



CHARACTERISATION AND COMPARISON OF HUMAN DENTAL PULP STEM CELLS OBTAINED FROM DIFFERENT TYPES OF TOOTH

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Abstract

Mesenchymal stem cells (MSCs) have been receiving a lot of attention in various pre-clinical and clinical studies owing to their multipotency property and self renewal ability. Although mesenchymal stem cells exist in many tissues, human tooth is regarded as one of the rich source of mesenchymal stem cells with distinct advantages over other sources. Ability to retain their stem cell properties post cryopreservation, ease of collection, wider window with multiple tooth options for accessing the stem cells have aroused interest in banking dental pulp stem cells for potential future. This has led to emergence of dental stem cell banks similar to umbilical cord blood across the world. All teeth are known to harbour stem cells in the pulp tissue and can be considered for stem cell banking. However, if one has to make a choice of any one tooth to be collected for banking there is no comparative analysis of the stem cells isolated from various tooth types like the incisors, canines, premolars or molars. This study focuses on the identification, characterisation and comparison of human dental pulp stem cells from different tooth types to evaluate if there are any distinctive differences between them and if any one tooth type has a significant edge over other as dental stem cell banking candidates.

Keywords- dental pulp stem cells, mesenchymal stem cells, stem cell banking, tooth types, dental pulp

I. INTRODUCTION

Dental tissues are an attractive source of mesenchymal stem cells (MSCs) and have gathered immense attention over the last few years [1]. Dental pulp stem cells (DPSCs) are mesenchymal multipotent cells coming from the 'cell rich zone' of the dental pulp, which is the soft tissue within the teeth. DPSCs represent an attractive adult stem cell source as they are recovered in large amount in dental pulps with non-invasive techniques compared to other adult stem cell sources [2,4]. Dental stem cells represent a gold standard for neural-crest-derived bone reconstruction in humans and can be used for the repair of body defects [3]. Significant progress has been made in dentin-pulp regeneration, maxillofacial bone regeneration, treatment of nervous system and immune system diseases with dental pulp stem cells for stem cell transplantation [5].

Owing to inherent stem cell advantages and proven retention of their stem cell properties following cryopreservation, dental pulp stem cells have become interesting candidates for long-term preservation and upscale production [1]. The concept of tooth/dental stem banking has gained wide acceptance and various companies have set up banks to tap the potential of this new and innovative approach for preserving SHED and stem cells from other dental sources [7]. With the increasing awareness amongst people to preserve the stem cells for future potential use, there are many dental stem banks storing dental stem cells, similar to umbilical cord blood banks.

Dental pulp stem cells can be harvested from both deciduous teeth - stem cells from human exfoliated deciduous teeth [SHED] and permanent teeth (teeth removed for orthodontic purposes, impacted teeth, and supernumerary teeth) [6]. The window available for banking healthy tooth stem

cells is a lot bigger since it can be done for children between 5-12 years old, children extracting premolars for orthodontic treatments and permanent teeth who are extracting their molar tooth.

While there are multiple studies demonstrating the presence of stem cells in both deciduous and permanent tooth dental pulps, there is no comparative analysis on the stem cells across the different tooth types to comprehend if there is any significant advantage of getting dental pulp stem cells from any one particular tooth type like incisors, canines, premolars or molars. This would have direct implication in the choice of the 'best tooth' for obtaining and preserving dental pulp stem cells for long term cryopreservation and banking. Hence this study focuses on selective parameters typically considered as acceptance / rejection criteria by stem cell banks for long term cryopreservation.

II. MATERIALS AND METHODS

2.1 Procurement of the donor samples of different tooth types

The research was conducted on 110 donor samples, comprising of both deciduous and permanent teeth. Deciduous teeth were collected from donors in the age group of 5 to 12 years. The permanent teeth like premolars were collected from donors in the age group of 13 – 18 years undergoing orthodontic extraction while the third molars (wisdom teeth) were collected from donors under the age group of 30 years of age. Only healthy teeth (without any caries) were collected for the study.

The deciduous teeth samples comprised of incisor, canines and molars. Permanent teeth samples comprised of Premolars and third molars. The donor sample distribution of the different tooth types was as provided in Table 1.

Table 1: Distribution of the donor samples in the study

Tooth	Tooth Type	Number of Donor Samples
Deciduous teeth	Canine	43
	Incisor	39
	Molar	18
Deciduous teeth samples - Total		100
Permanent teeth	Wisdom	5
	Premolar	5
Permanent teeth samples -Total		10
Total teeth samples		110

2.2 Collection of the tooth

The important stages in collection of tooth included preparation of transport medium for tooth, immersing tooth in transport medium and proper packing of tooth in transport kit and accomplishment of transport within 48-72 hours. The transport medium comprised of Penicillin G, Streptomycin and Amphotericin B. The collected tooth was immediately immersed in the transport medium which in turn was transported in a specially designed transport kit. The transport kit having multicomponent box ensured prevention of damage / deterioration during transport of the tooth to laboratory. This multicomponent box had amongst many other components, tooth collection tube containing transport medium. The tube was hermetically sealed after placing the tooth in it. This tube was then placed in tooth protector having thermobutton to record the temperature during transit. Further packing components comprised of biohazard bag, bubble wraps, gel packs and carton boxes. All the components of the multicomponent tool kit ensured safe delivery of tooth after extraction to laboratory. The gel/ice packs were kept at -20°C for 48 hrs and then kept in kit box so that tube protector is maintained at 2 to 8 °C which is most desired temperature during transport of tooth to the laboratory.

2.3 Processing of the dental pulp

2.3.1 Opening of the tooth and excavation of pulp tissue

In the laboratory, the tooth was washed with chlorhexidine solution. Post opening tooth with the help of piezoelectric device, the pulp tissue was excavated with the help of excavator.

2.3.2 Inoculation of chopped pulp tissue

The explant culture procedure was adopted for processing and comprised of the following steps: Chopped tissue (tissue pieces) was pasted at the bottom of each well in six- well plate. Further, culture media (alpha MEM supplemented with 20% FBS, Glutamine, Ascorbic acid, streptomycin+ penicillin) was added and tissue pieces were subjected to incubation in a CO₂ incubator at 37°C, 5 % CO₂ and 95% humidity. Microscopic observation was also done after a period of 5 – 10 days. Media changes were done at regular intervals till 60 – 70 % confluency was attained. Further, the cells were trypsinized by removing spent medium from each well of the six well plates, adding trypsin-EDTA to each well, incubating the cells in trypsin-EDTA and collecting cell suspension.

2.4 Characterisation of dental pulp stem cells

2.4.1 Cell Surface marker analysis using Flow Cytometry

The dental pulp stem cells were characterized with CD90 (Thy-1) and CD105 (SH2) expression typically after 30 days of culture or reaching confluency.

2.4.2 Cell count and viability using automated cell counter

10 µL of the cell suspension was mixed with 10 µL of trypan blue in a 0.5 mL microfuge tube. 10 µL was taken from this and loaded to the countess slide. The slide was loaded in the countess cell counter and the number of viable cells with % of viability was documented.

III. RESULTS AND DISCUSSION

The criteria for acceptance of dental pulp stem samples for long term cryopreservation have been summarised in Table 2. The processed dental pulps were analysed in light of these criteria.

Table 2: Product specification for DPSCs – explants technique

No	Analysis	Specification	Method
1	Cell morphology	Adherent Spindle Shaped Cells	Phase Contrast Microscopy
2	Viable Cell count	NLT 0.5 x 10 ⁶ Cells/mL	Automated Cell Counting
3	Viability %	NLT 50%	Automated Cell Counting
4	Sterility check	Negative	Gram Staining
5	Stem Cell Biomarker Analysis	CD105 ⁺ NLT 75% CD90 ⁺ NLT 75%	Flow Cytometry

3.1 Positive Migration of DPSCs in the culture (Success rate %)

Of the 110 donor samples studied, 108 cultures demonstrated normal fibroblastic morphology, small spindle-shaped cells dental pulp stem cells as shown in Figure 1. Only 2 samples failed to show any growth.

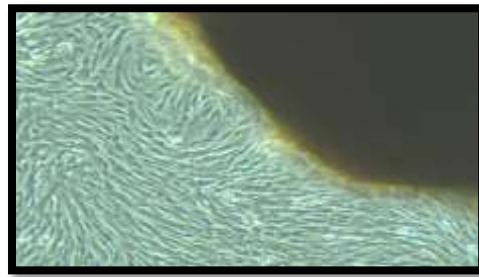


Figure 1: Spindle shaped morphology of dental pulp stem cells at passage 0 (P0)

This study did not research further to understand the reasons behind the negative growths. However the study conclusively proved that the protocol employed for the collection, transportation and processing of dental pulp was adequate enough for the growth of dental pulp stem cells in the culture.

3.2 Cell Surface marker analysis using Flow Cytometry:

The dental pulp stem cells were characterized with CD90 (Thy-1) and CD105 (SH2) expression typically after 30 days of culture. All the samples of dental pulp stem cells tested positive for these markers conclusively proving the mesenchymal nature of dental pulp stem cells.

3.3 Cell count and viability Analysis:

3.3.1 Cell Count

As mentioned in the product specifications for dental pulp stem cells, a cell count (cell yield) of 0.5 million cells from a donor sample is considered as the acceptance limit for cryopreservation/ banking of cells. All the positive growth samples, irrespective of the tooth type had a cell yield of over 0.5 million at P0 passage levels. 74% of the DPSC samples had a cell yield of over 1 million at P0 passage stage. There were variations across the tooth types in terms of cell yields as shown in Table 2. The molars incidentally, both in deciduous and permanent teeth recorded relatively lower percentages with cell yields above one million. One of the plausible reasons could be the age of the tooth. Molars among deciduous teeth fall down later in age as compared to the canines and incisors. However, to conclude on this aspect, more detailed study is recommended.

Table 3: Percent distribution of Cell yields of DPSCs across the tooth type at passage P0

Tooth	Tooth Type	0.5 - 1 Million	1 - 2 Million	2.5 - 5 Million	Total Samples
Deciduous teeth	Canine	21%	49%	30%	100%
	Incisor	16%	50%	34%	100%
	Molar	61%	17%	22%	100%
Deciduous teeth Samples - Total		26%	43%	30%	100%
Permanent teeth	Premolar	0%	40%	60%	100%
	Wisdom	50%	50%	0%	100%
Permanent teeth Samples - Total		22%	44%	33%	100%
Total dental samples		26%	44%	31%	100%

3.3.2 Cell Viability

Typically a viability of over 50% in a donor sample is considered as the acceptance limit for cryopreservation/ banking of cells. All the dental pulp stem cell samples irrespective of the tooth type qualified for these criteria. In fact all of them showed viability percentage of over 70% with close to 70% demonstrated the viability percentage of over 90%.

Table 4: Percent distribution of Viability of DPSCs across the tooth type at passage P0

Tooth Type	Tooth Name	Between 70% - 80%	Between 80% to 90%	Over 90%	Total samples
Deciduous teeth	Canine	14%	16%	70%	100%
	Incisor	0%	32%	68%	100%
	Molar	28%	17%	56%	100%
Deciduous teeth samples - Total		11%	22%	67%	100%
Permanent teeth	Premolar	0%	20%	80%	100%
	Wisdom	25%	25%	50%	100%
Permanent teeth samples -Total		11%	22%	67%	100%
Total dental samples		11%	22%	67%	100%

This study clearly indicates that stem cells were present in all the type of teeth evident from the high success rate across the samples. Irrespective of the tooth type, high levels of viability were observed. Typically 50% viability is the accepted specification for banking the stem cells, while close to 90% of the samples exhibited the viability of 80% and above and 67% of the samples showed viability of 90% and above. While 0.5 million cells at P0 at 70% confluency is considered as the acceptance criteria for banking, all the positive growth samples irrespective of the tooth type qualified this criteria of cell count. Variations in the cell yields were observed across the different types of tooth. Premolars across the permanent cell type and canines and incisors scored in terms of relative cell yields. Molars amongst the deciduous and permanent teeth showed relatively lower cell counts. This leads us to believe that the age of the tooth could be playing a role in the yield of cells. However the numbers analysed have been lower and more extensive study needs to be conducted to conclusively prove the results. However the study conclusively proves that all the tooth type can be good candidates for extracting dental pulp stem cells with high viability rate and acceptable cell yields.

IV. CONCLUSION

This study reinforced the presence of the DPSCs in the dental pulp through the spindle shaped morphology and surface cell marker analysis. The cell yields and the high viability percentages clearly indicate that DPSCs can be good candidates for long term cryopreservation. While variations were observed across the different tooth types in terms of cell yield and viability, all of them can be considered for dental stem banking when evaluated across the specified criteria.

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