RESEARCH ARTICLE

Fibroblast growth factor-6 enhances *CDK2* and *MAKT* expression in microvesicles derived from human stem cells extracted from exfoliated deciduous teeth [version 1; peer review: awaiting peer review]

Ahmed Othman ¹, Rabab Mubarak¹, Marwa Sameer¹, Dina Sabry ²

¹Department of Oral Biology, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt

²Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt

V1 First published: 22 May 2018, 7:622
<https://doi.org/10.12688/f1000research.14900.1>
 Second version: 07 Jun 2018, 7:622
<https://doi.org/10.12688/f1000research.14900.2>
 Third version: 14 Aug 2019, 7:622
<https://doi.org/10.12688/f1000research.14900.3>
 Fourth version: 28 Jan 2020, 7:622
<https://doi.org/10.12688/f1000research.14900.4>
 Fifth version: 21 May 2020, 7:622
<https://doi.org/10.12688/f1000research.14900.5>
 Latest published: 24 Jun 2020, 7:622
<https://doi.org/10.12688/f1000research.14900.6>

Abstract

Background: Stem cells from human exfoliated deciduous teeth (SHEDs) are considered one of the most convenient sources of adult stem cells. This study aimed to examine the effect of fibroblast growth factor 6 (FGF-6) on SHEDs and evaluate *CDK2* and *MAKT* gene expression in SHED-derived microvesicles (MVs). SHEDs were cultured from deciduous teeth pulp.

Methods: SHEDs were divided into two groups: the control group and test groups, with and without FGF-6 supplementation, respectively. After the third passage, SHED proliferation was assessed by MTT assay. MVs were purified and *CDK2* and *MAKT* gene expression was assessed by real time polymerase chain reaction. SHEDs were identified by their positivity for CD90 and CD73, and negativity for CD45 and CD34.

Results: SHEDs proliferation in the test group was significantly higher than in the control group ($P < 0.001$). mRNA from SHED-derived MVs from the test group exhibited a markedly elevated expression of *CDK2* and *MAKT*, ($P < 0.002$ and $P < 0.005$, respectively) in comparison with those of the control group. FGF-6 enhanced the proliferation of SHEDs. Proliferation enhancement is favorable for the production of a large number of stem cells, which will then be beneficial for cell-based therapies.

Open Peer Review

Approval Status

	1	2	3
version 6 (revision) 24 Jun 2020			
version 5 (revision) 21 May 2020		view	view
version 4 (revision) 28 Jan 2020	view	view	view
version 3 (revision) 14 Aug 2019	view		
version 2 (revision) 07 Jun 2018	view		
version 1 22 May 2018			

1. Asma Abdullah Nurul, University of Science
Malaysia, Kubang Kerian, Malaysia

2. Thanaphum Osathanon, Chulalongkorn

Conclusions: *CDK2* and *MAKT* genes in SHED-derived MVs can be used as molecular biomarkers for SHED proliferation.

Keywords

Stem cells from human exfoliated deciduous teeth, fibroblast growth factor 6, microvesicles, CDK2, MAKT

University, Bangkok, Thailand

3. **Abbas Ali Khademi**, Isfahan University of Medical Sciences, Isfahan, Iran

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Ahmed Othman (ahmed.alsaid@dentistry.cu.edu.eg)

Author roles: **Othman A:** Writing – Original Draft Preparation, Writing – Review & Editing; **Mubarak R:** Supervision; **Sameer M:** Supervision; **Sabry D:** Investigation, Methodology, Supervision, Validation

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2018 Othman A *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Othman A, Mubarak R, Sameer M and Sabry D. **Fibroblast growth factor-6 enhances *CDK2* and *MAKT* expression in microvesicles derived from human stem cells extracted from exfoliated deciduous teeth [version 1; peer review: awaiting peer review]** F1000Research 2018, 7:622 <https://doi.org/10.12688/f1000research.14900.1>

First published: 22 May 2018, 7:622 <https://doi.org/10.12688/f1000research.14900.1>

Introduction

Stem cells from human exfoliated deciduous teeth (SHEDs) are a type of adult stem cell acquired from the dental pulp of human exfoliated deciduous teeth. SHEDs stand out from other types of adult stem cells since they possess a remarkable growth and proliferation rate, providing an adequate stem cell source for any prospective clinical or laboratory use. The natural exfoliation of deciduous teeth provides a good chance to procure and isolate SHEDs without effort or complications, and with little or no trauma. SHEDs have the capacity to differentiate into many cell types, including chondrocytes, osteoblasts, adipocytes, cardiomyocytes and neurons¹⁻³.

Fibroblast growth factors (FGFs) are a family of secreted cytokine proteins that have a role in the regulation and direction of numerous cellular processes, including proliferation, differentiation, migration or metabolism⁴. FGF-6 is part of the FGF-4 subfamily of canonical FGFs. Like the other members of FGF-4 subfamily, FGF-6 is a secreted protein with a cleavable N-terminal signal peptide that binds and activates FGF receptors as an extracellular mediator⁴. Despite the fact that FGF-6 expression is almost completely limited to myogenesis⁵, it was found to exert a proliferating effect on human osteoblasts under specific conditions⁶.

Recently, microvesicles (MVs) have been identified as an approach deployed by stem cells as a mean of mediating intercellular interactions⁷. These phospholipid membrane-bound MVs partake in intercellular interactions, such as proliferation, differentiation and gene expression alteration, via their content of mRNA, miRNA and transfer proteins^{7,8}.

Human liver stem cell-derived MVs were found to have a role in hepatic regeneration, since they transfer proteins and mRNAs associated with the control of transcription, translation, proliferation, and apoptosis to hepatocytes⁹. Cyclin-dependent kinase 2 (*CDK2*) and megakaryocyte-associated tyrosine kinase (*MATK*) genes are associated with cellular proliferation as they were found in the mRNA of purified MVs at the time of microarray analysis and reverse transcription-quantitative PCR (RT-qPCR)⁹.

CDK2 is the catalytic subunit of the cyclin-dependent protein kinase complex, which controls advancement through the cell cycle via its involvement in the G₁ to S phase transition^{10,11}. *MATK* has been identified by Avraham *et al.* as an intracellular tyrosine kinase that participates in the proliferation and survival of megakaryocyte progenitors¹². Furthermore, Findings by Herrera *et al.* demonstrated that *MATK* conveyed by MVs was one of the genes responsible of liver stem cell proliferation⁹.

The current study was performed to evaluate SHED proliferation upon supplementation with FGF-6 in comparison with SHEDs in regular culture medium without FGF-6 supplementation; it also aimed to assess the effect of FGF-6 supplementation on *CDK2* and *MAKT* gene expression in purified proliferated stem cell MVs.

Methods

Sample collection

A total of 28 deciduous teeth indicated for extraction were collected from 25 patients at the Pediatric Dentistry Department in Faculty of Dental Medicine, Cairo University. Patient age ranged from 7 to 12 years. Collection was done at the pediatric clinic over 3 days, we looked for deciduous teeth indicated for extraction due to their natural shedding time in order to make room for their permanent successors, so no ethical concerns would arise. Deciduous tooth collection was conducted after obtainment of the guardians' written informed consent at Pediatric Dentistry Department in the Faculty of Dental Medicine Cairo University, with the approval of the Ethics Committee of the Faculty of Oral and Dental Medicine, Cairo University. Subjects were identified by their treating physician, following which we contacted the guardians of the subjects for consent to use the extracted teeth. Stem cell propagation (at the Medical Biochemistry Department in the Faculty of Medicine Cairo University) was performed in accordance with recommendations and with the approval of the Ethics Committee of the Faculty of Oral and Dental Medicine, Cairo University.

Deciduous tooth surfaces were washed several times with Dulbecco's PBS (Biowest, USA). Dental pulp was extracted delicately from teeth using a sterile endodontic barbed broach and placed in falcon tube containing PBS (Biowest, USA).

SHED culture and characterization

SHEDs culture and characterization were done after taking established procedures into account¹³. A total of 3 mg collagenase type II (Sigma Aldrich, USA) was dissolved in PBS to digest the extracted dental pulp tissues for 1 h at 37°C in a 5% CO₂ incubator and shaken well at 10 min intervals until the tissues were fully digested. The samples were strained using a cell strainer (40 µm nylon PP) (Bio Basic, Inc., Canada) to remove tissue debris and then centrifuged for 10 min at 3000 rpm at 5°C to obtain pellets of isolated cells. The supernatant fluid was discarded and cell suspension was obtained by pipetting cells in RPMI 1640 culture medium (Biowest, USA). Next, the isolated cell pellets were seeded in 75 cm³ tissue culture flasks for cell culture propagation. Culture medium (RPMI 1640) (was supplemented with 1% Pen/Strep solution (Lonza, USA) and 10% fetal bovine serum (FBS) (Lonza, USA) were supplemented to the culture media to achieve cell propagation at 37°C in humidified CO₂ incubator for 7–10 days, with medium changes every 3 days.

Cells were identified as being mesenchymal stem cells (MSCs) by their morphology and adherence to the plastic flask. In addition, quantification of several expressed MSCs markers was conducted using flow cytometry analysis. Adherent cells were trypsinized and subjected to centrifugation to form cell pellet. Next, 1×10⁵ cells were incubated with 10 µl monoclonal CD90 PE (catalog number FAB2067A; R&D Systems), CD73PE (catalogue number FAB5795P; R&D systems) CD34 PE (catalogue number FAB72271P; R&D Systems) and CD45 PE (catalog number DAB1430P; R&D Systems) antibodies, at 4°C

in the dark. Same species isotypes served as a negative control, Mouse IgG1 PE conjugated antibody (catalog number IC002P; R&D Systems). After a 20 min incubation, 2 ml PBS containing 2% FBS was added to a tube of monoclonal treated cells. The mixtures were then centrifuged for 5 min at 2500 rpm, followed by discarding the supernatant and re-suspending cells in 500 µl PBS containing 2% FBS. Cell analysis was performed using a CYTOMICS FC 500 Flow Cytometer and analyzed using CXP Software version 2.2.

SHEDs proliferation process and passaging

Passaging of SHEDs was done according to established protocols¹⁴, with modifications for this experiment. Sub-culturing and passaging was done when adherent cells primary culture (passage zero) have reached 80% confluence. 10^3 – 10^5 cells were seeded into 24-well plates prior to grouping and subsequent passaging till reached third passages. Seeded cells were divided into two groups: control group (SHEDs untreated with FGF-6) and test group (SHEDs treated with FGF6). FGF-6 was added at concentration 20 ng/ml for test group.

Cell viability

MTT reagent, supplied ready for use after the third passage of the SHEDs, was obtained from Tacs Trevigen (Gaithersburg, USA). For the cell viability assay, the two cell groups were seeded in three 96-well tissue culture plates each, at 10^3 cells/ml per well. The MTT reagent was added and the plate was incubated in the dark for 2–4 h. Detergent reagent (catalog number # 4890-25-02, TACS) was added to each well to solubilize formazan dye prior to absorbance measurement. The absorbance in each well was measured at a range from 490 to 630 nm using an enzyme-linked immunosorbent assay plate reader (Stat Fax 2200, Awareness Technologies, Florida, USA)¹⁵.

MV isolation

MVs were obtained from supernatants of third-passage MSCs (5×10^6 cells/ml) cultured in RPMI-1640 deprived of FBS and supplemented with 0.5% of bovine serum albumin (BSA) (Sigma Aldrich, USA). After centrifugation at 2000g for 20 min to remove debris, cell-free supernatants were centrifuged at 100,000g for 1 h at 4°C, washed in serum-free medium 199 containing 25 mM HEPES (Sigma) and submitted to a second ultracentrifugation under the same conditions¹⁶. MVs were then prepared for electron microscopy characterization. Briefly MVs were diluted in 145 µL PBS containing 0.2% paraformaldehyde (w/v). 10 µl was administered to a formvar-carbon-coated 300 mesh grid (Electron Microscopy Sciences, Hatfield, USA) for 7 min, followed by staining with 1.75% uranyl acetate (w/v). Samples were left to dry at room temperature for 2 h and imaged by transmission electron microscopy (TEM) (CM-10, Philips, Eindhoven, The Netherlands) at 100 kV afterwards¹⁷.

Gene expression profile

Total RNA was isolated from MVs using an RNA purification kit (Gene JET, Kit, #K0731, Thermo Fisher Scientific, Inc.). RNA quantification using RT-qPCR was achieved using a one-step reaction (SensiFAST™ SYBR® Hi-ROX One-Step Kit, catalog no. PI-50217 V; Bioline, UK). Sequence-specific

primers (Bio Basic, USA) for the studied target genes (*CDK2* and *MATK*) and reference housekeeping gene (β -actin) were used. The prepared reaction mix samples were applied in real time PCR (StepOne Applied Biosystem, Foster city, USA). The cDNA was subsequently amplified using a SYBRGreen I PCR Master kit (Fermentas) in a 48-well plate as follows: 10 min at 95°C for enzyme activation, followed by 40 cycles of 15 s at 95°C, 20 s at 60°C and 30 s at 72°C for the amplification step. Changes in the expression of each target were normalized relative to the mean Cq values of β -actin as housekeeping gene by the $2^{-\Delta\Delta Cq}$ method. We used 1 µM of both primers specific for each target gene. Primers sequences were as follows: *CDK2* sense, 5'-AATCCGCTGGACACTGAGA-3' and antisense, 5'-CCAGCAGCTTGACAATATTAGGA-3' (Genbank accession number [XM011537732.1](#)); *MATK* sense, 5'-CCGCGACGTCATCCACTAC-3' and antisense, 5'-TTGTAATGCTCCACCATGTCCAT-3' (Genbank accession number [AH006874.3](#)); and *GAPDH* sense, 5'-GGCGGACACCACCATGTACCCT-3' and antisense, 5'-AGGGCCGGGACTCGTCATACT-3' (Genbank accession number [AAG01996.1](#)).

Statistical analysis

Data were coded and entered using SPSS version 23. Data are presented as the median and interquartile range for quantitative data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test. Correlations between quantitative variables were done using Spearman's correlation coefficient. P-values less than 0.05 were considered as statistically significant.

Results

SHED characterization

The SHEDs in culture were identified morphologically by their fusiform fibroblast like form for both groups. SHEDs in the test group exhibited an increase in proliferation during culture and passaging, when compared with the control group ([Figure 1](#)). Flow cytometric analysis SHEDs were identified as they were negative for CD34 and CD45 and positive for CD90 and CD73 ([Figure 2A](#)).

Cell viability

The proliferated SHEDs were assessed using MTT assay. The viability of the cells in the test group was significantly higher ($P < 0.001$) in comparison with that of the control group ([Table 1](#)).

TEM

TEM detected MVs purified from SHED after ultracentrifugation ([Figure 2B](#)). MVs were characterized by their size (500 nm), as detected by TEM.

RT-qPCR

Purified MVs demonstrated a significant positive expression intensity of *CDK2* ($P = 0.002$) and *MATK* ($P = 0.005$) in the test group in relation with the control group. A box plot ([Figure 3](#)) shows that expression of *CDK2* and *MATK* is higher in the test group than the control group, as they display a higher interquartile range (IQR) and median. The correlation coefficients of *CDK2* and *MATK* expression to cell viability were positive for both genes in the test group ([Figure 4](#)).

Figure 1. Isolation, morphological observation of stem cells from human exfoliated deciduous teeth through phase contrast microscopy. (A) Passage one shows stem cells with spindle-like morphology as grow from human exfoliated deciduous teeth pulp in few number. (B and C) Passages two (B) and three (C) show an increase in number of stem cells with spindle-like morphology. Isolation, morphological observation of stem cells from human exfoliated deciduous teeth in the test group through phase contrast microscopy. (D) Passage one shows a marked increase in number and confluency of stem cells with spindle-like morphology in comparison with control group in passage one. (E and F) Passages two (E) and three (F) show a pronounced, confluent and expanded SHED with fibroblast like morphology in relation to control groups of second and third passages.

Figure 2. Flow cytometry and transmission electron microscopy. (A) Flow cytometry analysis of CD90, CD73, CD34 and CD45 for stem cell characterization. (B) Electron microscopy ultrastructure of released microvesicles (black arrow) from the mesenchymal stem cells of dental pulp.

Table 1. Cell proliferation assay for both studied groups. Data presented as median (IQR).

Variable	SHEDs	SHEDs supplemented with FGF-6	P-value
Absorbance (450 nm)	0.90 (0.77-1.36)	2.55 (1.63-2.98)	<0.001

SHEDs, stem cells from human exfoliated deciduous teeth; FGF-6, fibroblast growth factor-6.

Figure 3. Box-and-whiskers plot showing number distribution for *CDK2* and *MATK* expression in both test and control groups. (A and B) Expression of each gene is higher in the FGF-6-supplemented group than in the control group, since higher interquartile range (IQR) and median values are observed.

Figure 4. Assessment of cell proliferation. (A) Expression of *CDK2* is positively correlated with cell proliferation in the test group. (B) Expression of *MATK* is positively correlated with cell proliferation in the test group.

Dataset 1. Raw data for the MTT cell viability assay and for reverse transcription-quantitative PCR

<http://dx.doi.org/10.5256/f1000research.14900.d203796>¹⁸

Discussion

The present study was developed to deal with difficulties sourcing stem cells and a lack of sufficient stem cells for reliable tissue formation. The study focused on stem cells isolated from human deciduous teeth (SHEDs) and tissue-inducing

substances, which in this case is FGF-6. The reason we chose SHEDs for the isolated stem cells is that they present an opportune source of adult stem cells; the deciduous teeth are naturally exfoliating so there are no ethical problems surrounding their use, and the isolation of cells is simple, painless, convenient and time-efficient¹⁹.

There are several criteria for SHED identification; they were identified as stem cells by their morphology under the inverted microscope, as they appeared as fibroblast-like cells. Another feature exhibited was that they have a plastic adherence feature under our normal culture conditions¹. SHEDs were also identified as ectomesenchymal stem cells through the quantification of several expressed mesenchymal stem cell markers using flow cytometry; they were shown to be positive for CD90 and CD73, and negative for CD45 and CD34²⁰.

In this case, the stem cells are quiescent²¹, unlike progenitor cells, meaning growth factor treatment is required to produce a large amount of cells. In our experiment, FGF-6 was chosen as it has, to our knowledge, never used on SHEDs before, and would avoid the conflicting reports of the effects of bFGF on SHEDs^{22,23}.

MVs were isolated from the proliferated SHEDs for three reasons. The first reason was to assess the two groups of proliferated SHEDs (i.e. the control group and the group supplemented with FGF-6) via the expression of *CDK2* and *MATK*. The second reason was to explore the MVs RNA since the physiological functions of MVs in many tissues remain mostly unknown; on the other hand, their potential roles in pathological settings have been studied largely in the fields of oncology and immunology. The third reason was that the MVs have great potential for use in research²⁴. It was demonstrated that the cell-derived MVs are found to be able to change phenotypes of different cells to become stem cells via epigenetic reprogramming or infectious particle transfer²⁴.

In this study, the *CDK2* gene was used to observe SHEDs proliferation since it has been used to monitor proliferation

in many types of stem cells, such as neural progenitor stem cells²⁵ and liver stem cells⁹. *CDK2* also encodes a serine/threonine protein kinase family member, with receptors in this family having a role in the regulation of cell proliferation, programmed cell death, cell differentiation, and embryonic development²⁶.

Megakaryocyte-associated tyrosine kinase is the enzyme which is encoded by *MATK* in humans. This enzyme possesses a similar amino acid sequence to tyrosine-protein kinase CSK. It was chosen for our experiment as it is not frequently used for the assessment of SHED proliferation, to evaluate whether this ambiguous gene can be studied in further research to assess the proliferation rate of SHEDs and other types of stem cells it is known to be capable of phosphorylating and inactivating Src family kinases, and may inhibit T-cell proliferation¹².

Conclusion

From the present study, it can be concluded that SHED proliferation was significantly higher in culture medium with FGF-6 present than in culture medium with no FGF-6; there was a corresponding increase in positive expression of *CDK2* and *MATK* mRNA from extracted MVs. The intensity of *CDK2* and *MATK* gene expression in SHEDs supplemented with FGF-6 was significantly higher than SHEDs not supplemented with FGF-6.

Data availability

Dataset 1. Raw data for the MTT cell viability assay and for reverse transcription-quantitative PCR. DOI: [10.5256/f1000research.14900.d203796](https://doi.org/10.5256/f1000research.14900.d203796)¹⁸.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

- Miura M, Gronthos S, Zhao M, *et al.*: **SHED: stem cells from human exfoliated deciduous teeth.** *Proc Natl Acad Sci U S A.* 2003; **100**(10): 5807–5812.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rai S, Kaur M, Kaur S: **Applications of stem cells in interdisciplinary dentistry and beyond: an overview.** *Ann Med Health Sci Res.* 2013; **3**(2): 245–254.
[PubMed Abstract](#) | [Free Full Text](#)
- Yin Z, Wang Q, Li Y, *et al.*: **A novel method for banking stem cells from human exfoliated deciduous teeth: lentiviral TERT immortalization and phenotypical analysis.** *Stem Cell Res Ther.* 2016; **7**: 50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ornitz DM, Itoh N: **The Fibroblast Growth Factor signaling pathway.** *Wiley Interdiscip Rev Dev Biol.* 2015; **4**(3): 215–266.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Armand AS, Laziz I, Chanoine C: **FGF6 in myogenesis.** *Biochim Biophys Acta.* 2006; **1763**(8): 773–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bosetti M, Leigheb M, Brooks RA, *et al.*: **Regulation of osteoblast and osteoclast functions by FGF-6.** *J Cell Physiol.* 2010; **225**(2): 466–471.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Sabin K, Kikyo N: **Microvesicles as mediators of tissue regeneration.** *Transl Res.* 2014; **163**(4): 286–295.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ettelaie C, Collier ME, Maraveyas A, *et al.*: **Characterization of physical properties of tissue factor containing microvesicles and a comparison of ultracentrifuge-based recovery procedures.** *J Extracell Vesicles.* 2014; **3**(1): 23592.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Herrera MB, Fonsato V, Gatti S, *et al.*: **Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomized rats.** *J Cell Mol Med.* 2010; **14**(6 B): 1605–1618.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Julian LM, Carpenedo RL, Rothberg JL, *et al.*: **Formula G1: Cell cycle in the driver's seat of stem cell fate determination.** *BioEssays.* 2016; **38**(4): 325–332.
[PubMed Abstract](#) | [Publisher Full Text](#)

11. Wierød L, Rosseland CM, Lindeman B, *et al.*: **CDK2 regulation through PI3K and CDK4 is necessary for cell cycle progression of primary rat hepatocytes.** *Cell Prolif.* 2007; **40**(4): 475–487.
[PubMed Abstract](#) | [Publisher Full Text](#)
12. Avraham S, Jiang S, Ota S, *et al.*: **Structural and functional studies of the intracellular tyrosine kinase MATK gene and its translated product.** *J Biol Chem.* 1995; **270**(4): 1833–1842.
[PubMed Abstract](#) | [Publisher Full Text](#)
13. Huang GT, Gronthos S, Shi S: **Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine.** *J Dent Res.* 2009; **88**(9): 792–806.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. Zhang N, Chen B, Wang W, *et al.*: **Isolation, characterization and multi-lineage differentiation of stem cells from human exfoliated deciduous teeth.** *Mol Med Rep.* 2016; **14**(1): 95–102.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Shabbir A, Cox A, Rodriguez-Menocal L, *et al.*: **Mesenchymal Stem Cell Exosomes Induce Proliferation and Migration of Normal and Chronic Wound Fibroblasts, and Enhance Angiogenesis *In Vitro*.** *Stem Cells Dev.* 2015; **24**(14): 1635–1647.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Collino F, Deregibus MC, Bruno S, *et al.*: **Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs.** *PLoS One.* 2010; **5**(7): e11803.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. van der Pol E, Coumans FA, Grootemaat AE, *et al.*: **Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing.** *J Thromb Haemost.* 2014; **12**(7): 1182–1192.
[PubMed Abstract](#) | [Publisher Full Text](#)
18. Othman A, Mubarak R, Sameer M: **Dataset 1 in: Fibroblast growth factor-6 enhances CDK2 and MAK7 expression in microvesicles derived from human stem cells extracted from exfoliated deciduous teeth.** *F1000Research.* 2018.
[Data Source](#)
19. Ma L, Makino Y, Yamaza H, *et al.*: **Cryopreserved Dental Pulp Tissues of Exfoliated Deciduous Teeth Is a Feasible Stem Cell Resource for Regenerative Medicine.** *Câmara NOS ed. PLoS One.* 2012; **7**(12): e51777.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Vishwanath VR, Nadig RR, Nadig R, *et al.*: **Differentiation of isolated and characterized human dental pulp stem cells and stem cells from human exfoliated deciduous teeth: An *in vitro* study.** *J Conserv Dent.* 2013; **16**(5): 423–428.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Cheng T, Scadden D: **Handbook of Stem Cells, Two-Volume Set: Volume 1- Embryonic Stem Cells...** Chapter 8-Google Books. 2014.
22. Sukarawan W, Nowwarote N, Kerdpon P, *et al.*: **Effect of basic fibroblast growth factor on pluripotent marker expression and colony forming unit capacity of stem cells isolated from human exfoliated deciduous teeth.** *Odontology.* 2014; **102**(2): 160–166.
[PubMed Abstract](#) | [Publisher Full Text](#)
23. Nowwarote N, Pavasant P, Osathanon T: **Role of endogenous basic fibroblast growth factor in stem cells isolated from human exfoliated deciduous teeth.** *Arch Oral Biol.* 2015; **60**(3): 408–415.
[PubMed Abstract](#) | [Publisher Full Text](#)
24. Quesenberry PJ, Aliotta JM: **Cellular phenotype switching and microvesicles.** *Adv Drug Deliv Rev.* 2010; **62**(12): 1141–1148.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Jablonska B, Aguirre A, Vandenbosch R, *et al.*: **Cdk2 is critical for proliferation and self-renewal of neural progenitor cells in the adult subventricular zone.** *J Cell Biol.* 2007; **179**(6): 1231–1245.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. De Bondt HL, Rosenblatt J, Jancarik J, *et al.*: **Crystal structure of cyclin-dependent kinase 2.** *Nature.* 1993; **363**(6430): 595–602.
[PubMed Abstract](#) | [Publisher Full Text](#)

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research