, D.T. Differentiation of MSCs isolated from cryopreserved cord t issue. Abstract Presentations from the AABB Annual Meeting. *Transfusion*. (2012)52:10A–229A.
2. Iftimia-Mander A., Hourd P., Dainty R., Thomas R.J. Mesenchymal stem cell isolation from human umbilical cord tissue: understanding and minimizing variability in cell yield for process optimization. *Biopreserv Biobank*. (2013)11(5):291-8.