COLLECTION, CRYOPRESERVATION AND CHARACTERIZATION OF HUMAN TOOTH-DERIVED MSC FOR BANKING AND CLINICAL USE

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ABSTRACT

Recent studies investigating extracted teeth as a source of post-natal MSC have identified a population of clonogenic and highly proliferative cells derived from enzymatically digested dental pulp tissue. Results from these studies indicate that these cells have potential for stem cell-mediated therapies as well as tissue engineering applications. We investigated the collection, processing and cryobiological characteristics of mesenchymal stem cells recovered from extracted human teeth, with the goal of developing a cryobank of dental pulp-derived MSC for research and/or clinical utilization as a human cell product processed under current good tissue practices (cGTP). We first examined the medium 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. Optimal sterilization of the exterior of extracted teeth was achieved using a low-concentration povidone-iodine solution prior to removal of the pulp. Cryopreservation of intact teeth, enzymatically digest dental pulp, and first-passage MSC was achieved, with recovery of cryopreserved MSC in 76% of preserved specimens. Finally, we characterized low-passage cultured cells to ensure that MSC were isolated. Of six cultures extensively tested, all were >95% positive for CD73, CD90 and CD105 and <5% positive for CD34, CD45, CD11b, CD19 and HLA-DR by flow cytometry. All cultures underwent osteogenic, adipogenic and chondrogenic differentiation after three weeks' culture in the appropriate differentiation medium. Thus, these dental pulp-derived cells meet minimal criteria to be called MSC. These studies indicate that 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. Furthermore, 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 cGTP protocols for the clinical banking of MSC.

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 tissues;
- 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 (HypoThermosol (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

SCHEMA FOR TOOTH PROCESSING

Place extracted tooth in one of three transport solutions for various time periods

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Decontaminate in povidone-iodine solution; isolate pulp; enzymatically digest pulp and plate cells for culture

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Freeze intact tooth; isolated pulp; or cultured MSC

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Determine post-freeze recovery of MSC from frozen products

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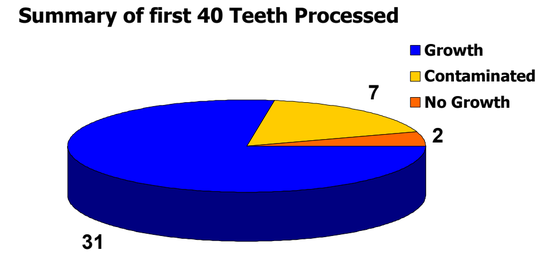
Characterize cultured MSC

RESULTS



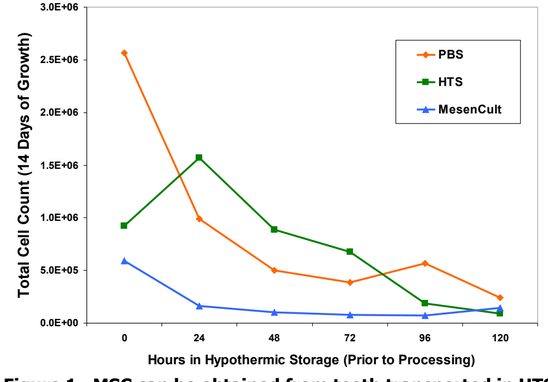


Figure 1. MSC can be obtained from teeth transported in HTS, medium or saline up to 120 hours post-extraction. Extracted teeth were placed in either HTS, MesenCult basal medium or PBS and stored at 4°C for various periods until processing. MSC lines were established from teeth stored in all three transport media after as long as 120 hours post-extraction. Shown here are MSC cell counts 14 days after the cultures were established. N = 18 teeth for each group.

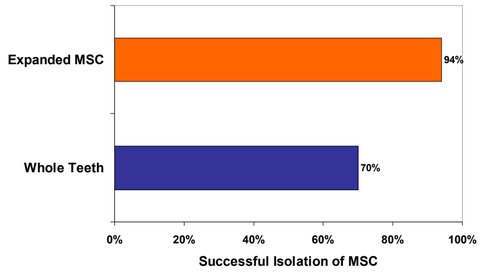


Figure 2. Viable MSC can be recovered from frozen whole teeth as well as from cultured MSC. Viable MSC cultures were recovered following cryopreservation of 29/31 expanded low-passage MSC (orange bar) and 7/10 frozen whole teeth (blue bar). MSC were also recovered from frozen dental pulp (N = 2; data not shown).

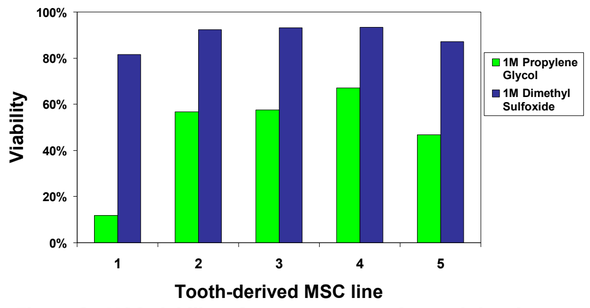


Figure 3. MSC viability after cryopreservation and thawing. Five MSC lines were expanded and passage 2-3 MSC were frozen in either 1 M propylene glycol (green bars) or dimethyl sulfoxide (blue bars) and stored in liquid nitrogen. Upon thawing, MSC viability was assessed using trypan blue staining. Viability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

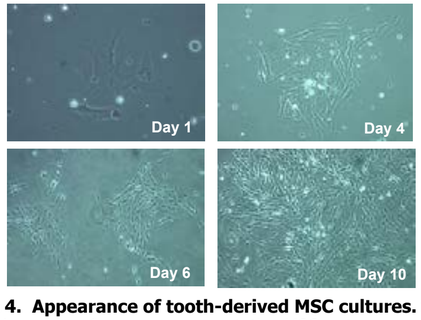
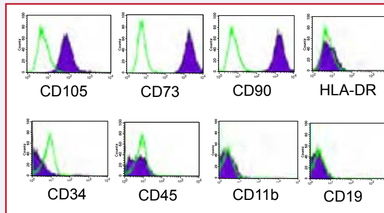


Figure 4. Appearance of tooth-derived MSC cultures. Shown are photomicrographs from one representative MSC culture one, four, six and ten days after enzymatic digestion and plating.



ANTIGEN	% EXPRESSION
CD73	99.7 ± 0.2
CD90	98.8 ± 0.9
CD105	95.6 ± 2.7
CD34	0.2 ± 0.3
CD45	0.1 ± 0.1
CD11b	1.4 ± 1.4
CD19	2.3 ± 1.1
HLA-DR	0.1 ± 0.1

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 from eight MSC lines is shown on the right.

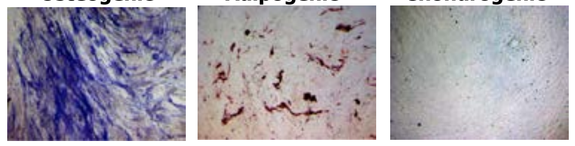


Figure 6. Tooth-derived MSC have the potential to undergo osteogenic, adipogenic and chondrogenic differentiation. Passage 3-4 MSC were cultured in osteogenic, adipogenic (both from StemCell Technologies) or chondrogenic differentiation medium for three weeks before appropriate staining. Eight MSC lines were tested, and all were capable of undergoing trilineage differentiation. Shown are photographs of one representative MSC line.

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

• Tooth-derived MSC meet minimum flow cytometry and trilineage differentiation criteria to be called 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