Chelonian Conservation And Biology

Vol. 17 No.2 (2022) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI:doi.org/10.18011/2022.04(1) 2608.2615

ASSESSING THE VIABILITY AND PROPERTIES OF DENTAL PULP STEM CELLS FROM HUMAN THIRD MOLARS FOR POTENTIAL REGENERATIVE ENDODONTICS APPLICATIONS

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Abstract

Dental pulp stem cells (DPSCs) isolated from extracted teeth present an exciting stem cell source for potential applications in regenerative endodontics to restore damaged or diseased pulp tissue. However, the long-term viability and properties of DPSCs obtained from different tooth sources over extended culture periods remain incompletely characterized. This study aimed to address this research gap by isolating and culturing stem cells from human third molar dental pulp over six passages. Cell viability, morphology, proliferation, expression of mesenchymal stem cell surface markers, and differentiation capacity into odontoblasts and adipocytes were analyzed at each passage. DPSCs extracted from 15 impacted third molars from patients age 18-25 years were cultured and monitored over six serial passages. Cell viability remained excellent through passage 6 with 98% viability. Spindle-shaped fibroblastic morphology was maintained consistently. Doubling time averaged between 30-36 hours across passages. Immunophenotyping revealed positive expression of mesenchymal stem cell surface markers CD73, CD90, and CD105 that was sustained consistently through the final passage. Multilineage differentiation capacity into both osteogenic and adipogenic lineages was demonstrated. Results indicate DPSCs derived from human third molars represent a viable and stable stem cell population for investigation in regenerative endodontics approaches to restore damaged dental pulp tissue and whole tooth structures. Findings support their further study in 3D scaffold cultures and in vivo transplantation models.

Introduction

Root canal treatment is an endodontic procedure commonly used to treat infection and inflammation of the dental pulp. However, while it addresses immediate disease, the root canal space is filled with inorganic materials lacking cellular components or bioactivity. In cases of severe infection, pulp necrosis, or trauma, the tooth is fully devitalized requiring future extraction once the crown cracks or root fractures. This has driven interest in regenerative

endodontics using stem cell-based pulp regeneration approaches to restore vascularized, innervated pulp tissue capable of generating new dentin (Demarco et al., 2017).

Dental pulp stem cells (DPSCs) derived from extracted teeth have emerged as a promising stem cell source for endodontic regeneration (Demarco et al., 2017). DPSCs possess key properties of self-renewal and multilineage differentiation capacity. Studies indicate they can differentiate into odontoblasts capable of producing dentin-like mineralized tissue when transplanted in vivo, demonstrating feasibility to regenerate dental pulp (Demarco et al., 2017). However, most studies have focused on short-term culture, while long-term proliferative capacity and stemness over serial passaging remains unclear.

This study aimed to address this gap by isolating and characterized DPSCs from human third molars over six cell culture passages. Cell viability, morphology, proliferation, stem cell surface markers, and differentiation potential were assessed at each passage. Findings provide important insights on the long-term regenerative capacity of third molar DPSCs to guide development of regenerative endodontics therapies.

Background

Dental Pulp Stem Cell Sources

Viable stem cells can be isolated from the dental pulp of both primary and permanent teeth (Estrela et al., 2011). Common sources include impacted third molars, premolars extracted for orthodontics, and exfoliated deciduous teeth (Estrela et al., 2011). DPSCs reside among other cell types including fibroblasts, endothelial cells, and odontoblasts within the pulp chamber's loose connective tissue. They are theorized to play a role in regular pulp homeostasis and initial reparative dentin formation after injury (Demarco et al., 2017). When cultured, DPSCs exhibit clonogenic and self-renewal capabilities while maintaining multilineage differentiation potential similar to mesenchymal stem cells from other tissues (Estrela et al., 2011).

Multipotential Differentiation Capacity

Studies have demonstrated DPSCs differentiate into various cell lineages in vitro when cultured under specific induction conditions (Estrela et al., 2011; Ponnaiyan & Jegadeesan, 2018). Confirmed lineages include:

- Odontoblastic: Forming dentin-like mineralized tissue
- Osteoblastic: Forming bone-like mineralized tissue
- Adipocytic: Lipid vacuole formation
- Chondrocytic: Cartilage formation
- Myocytic: Muscle formation
- Neurogenic: Neural cell formation

This highlights their diverse regenerative applications.

Importance of Assessing Proliferative Capacity

A key property of stem cells is sustained self-renewal allowing exponential cell expansion while retaining multidifferentiation ability (Sete et al., 2012). This proliferative capacity over successive passages indicates duration of stemness during in vitro cultivation. High proliferative potential provides sufficient cell numbers for downstream translational applications. This underscores the need to assess growth kinetics and population doubling of DPSC cultures over long-term passage (Sete et al., 2012).

Rationale for Studying Third Molar DPSCs

Most DPSC research has focused on short-term cultures, while characteristics over extended passaging remain poorly defined (Sete et al., 2012; Ponnaiyan & Jegadeesan, 2018). Furthermore, how DPSC properties and viability vary by tooth type and patient age requires elucidation (Eubanks et al., 2014). Third molars extracted for clinical indications represent an easily accessible immature permanent tooth source from young adults. This study examined DPSCs isolated from impacted third molars over serial subcultivation to assess longevity and regenerative capacity. Findings can help determine suitability for regenerative endodontics.

Aim

This study aimed to isolate and characterize dental pulp stem cells from human third molars over six cell passages. Specific objectives were:

- To quantitatively assess DPSC viability at each passage
- To evaluate morphology and growth kinetics
- To examine expression of mesenchymal stem cell surface markers
- To assess multilineage differentiation capacity

Hypotheses

- DPSCs will remain viable through passage 6
- Spindle-shaped morphology and doubling time will be consistent
- Mesenchymal surface antigens will be positively expressed
- Multipotency will be maintained through final passage

Methods

Study Design

DPSC cultures were established from 15 extracted human third molars. Cells were maintained in culture through 6 serial passages. At each passage, quantitative and qualitative assays were conducted.

Tooth Sample Collection

15 impacted third molars were collected from patients age 18-25 years undergoing extraction for clinical indications at a dental clinic in Riyadh. Only asymptomatic non-carious teeth were included. Patients provided informed consent.

DPSC Isolation and Culture

Teeth were scrubbed with povidone-iodine and the pulp chamber opened using a sterilized dental handpiece. The pulp was gently extracted and enzymatically digested with 3 mg/mL collagenase and 4 mg/mL dispase for 1 hour at 37°C. Cell suspensions were passed through a 70 µm cell strainer and cultured in flasks with DMEM/F12 medium containing 20% fetal bovine serum and antibiotics at 37°C and 5% CO2 with media changes every 2-3 days. Upon reaching 90% confluence, cells were sub-cultured up to passage 6.

Cell Morphology

Cell morphology was evaluated at each passage using an inverted phase contrast microscope. Shape and adherence were noted.

Cell Viability

The number of viable versus non-viable cells was quantified at each passage using the Trypan Blue dye exclusion method. Viability was calculated as the percentage of live cells out of the total cell population.

Doubling Time

Population doubling time was calculated at each passage using the formula:

Doubling Time = Duration x log(2) / log(final cell concentration) – log(initial cell concentration)

Immunophenotyping

Flow cytometry assessed surface marker expression of mesenchymal stem cell antigens CD73, CD90, and CD105 at every passage. Cells were incubated with fluorescent antibodies targeting each surface marker and run through a flow cytometer to quantify the percentage of positively expressing cells.

Multilineage Differentiation

Osteogenic and adipogenic differentiation capacity was assessed at each passage. For osteogenesis, DPSCs were cultured in induction medium containing glycerol phosphate, ascorbate, and dexamethasone for 3 weeks then analyzed for alkaline phosphatase activity and calcium deposition. For adipogenesis, cells were cultured in adipogenic medium containing indomethacin, dexamethasone, and insulin for 3 weeks then evaluated for intracellular lipid accumulation using Oil Red O staining.

Statistical Analysis

Mean and standard deviation were calculated for quantitative data. Repeated measures ANOVA analyzed variation in parameters across passages. $p<0.05$ was considered statistically significant.

Results

Morphology

Cultured DPSCs demonstrated a spindle-shaped fibroblastic morphology at initial isolation from dental pulp tissue (Figure 1A). This morphology was maintained consistently through passage 6 (Figure 1B-1F). Cells remained adhered and continued proliferating.

Figure 1. DPSC Morphology was Consistently Maintained from Passage 1 (A) to Passage 6 (F)

Viability and Proliferation

Trypan blue exclusion showed excellent viability that was sustained through the final passage, ranging from 95-98% (Table 1). No significant decline was seen over successive passages ($p>0.05$).

Average population doubling time ranged from 30-36 hours across passages, indicating a high proliferative rate that was retained throughout long-term culture (Table 1). Doubling time did not significantly vary across passages ($p>0.05$).

Table 1. Viability and Doubling Time of DPSCs by Passage

Passage	Viability (%)	Doubling Time (Hours)
1	98 ± 1.2	32 ± 2.8
2	97 ± 1.5	34 ± 4.1
3	96 ± 1.8	36 ± 3.7
4	95 ± 1.6	35 ± 2.9
5	96 ± 1.3	33 ± 3.1
6	98 ± 1.0	30 ± 2.6

Immunophenotyping

Flow cytometry indicated consistent positive expression of mesenchymal stem cell surface markers CD73, CD90, and CD105 across all passages, with greater than 90% of cells expressing each marker through passage 6 (Figure 2).

Figure 2. Positive DPSC Expression of CD73, CD90, and CD105 Surface Markers was Maintained Through Passage 6

Multilineage Differentiation

Osteogenic and adipogenic differentiation capacity was demonstrated at all passages when DPSCs were cultured under induction media conditions. Osteogenesis was evidenced by alkaline phosphatase activity and mineralized calcium deposition (Figure 3A-B). Adipogenesis was confirmed through intracellular lipid vacuole formation detected with Oil Red O staining (Figure 3C-D).

Figure 3. DPSCs Showed Osteogenic Differentiation with Calcium Deposition (A-B) and Adipogenic Differentiation with Lipid Vacuoles (C-D) Through Passage 6

Discussion

This study isolated and characterized DPSCs from human third molars over long-term culture through six passages, assessing viability, proliferative capacity, stem cell marker expression, and multilineage differentiation potential at each passage. Results demonstrated excellent DPSC viability up to 98%, maintained fibroblast-like morphology, stable population doubling times averaging 32 hours, consistent expression of mesenchymal stem cell surface markers CD73, CD90, and CD105, and retention of multipotential differentiation ability throughout all passages. Findings support the feasibility of utilizing third molar DPSCs for dental pulp regenerative approaches even after extensive in vitro cultivation.

Sustained proliferative rates with cell doubling occurring approximately every 30-36 hours throughout the culture period indicate substantial proliferative capacity comparable to DPSCs derived from other tooth sources (Sete et al., 2012; Ponnaiyan & Jegadeesan, 2018). This exponential cell expansion could provide sufficient cell numbers for clinical use. Positive staining for CD73, CD90, and CD105 surface antigens confirms the isolated cell populations maintained mesenchymal stem cell identity even following repeated passaging (Dominici et al., 2006). Importantly, the consistency of osteogenic and adipogenic differentiation capacity through passage 6 demonstrates retention of multipotentiality necessary for pulp tissue regeneration. Results align with and extend previous studies by demonstrating durable stem-like properties of DPSCs specifically from third molars over long-term culture (Eubanks et al., 2014).

Findings have key implications for translating the use of DPSCs into clinical regenerative endodontic approaches seeking to regrow functional dental pulp tissue capable of generating new dentin. This could dramatically improve the prognosis of compromised or necrotic teeth and eliminate the need for extraction. Immature third molars typically extracted for orthodontics or impaction represent an easily accessible source of DPSCs for autologous use with high viability and stemness. Results support their further investigation using 3D scaffold cultures and in vivo transplantation models to confirm capacity to regenerate dentin-pulp-like structures.

Limitations include lack of genetic analysis to confirm cell populations were homogeneous without contamination. The culture conditions may have influenced behavior. As an in vitro study, translational effectiveness remains unconfirmed. However, rigorously assessing longevity

and stemness provides key insights on viability that help validate third molar DPSCs as having substantial promise for pulp regenerative cell-based endodontic therapies. Findings warrant further study of their regenerative applications.

Conclusion

This in vitro study isolated and characterized dental pulp stem cells obtained from human third molars over long-term culture through six cell passages. DPSCs demonstrated sustained excellent viability up to 98%, stable morphology, consistent population doubling times averaging 32 hours, positive expression of mesenchymal stem cell surface markers CD73, CD90 and CD105, and retention of multipotential differentiation capacity through the final passage. Results indicate dental pulp stem cells derived from human third molars remain viable and preserve stem cell characteristics even after extensive subcultivation. Findings support their continued investigation as a feasible stem cell source for cell-based regenerative endodontics therapies seeking to restore damaged or necrotic dental pulp tissue. Further research using 3D culture models and in vivo transplantation is warranted to confirm their capacity to regenerate functional dentin-pulp tissue.

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