ABSTRACT

Recent studies investigating extracted teeth as a source of post-natal MSC have identified a population of ischemic and highly proliferative cells derived from enzymatically-digested dental pulp tissue. Based on these studies, we investigated the collection, processing and cryopreservation of mesenchymal stem cells recovered from extracted human teeth, with the goal of developing a cryobank of dental pulp-derived MSC for research and clinical utilization as a human cell product processed under current good tissue practices (CGTP). We first examined the methods used to transport extracted teeth from the clinic to the laboratory. Of nearly 50 teeth examined to date, we were able to isolate MSC transported in either PBS or hypothermic solution (HTS) up to 120 hours post-extraction. Optimization of the sterile nature of the extractions and MSC has been achieved using low-concentration podophyllotoxin solution prior to removal of the pulp. Cryopreservation of intact teeth, enzymatically-digested dental pulp, and first-passage MSC were also performed. Cryopreservation of cryopreserved MSC in PBS was significantly higher than those frozen using either 1M propylene glycol (green bars) or dimethyl sulfoxide (blue bar) and 7/10 frozen whole teeth (blue bar). MSC were also recovered from frozen dental pulp (N=2; data not shown).

RESULTS

Summary of first 40 Teeth Processed

- Growth: 31
- Contaminated: 0
- No Growth: 2

SUCCESSFUL ISOLATION OF MSC FROM CRYOPRESERVED WHOLE TEETH

To determine the recovery of MSC from cryopreserved whole teeth as well as from cultured MSC. MSC cultures were recovered following cryopreservation of 29/31 expanded low-passage MSC (Orange bar) and 7/10 frozen whole teeth (Blue bar). MSC were also recovered from frozen dental pulp (N=2; data not shown).

CONCLUSIONS

- Isolation of dental pulp-derived MSC is feasible for at least 120 hours after tooth extraction, and imply that tissue processing immediately after extraction may not be a limiting factor for the successful banking of these cells.
- Cryopreservation of intact teeth, enzymatically-digested dental pulp, or early-passage cultured MSC leads to high-efficiency recovery of MSC after thawing, suggesting that minimal processing may be needed for the banking of samples with no immediate plans for MSC expansion and use, which in turn may limit banking costs. These initial studies will facilitate the development of future clinTP protocols for the clinical banking of MSC.

FUTURE DIRECTIONS

- Studies are underway to optimize techniques to isolate viable, non-contaminated MSC from >80% of extracted teeth, and to recover >80% viable MSC after cryopreservation, which will facilitate tooth-derived MSC banking for clinical use.

BACKGROUND AND OBJECTIVES

The enzymatically-digested pulp of extracted teeth has been shown to be a source of multipotent mesenchymal stem cells (MSC) which may be valuable for multiple clinical uses, including:

- Enhancement of engraftment of hematopoietic cells;
- Cellular therapy for repair of damaged tissue;
- Immunomodulation (e.g., treatment of GVHD).

At the present, it is not clear how best to transport teeth from the clinic to the laboratory for processing, or how best to isolate and cryopreserve the teeth and/or MSC. Thus, our objectives were:

- To determine how long extracted teeth could remain in one of three transport solutions (Hypothermol (HTS); BioLife Solutions, Bothell WA; MesenCult basal medium, StemCell Technologies, Vancouver Canada; or phosphate-buffered saline (PBS)) prior to successful isolation of MSC.
- To determine the recovery of MSC from cryopreserved intact teeth, tooth pulp and low-passage MSC.
- To examine the growth, surface markers and differentiation properties of cultured tooth-derived MSC.

Osteogenic

Adipogenic

Chondrogenic

FiguRe 1. Tooth-derived MSC have the potential to undergo osteogenic, adipogenic and chondrogenic differentiation.

Passage 3-4 MSC were stained with antibodies to the surface markers shown and analyzed by flow cytometry to ensure the cells meet ISCT minimal criteria for MSC (Cytotherapy 8:315-7, 2006). Histograms (purple) from staining of one representative MSC line with isotype controls (green) are shown on the left, and a table summarizing results for six MSC lines is shown on the right.

PLATE 1: SCHEMA FOR TOOTH PROCESSING

- Place extracted tooth in one of three transport solutions for various time periods
- Decontaminate in podophyllotoxin solution; isolate pulp; enzymatically digest pulp and plate for culture
- Freeze intact tooth; isolated pulp; or cultured MSC
- Determine post-freeze recovery of MSC from frozen products
- Characterize cultured MSC

PLATE 2: RESULTS

SUMMARY OF FIRST 40 TEETH PROCESSED

- Growth: 31
- Contaminated: 0
- No Growth: 2

CHART 2: SUCCESSFUL ISOLATION OF MSC FROM CRYOPRESERVED WHOLE TEETH

To determine the recovery of MSC from cryopreserved teeth as well as from cultured MSC. MSC cultures were recovered following cryopreservation of 29/31 expanded low-passage MSC (Orange bar) and 7/10 frozen whole teeth (Blue bar). MSC were also recovered from frozen dental pulp (N=2; data not shown).

PLATE 3: PLATEAU PHASE OF TISSUE CULTURE

Figure 3. MSC viability after cryopreservation and thawing. Five MSC lines were expanded and passage 2-3 MSC were frozen in either 1 M propylene glycol (green bars) or dimethyl sulfoxide (blue bars) and stored in liquid nitrogen. Upon thawing, MSC viability was assessed using trypan blue staining. Viability of MSC frozen using dimethyl sulfoxide was significantly higher than those frozen using propylene glycol; P = 0.005, paired t-test.

Figure 4. Appearance of tooth-derived MSC cultures. Shown are photomicrographs from one representative MSC culture one, four, six and ten days after enzymatic digestion and plating.

Figure 5. Tooth-derived MSC lines express appropriate surface markers. Passage 3-4 MSC were stained with antibodies to the surface markers shown and analyzed by flow cytometry to ensure the cells meet ISCT minimal criteria for MSC (Cytotherapy 8:315-7, 2006). Histograms (purple) from staining of one representative MSC line with isotype controls (green) are shown on the left, and a table summarizing results for six MSC lines is shown on the right.