BACKGROUND

Umbilical cord tissue (CT) can be readily collected in a non-invasive procedure and is a rich source of mesenchymal stem cells (MSCs) and other progenitor cell populations with potential therapeutic value. A systematic analysis of the reliability and robustness of explant outgrowth and engraftment of fresh umbilical cord tissue (CT) for the purpose of MSCs isolation concluded that explant outgrowth is highly amenable to large-scale biobanking operations. Furthermore, explant outgrowth can be utilized to isolate MSCs from either fresh cord tissue or tissue cryopreserved as a composite, or raw, material. Cryopreservation of CT as a composite tissue maintains native architecture and biochemical properties of the inter-cell movement/stromal matrix, storage, qualities that are necessary for the successful isolation of MSCs.

In a typical explanting procedure, tissue is placed in cultureware in the presence of a supportive medium until the cells of interest have migrated onto the growth surface. The tissue is then collected and the cells are either harvested or allowed to continue in culture. Several studies have shown the feasibility of a repeated explant culture scheme in which explanted tissue is not discarded but instead plated as a new explant to isolate additional primary cell material. However, to our knowledge, no study has not systematically examined the consistency of cell outgrowth from each individual explant as opposed to CT as a whole unit. Here, we evaluated the use of a repeated CT explant culture for cell isolation with an assay that allows for characterization and quantitative measurement of outgrowth from individual explants.

METHODS

Donated CT (n=4) was collected from consenting mothers, transported to a processing facility, washed, and segmented into small pieces. A portion of each piece was explanted fresh to cryopreserved/while another portion was cryopreserved and stored at -196°C in a clinical-grade, CryoStor® Biodegradable freeze medium (CryoStor® BioSafe Solution) for at least 1.5 years then thawed and explanted. For explant cultures, smaller tissue pieces were cut from the fresh or frozen tissue and placed on tissue culture plates in a gelled pattern with 24 explant locations for each plate. After 7 days, the tissue pieces were removed from the plate and the medium exchanged. For explant cultures, the tissue pieces were discarded. For thawed platings, the tissue pieces were transferred to 7 tissue wells of a 25-mm plate (1 tissue piece per well) and medium was added. Fold cell recovery for each plating, from the initial thawed plating through four serial replatings, the average change in score did not differ significantly from the average score of fresh or initial thawed platings.

RESULTS

All fresh and cryopreserved CT units yielded proliferating, plastic-adherent cells with fibroblastic morphology, yielding a 100% rate of success for isolation. Fresh and frozen CT from the same donor were comparable, as evident from the ratio of fresh to frozen (“Thawed, Plate 1”) scores (Table 1), similar to what has previously been described. When compared to the average score of initial thawed platings, the average score of explant platings did not differ significantly from the average score of fresh or initial thawed platings.

Outgrowth of adherent cells from individual tissue pieces was fairly consistent, as shown in Tables 2 and 3. On average for each plating, from the initial thawed plating through four serial replatings, the average change in score for a single tissue piece was 0.02±0.37 points. Outlining the comparison between the initial thawed platings and the first replating, in which the average score was 0.02±0.37 points, there was an average change in score to a single tissue piece was 0.05±0.16 points. The overall average score range for individual tissue pieces was less than 2 points (1.50±2.23) and nearly 75% of wells exhibited minimal change in score over five serial platings.

Total cumulative cell yield per CT unit was increased on average by 25 fold through serially replating and harvesting of cells that continued to migrate out of explanted tissue pieces, as shown in Figure 2.

CONCLUSION

Using an enzyme-free approach, we show consistent outgrowth from individual explants of cryopreserved CT over five serial platings. The capacity of explants to generate cell outgrowth did not appear to diminish over multiple replatings. Furthermore, explant score for individual tissue pieces, representative of the degree of cell outgrowth and proliferation, displayed minimal variation. The ability to explain CT explants provides an opportunity to increase cell recovery from a given tissue under processes that are driven by the cells themselves and the advantage of retaining the natural matrix, providing a stem cell niche in vitro. Also, cryopreserving CT as composite material did not appear to diminish over multiple replatings. Furthermore, explant outgrowth can be utilized to isolate MSCs from either fresh cord tissue or tissue cryopreserved as a composite, or raw, material. Cryopreservation of CT as a composite tissue maintains native architecture and biochemical properties of the inter-cell movement/stromal matrix, storage, qualities that are necessary for the successful isolation of MSCs.

REFERENCES