

World Journal of *Stem Cells*

World J Stem Cells 2021 November 26; 13(11): 1610-1812



Contents

Monthly Volume 13 Number 11 November 26, 2021

REVIEW

- 1610 Application of dental stem cells in three-dimensional tissue regeneration
Hsiao HY, Nien CY, Hong HH, Cheng MH, Yen TH
- 1625 Priming strategies for controlling stem cell fate: Applications and challenges in dental tissue regeneration
Zhang SY, Ren JY, Yang B
- 1647 Epigenetic regulation of dental pulp stem cells and its potential in regenerative endodontics
Liu Y, Gan L, Cui DX, Yu SH, Pan Y, Zheng LW, Wan M
- 1667 Effects of immune cells on mesenchymal stem cells during fracture healing
Ehnert S, Relja B, Schmidt-Bleek K, Fischer V, Ignatius A, Linnemann C, Rinderknecht H, Huber-Lang M, Kalbitz M, Histing T, Nussler AK
- 1696 Regulation of the mesenchymal stem cell fate by interleukin-17: Implications in osteogenic differentiation
Krstić J, Mojsilović S, Mojsilović SS, Santibanez JF
- 1714 Why stem/progenitor cells lose their regenerative potential
Picerno A, Stasi A, Franzin R, Curci C, di Bari I, Gesualdo L, Sallustio F
- 1733 Nanofat: A therapeutic paradigm in regenerative medicine
Jeyaraman M, Muthu S, Sharma S, Ganta C, Ranjan R, Jha SK

MINIREVIEWS

- 1747 Application of adipose-derived stem cells in treating fibrosis
Li ZJ, Wang LQ, Li YZ, Wang CY, Huang JZ, Yu NZ, Long X

ORIGINAL ARTICLE

Basic Study

- 1762 Exosomes derived from inflammatory myoblasts promote M1 polarization and break the balance of myoblast proliferation/differentiation
Luo ZW, Sun YY, Lin JR, Qi BJ, Chen JW
- 1783 ARPE-19 conditioned medium promotes neural differentiation of adipose-derived mesenchymal stem cells
Mannino G, Cristaldi M, Giurdanella G, Perrotta RE, Lo Furno D, Giuffrida R, Rusciano D
- 1797 MKK7-mediated phosphorylation of JNKs regulates the proliferation and apoptosis of human spermatogonial stem cells
Huang ZH, Huang C, Ji XR, Zhou WJ, Luo XF, Liu Q, Tang YL, Gong F, Zhu WB

ABOUT COVER

Editorial Board Member of *World Journal of Stem Cells*, Pietro Gentile, MD, PhD, Associate Professor, Surgical Science Department, Plastic and Reconstructive Surgery Unit, University of Rome "Tor Vergata", Rome 00133, Italy. pietrogentile2004@libero.it

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells (WJSC, World J Stem Cells)* is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc.*

INDEXING/ABSTRACTING

The *WJSC* is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, BIOSIS Previews, Scopus, PubMed, and PubMed Central. The 2021 Edition of Journal Citation Reports® cites the 2020 impact factor (IF) for *WJSC* as 5.326; IF without journal self cites: 5.035; 5-year IF: 4.956; Journal Citation Indicator: 0.55; Ranking: 14 among 29 journals in cell and tissue engineering; Quartile category: Q2; Ranking: 72 among 195 journals in cell biology; and Quartile category: Q2. The *WJSC*'s CiteScore for 2020 is 3.1 and Scopus CiteScore rank 2020: Histology is 31/60; Genetics is 205/325; Genetics (clinical) is 64/87; Molecular Biology is 285/382; Cell Biology is 208/279.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Hua-Ge Yu; Production Department Director: Yun-Jie Ma; Editorial Office Director: Ze-Mao Gong.

NAME OF JOURNAL

World Journal of Stem Cells

ISSN

ISSN 1948-0210 (online)

LAUNCH DATE

December 31, 2009

FREQUENCY

Monthly

EDITORS-IN-CHIEF

Shengwen Calvin Li, Tong Cao, Carlo Ventura

EDITORIAL BOARD MEMBERS

<https://www.wjnet.com/1948-0210/editorialboard.htm>

PUBLICATION DATE

November 26, 2021

COPYRIGHT

© 2021 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

<https://www.wjnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>

Priming strategies for controlling stem cell fate: Applications and challenges in dental tissue regeneration

Si-Yuan Zhang, Jia-Yin Ren, Bo Yang

ORCID number: Si-Yuan Zhang 0000-0002-6214-3459; Jia-Yin Ren 0000-0003-4289-0462; Bo Yang 0000-0003-0127-9632.

Author contributions: Zhang SY drafted the manuscript; Ren JY drew the figures and revised the manuscript; Yang B proposed the ideas and revised the manuscript; All authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Supported by Applied Basic Research Programs of Science and Technology Department of Sichuan Province, No. 2021YJ0147; and National Natural Science Foundation of China, No. 31600789.

Country/Territory of origin: China

Specialty type: Dentistry, oral surgery and medicine

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0

Si-Yuan Zhang, Bo Yang, State Key Laboratory of Oral Diseases and National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, Sichuan Province, China

Jia-Yin Ren, Department of Oral Radiology, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, Sichuan Province, China

Bo Yang, Department of Oral and Maxillofacial Surgery, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, Sichuan Province, China

Corresponding author: Bo Yang, DDS, PhD, Academic Fellow, State Key Laboratory of Oral Diseases and National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, No. 14 3rd Section, Renmin South Road, Chengdu 610041, Sichuan Province, China. dr.yangbo@scu.edu.cn

Abstract

Mesenchymal stromal cells (MSCs) have attracted intense interest in the field of dental tissue regeneration. Dental tissue is a popular source of MSCs because MSCs can be obtained with minimally invasive procedures. MSCs possess distinct inherent properties of self-renewal, immunomodulation, proangiogenic potential, and multilineage potency, as well as being readily available and easy to culture. However, major issues, including poor engraftment and low survival rates *in vivo*, remain to be resolved before large-scale application is feasible in clinical treatments. Thus, some recent investigations have sought ways to optimize MSC functions *in vitro* and *in vivo*. Currently, priming culture conditions, pretreatment with mechanical and physical stimuli, preconditioning with cytokines and growth factors, and genetic modification of MSCs are considered to be the main strategies; all of which could contribute to improving MSC efficacy in dental regenerative medicine. Research in this field has made tremendous progress and continues to gather interest and stimulate innovation. In this review, we summarize the priming approaches for enhancing the intrinsic biological properties of MSCs such as migration, antiapoptotic effect, proangiogenic potential, and regenerative properties. Challenges in current approaches associated with MSC modification and possible future solutions are also indicated. We aim to outline the present understanding of priming approaches to improve the therapeutic effects of MSCs on dental tissue regeneration.

Key Words: Mesenchymal stem cells; Priming; Dental regeneration; Culture conditions;

Grade E (Poor): 0

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Received: February 27, 2021**Peer-review started:** February 27, 2021**First decision:** April 20, 2021**Revised:** May 14, 2021**Accepted:** August 27, 2021**Article in press:** August 27, 2021**Published online:** November 26, 2021**P-Reviewer:** Naserian S, Ren N, Song S**S-Editor:** Ma YJ**L-Editor:** Kerr C**P-Editor:** Liu JH

Cytokines; Growth factors; Genetic modification

©The Author(s) 2021. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Undoubtedly, the efficacy associated with the survival and regenerative properties of unmodified mesenchymal stromal cells (MSCs) cannot be overemphasized. These properties cannot be augmented until these cells are enhanced by priming approaches to protect MSCs against an inhospitable microenvironment *in vivo*. In this review, we focus on discussing the current MSC priming approaches in the field of dental tissue regeneration. As a promising outcome, primed MSCs can exhibit prolonged therapeutic efficacy and can be applied to the clinical treatment of some dental diseases in the near future.

Citation: Zhang SY, Ren JY, Yang B. Priming strategies for controlling stem cell fate: Applications and challenges in dental tissue regeneration. *World J Stem Cells* 2021; 13(11): 1625-1646

URL: <https://www.wjgnet.com/1948-0210/full/v13/i11/1625.htm>

DOI: <https://dx.doi.org/10.4252/wjsc.v13.i11.1625>

INTRODUCTION

Research on mesenchymal stromal cell (MSC)-based therapy has made rapid strides over recent decades due to the beneficial biological effects of these cells. MSCs, which are also known as mesenchymal stem cells, are spindle-shaped cells located at perivascular sites in various human tissues and organs, including bone marrow, adipose tissue, umbilical cord, and dental tissue. Each of these MSC sources has its own advantages and disadvantages[1]. In general, MSCs are readily available and easy to culture *in vitro*, with genetic stability. MSCs can be characterized based on their specific properties: adherence to plastic and a typical immunophenotypic profile (expression of the surface markers CD44, CD73, CD90 and CD105, and a lack of CD34, CD45, CD14 and HLA-DR)[2]. MSCs possess multilineage-differentiation potential into osteoblasts, chondrocytes, adipocytes, and even highly specialized cells, such as myoblasts[3], neurons[4], endothelial cells[5], and hepatocytes[6,7]. In addition, the low immunogenicity and outstanding immunomodulatory properties of MSCs make them ideal therapeutic cell candidates[8]. To date, the experimental and preclinical applications of MSCs span various diseases and conditions, accompanied by promising outcomes.

Over the last few decades, the search for MSC-like cells in specific tissues has led to the discovery of distinct populations of MSCs from various human dental tissues. Dental tissue is a popular MSC source. Compared to cells from other tissues, MSCs from dental tissue can be obtained through minimally invasive procedures. Currently, five main populations of dental tissue-derived MSCs have been successfully isolated and characterized. Postnatal dental pulp stem cells (DPSCs)[9] were the first human dental MSCs identified in pulp tissue. Later, other types of dental tissue-derived MSCs were gradually discovered, including stem cells from human exfoliated deciduous teeth (SHEDs)[10], periodontal ligament stem cells (PDLSCs)[11], dental follicle precursor cells (DFCs)[12] and stem cells from the apical papilla (SCAPs)[13]. In dental tissue, these five types of MSCs are accessible MSC-like populations with superior self-renewal capacities, immunomodulatory functions, and multilineage-differentiation potential for tissue regeneration.

Nevertheless, the beneficial effects of MSC-based regeneration are not always fulfilled. MSC properties can be influenced by *in vitro* and *in vivo* biological, biochemical and biophysical factors *via* reciprocal cell-to-cell interactions, the extracellular matrix (ECM), and soluble bioactive factors[14]. MSCs *in vivo* interact with surrounding cells and tissues in a three-dimensional (3D) microenvironment, producing anti-inflammatory molecules, promoting angiogenesis, preventing cell death, and reconfiguring the ECM[15]. Moreover, MSCs reside in a microenvironment with relatively low oxygen tension (*i.e.*, 1%-5% O₂) *in vivo*, while the O₂ tension *in vitro* (*i.e.*, 20%-21%) is much higher than in the original MSC microenvironment[16]. The

different O₂ tension associated with *in vitro* culture can decrease cell-regenerative capacities, including proliferation, differentiation, and anti-inflammatory responses. MSCs are generally cultured in nutrient- and O₂-enriched environments *in vitro*. In contrast, transplanted MSCs are confronted with harsh conditions such as lack of blood supply and inflammation induced by tissue damage; both of which cause apoptosis and senescence, leading to the failure of regeneration. In this context, MSCs have shown promising regenerative properties for tissue repair; however, exploring more effective strategies is still necessary to improve their therapeutic efficacy.

MSC properties cannot be augmented without further enhancement through by priming approaches to protect MSCs against an inhospitable microenvironment *in vivo*. To this end, some recent investigations have sought ways to improve MSC functions *in vitro* and *in vivo*. Optimizing culture conditions, preconditioning with cytokines and growth factors, and genetic modifications of MSCs are considered to be the main strategies; all of which contribute to improving MSC transplantation efficiency for tissue regeneration[17]. Several studies have revealed that pretreated MSCs exhibit better cell survival, augmented homing abilities to injury sites, enhanced immunomodulatory properties, optimized proangiogenic abilities, and increased multilineage-differentiation capabilities[18-20]. Figure 1 shows the six focal improvements in MSCs that contribute to improved therapeutic effects. Research in this field has made tremendous progress and continues to gather interest and spur innovation. In this review, we summarize the approaches proposed to improve dental tissue-derived MSC functions for dental tissue regeneration and divide these approaches into four categories: (1) Culture condition manipulation; (2) Pretreatment with mechanical and physical stimuli; (3) Preconditioning with growth factors and cytokines; and (4) Gene modification. We further discuss these approaches mainly in the context of enhancing the intrinsic biological properties of MSCs such as migration, anti-apoptotic effect, immunomodulation, proangiogenic potential, and regenerative properties.

DENTAL TISSUE-DERIVED MSCS

Characteristics of dental MSCs

DPSCs are MSC-like cells in the pulp chamber of permanent teeth that originate from the cranial neural crest. Figure 2 shows the different dental regions from which DPSCs originate, together with other dental tissue-derived MSCs. Similar to bone-marrow-derived MSCs, DPSCs have enriched expression of the surface markers Stro-1, CD29, CD73, CD90, CD105 and CD166, while they are negative for hematopoietic markers such as CD14, CD45, CD34, CD25 and CD28[9]. DPSCs exhibit rapid proliferation and superior immunosuppressive properties, and they are prone to forming dentin/pulp-like complexes. Aside from their odontogenic potential, DPSCs can be reprogrammed into adipocytes, chondrocytes, myocytes and neural cells[21].

SHEDs were isolated from the dental pulp tissue of exfoliated deciduous teeth. SHEDs share similar MSC regenerative capacities such as self-renewal and multilineage differentiation potential[10]. However, these cells exhibit increased proliferation rates and the spontaneous formation of sphere-like cell clusters. SHEDs are distinct from DPSCs[22]. In addition to the expression of DPSC surface markers, SHEDs also highly express the embryonic stem cell markers Oct4 and Nanog, stage-specific embryonic antigen (SSEA)-3 and SSEA-4, and the neural stem cell marker nestin. After the induction of neurogenesis, SHEDs show higher expression than DPSCs of neuronal and glial cell markers, such as β -III-tubulin, tyrosine hydroxylase, microtubule-associated protein (MAP)2, and nestin[23].

SCAPs and DFCs are MSCs that are derived only from developing permanent teeth. SCAPs are found at the apices of growing teeth, and DFCs are located in connective tissue sacs surrounding the enamel organ[13,24]. These two types of dental MSCs can form adherent clonogenic clusters and differentiate into adipocytes, odontoblasts/osteoblasts, cementoblasts, and periodontal ligament. SCAPs have been reported to possess greater potential to form dentin than DPSCs, due to their higher proliferation capacity and greater telomerase activity. DFCs are regarded as the parent cells of periodontal tissue and can form periodontal tissues, including alveolar bone, periodontal ligament, and cementum.

PDLSCs are derived from the human periodontal ligament, which is a connective tissue that lies between the cementum and the alveolar bone socket. PDLSCs have been demonstrated to be a reliable source of periodontal tissue regeneration. Compared to DPSCs, PDLSCs exhibit higher expression of scleraxis, a tendon-specific transcription factor[11]. These cells can be readily expanded *in vitro* and generate

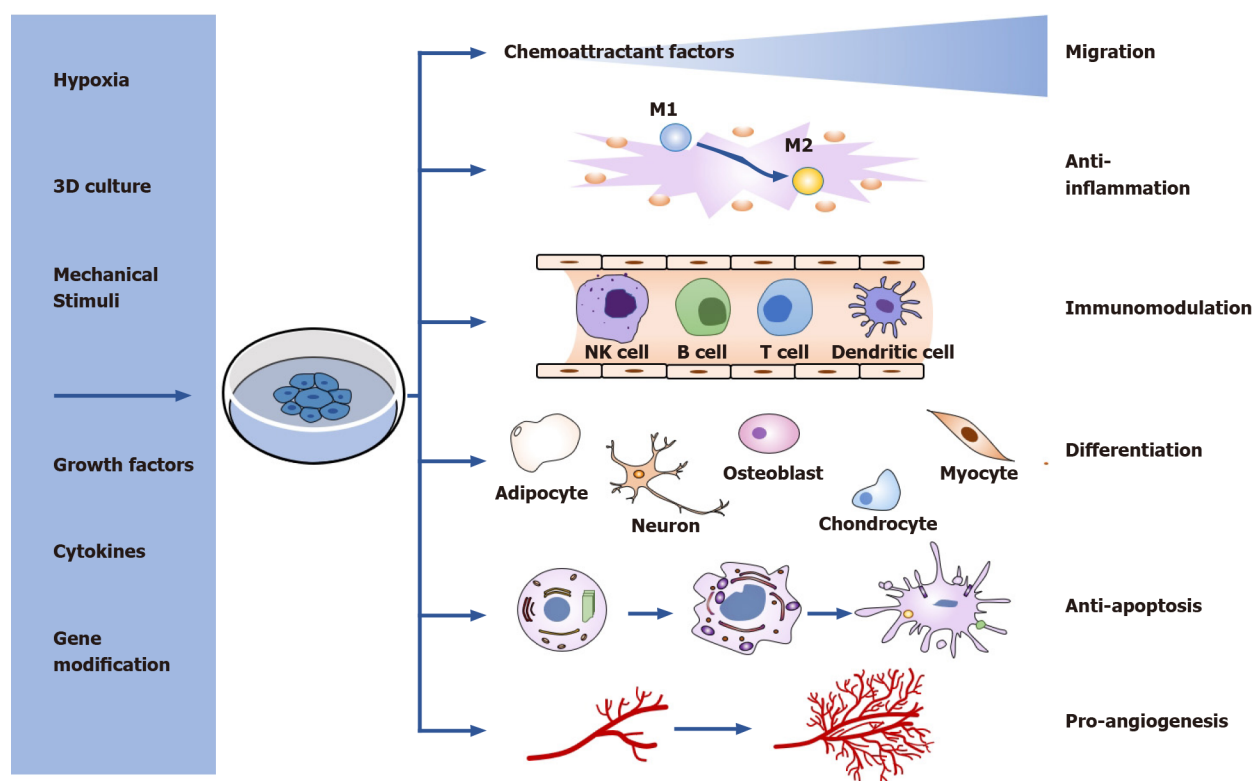


Figure 1 Overview of the functional improvements of mesenchymal stromal cell (MSC) properties by priming strategies. MSCs can be primed via different approaches, such as priming culture conditions, pretreatment with mechanical and physical stimuli, preconditioning with cytokines and growth factors, and genetic modification. As promising outcome, primed MSCs can exhibit prolonged therapeutic efficacy, including migration, anti-apoptosis, anti-inflammation, immunomodulation, proangiogenesis, and regenerative properties. The improved capabilities are interconnected and greatly influenced by priming approaches.

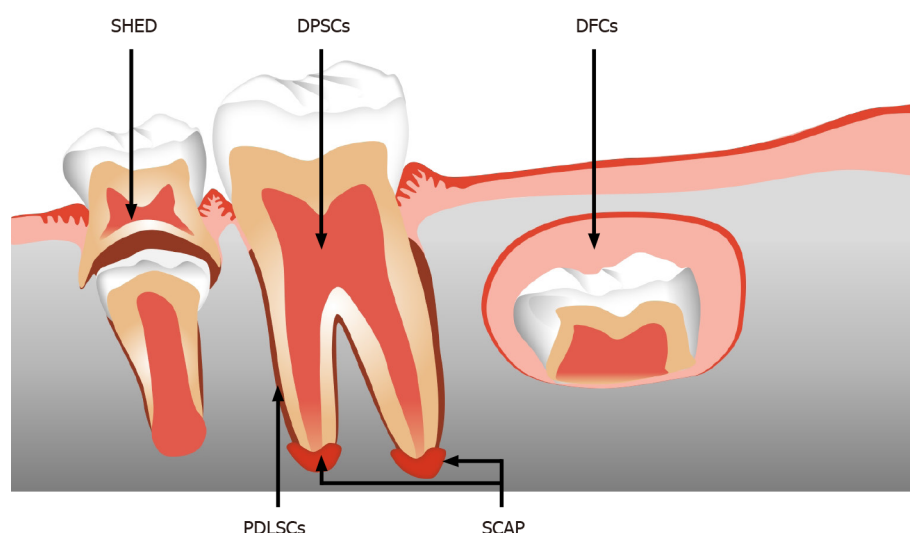


Figure 2 Schematic image of dental mesenchymal stromal cells from different tissue regions. SHED: Stem cells from exfoliated deciduous teeth; DPSCs: Dental pulp stem cells; DFCs: Dental follicle precursor cells; PDLSCs: Periodontal ligament stem cells; SCAP: Stem cells from apical papilla.

cementum/periodontal ligament-like complexes *in vivo*. In addition, the osteogenic differentiation capability of PDLSCs was demonstrated by the formation of calcified nodules and the expression of alkaline phosphatase (ALP), matrix extracellular protein (MEPE), bone sialoprotein (BSP), osteocalcin (OCN), and transforming growth factor (TGF)- β receptor I.

Dental MSC applications and limitations

Dental MSCs have been extensively investigated in preclinical studies. Moreover,

several clinical trials have been reported in recent years[25-27]. Some reviews have already summarized the benefits of dental MSCs in regenerative medicine[28-30]. In short, dental MSCs have been reported to promote the regeneration of dental tissues, bone, cartilage, muscle, and nerves[31-34]. Moreover, dental MSCs have also been implicated in the treatment of various diseases, such as brain ischemia[35], liver fibrosis[36], diabetes[37], rheumatoid arthritis[38], and Alzheimer's disease[39]. However, dental MSCs have thus far exhibited only moderate benefits in clinical studies, and researchers are still struggling to move forward to advanced phases (III and IV) of clinical trials. To secure efficient and successful translation of clinical procedure for dental MSCs, substantial approaches concerning functional improvements must be established.

CELL FUNCTION OPTIMIZATION STRATEGIES

Culture condition manipulation

In general, MSCs reside in a confined microenvironment called the stem cell niche *in vivo*, which includes not only MSCs themselves but also other supporting cells and ECM. The stem cell niche is the basis of tissue homeostasis. Dental MSCs are isolated from individuals; these cells must be cultured and expanded *in vitro* to obtain a sufficient number of cells before transplantation. However, *in vitro* culture does not entirely replicate *in vivo* cell behavior, and cells may lose their tissue-specific functions. Cell culture conditions, such as O₂ tension and 3D culture, influence cell behavior. Recreating the physical and mechanical microenvironment experienced by MSCs *in vivo* is important in reproducing the stem cell niche. Several studies have reported that manipulation of conventional culture conditions could enhance the regenerative efficacy of MSCs. Here, we introduce two widely used alternative culture methods: hypoxic preconditioning and 3D spheroid culture. The enhancement of intrinsic biological properties of MSCs by culture condition manipulation and other priming strategies are summarized in Table 1.

Hypoxic preconditioning

O₂ is one of the critical factors associated with cellular homeostasis, as a lack of O₂ could result in disease pathogenesis. O₂ tension varies from 1% to 12% in the physiological state in adult organs and tissue, depending on the degree of tissue vascularization and metabolic activities[16,40]. MSCs residing in the general dental microenvironment are exposed to low O₂ tension (*i.e.*, 3%–6% O₂)[41], while MSCs *in vitro* are typically exposed to higher O₂ concentrations (*i.e.*, 20%–21% O₂). The negative impact of ambient O₂ tension on MSCs cultured *in vitro*, such as decreased proliferative capacity, DNA damage, and senescence, was reported by a number of studies [42-46]. When MSCs are expanded in normoxia and then transplanted into injured tissue, they face hypoxic conditions and undergo apoptosis. Hypoxic preconditioning of MSCs is regarded as a better way to mimic the naïve MSC niche and improve their therapeutic potential than normoxic culture conditions[47].

In general, hypoxic conditions require a reliable experimental device to maintain stable O₂ tension for cell culture. Commonly used CO₂ incubators have difficulty producing low O₂ levels. Currently, there are several approaches to achieve hypoxic conditions for cultured cells[48]. One approach involves using a specialized hypoxic chamber inside a standard CO₂ incubator. This is a convenient and low-cost method. However, the major drawback is gas leakage that may disrupt the experimental processes and cause fluctuations in O₂ concentrations in the incubator. Moreover, O₂ concentrations can be temporarily disturbed by every time incubator doors are opened and take time to stabilize. Hypoxic culture can also be performed in a tri-gas incubator, which is an effective device to generate stable experimental O₂ concentrations. CO₂ and nitrogen (N₂) are supplied to reduce the O₂ levels in the incubator. Because of ability to control the gas mixture (CO₂, O₂ and N₂), the tri-gas incubator is currently considered to be a practical approach to provide the closest conditions to those in the body. A third method is pharmacological agents that stimulate hypoxia. Cobalt chloride (CoCl₂)[49,50] and deferoxamine (DFO)[51] are well-known hypoxia mimetics that act by inducing the expression of hypoxia inducible factors (HIFs), which play vital roles in the hypoxia signaling pathway and guide the cellular response to hypoxia. Stabilization of HIF-1 can also be achieved by propyl hydroxylase inhibitors (PHDs), which block enzymatic activity to inhibit HIF-1 degradation during hypoxia[52].

Table 1 Priming strategies for controlling mesenchymal stromal cell fate

Priming strategies	Cell type	Benefits	Mechanisms	Reference	
Culture conditions	Hypoxia:				
	1%–3% O ₂	DPSCs; PDLSCs	Improve survival	Upregulation of stem cell markers; Regulation of metabolic activities; Activation of the p38/MAPK and ERK/MAPK pathways	[66,67,70]
		DPSCs; SHEDs; SCAPs	Promote angiogenesis	Increase proangiogenic factors releasing	[60,62,63]
		DPSCs; SCAPs; PDLSCs	Enhance differentiation potential	Upregulation of odontoblastic markers	[68,69,71,72]
		PDLSCs	Enhance anti-inflammation effect	Upregulation of IL-37	[73]
	Pharmacological stimulation	DPSCs	Promote angiogenesis	Increase intracellular levels of HIF-1α	[51,64,65]
		PDLSCs	Improve survival but inhibit differentiation potential		[49]
	3D culture:				
	Single cell type	DPSCs; PDLSCs	Enhance differentiation potential	Upregulation of odontoblastic markers	[83,84,87]
	Coculture	DPSCs and ECs	Promote angiogenesis		[86]
	Mechanical and physical stimuli:				
	LIPUS	DPSCs; PDLSCs	Increase proliferation	Activation of MAPK pathway	[94,95]
	Cyclic mechanical tension	DPSCs	Promote osteogenic differentiation; Increase cytokines release	Upregulation of osteoblastic markers	[98,99]
	Uniaxial stretch	DPSCs	Increase proliferation but inhibit osteo/odontogenic differentiation		[96,97]
Cytokines					
SDF-1	DPSCs; PDLSCs	Promote cell migration	Activation of SDF-1/CXCR4 axis; Autophagy; Activation of AKT and GSK3β/β-catenin pathways	[104,107,109,110]	
	PDLSCs	Anti-apoptosis	Activation of ERK pathway	[105]	
	DPSCs; PDLSCs	Enhance differentiation potential	Upregulation of odontoblastic markers; Upregulation of osteoblastic markers	[106,108,111]	
TNF-α		Enhance immunomodulatory effects	Mediated by TNF/TNFR2 signaling	[124-126]	
		Enhance osteogenic differentiation	Activation of p38 pathway; Activation of miR-21/STAT3 and NF-κB pathway	[127-129]	
		Inhibit differentiation potential (50-100 ng/mL)	Activation Wnt/β-catenin pathway	[132]	
G-CSF; IFN-γ	DPSCs	Promote cell migration		[114,121,122]	
	DPSCs	Enhance or inhibit differentiation potential depend on cytokines concentration		[116,120,121]	
Preconditioning mediators	growth factors				
	bFGF	DPSCs	Promote angiogenesis		[136,139-141]
		DPSCs; PDLSCs	Enhance differentiation potential on dose dependent (20-50 ng/mL in vitro; 15 μg/mL-5 mg/mL in vivo)	Upregulation of odontoblastic markers; Upregulation of osteoblastic markers; Upregulation of neural markers; Activation of FGFR/MEK/ERK1/2 and BMP/BMPR signaling pathways	[137,138,141,144,149-151]

Genetic modification	IGF-1	DPSCs	Promote anti-inflammation effect	Altered cytokines expression;	[146-148]
		PDLSCs	Promote cell survival		[165]
		DPSCs	Anti-apoptosis		[164]
		DPSCs; PDLSCs	Enhance differentiation potential	Upregulation of osteoblastic markers; Upregulation of odontoblastic markers; Activation of mTOR pathway; Target of EphrinB1	[160-162,166]
	Sox-2	DPSCs	Improve cell migration		[171]
	Bcl-2; Oct-4	DPSCs	Improve cell survival	Upregulation of stemness-rated genes;	[168,169]
	Foxo-1	PDLSCs	Promote anti-inflammation effect	Resistance to oxidative stress	[175]
	BMP family; Runx2	DPSCs; SCAP; DFCs	Enhanced differentiation potential	Upregulation of osteoblastic markers; Upregulation of odontoblastic markers	[178-184]

DPSCs: Dental pulp stem cells; PDLSCs: Periodontal ligament stem cells; SHED: Stem cells from human exfoliated deciduous teeth; SCAP: Stem cells from the apical papilla; LIPUS: Low-intensity pulsed ultrasound; SDF-1: Stromal cell-derived factor-1; TNF- α : Tumor necrosis factor- α ; G-CSF: Granulocyte-colony stimulating factor; IFN- γ : Interferon- γ ; bFGF: Basic fibroblast growth factor; IGF-1: Insulin-like growth factor-1; Bcl-2: B-cell lymphoma 2.

Hypoxic preconditioning was reported to have positive impacts on the survival and proangiogenic properties of MSCs. When cultured in low-serum medium, 1% O₂ pretreated MSCs can prevent damage through increased paracrine secretion of proangiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)[53]. Low O₂ concentrations also increased metabolic activity and decreased caspase-3/7 activity, thus reducing the sensitivity of MSCs to the ischemic microenvironment[54]. MSCs induced by 2% O₂ showed decreased tumorigenic potential, as indicated by significantly reduced transformation into tumor-associated fibroblasts[55]. Compared to normoxic conditions at 20% O₂, hypoxic conditions at 3% O₂ improved genetic and chromosomal stability, ensuring the safety of MSCs[56]. Regarding MSCs derived from dental tissue, hypoxic preconditioning has been evaluated in DPSCs[57,58], SHEDs[59], SCAPs[60] and PDLSCs[61].

DPSCs, SHEDs and SCAPs are involved in pulp regeneration. These cells have been demonstrated to support the process of pulp revascularization under hypoxia by releasing proangiogenic molecules[60,62,63]. DFO, CoCl₂, and PHD inhibitors induce hypoxia-stimulated VEGF production in explanted dental pulp by increasing intracellular levels of HIF-1 α [51,64,65]. The mesenchymal stem cell marker STRO-1 is reported to be increased in dental pulp cells under hypoxic conditions[66]. Fukuyama *et al*[67] revealed that the proliferation of dental pulp cells was initially suppressed under hypoxia but increased afterward because of activation of the metabolism-related enzyme AMP-activated protein kinase. Hypoxic conditions can modulate the mineralization potential of DPSCs. Higher expression of odontoblastic markers such as OCN, dentin sialophosphoprotein (DSPP), and dentin matrix acidic phosphoprotein-1 (DMP1) was observed under hypoxia[68]. SCAPs preconditioned with 1% O₂ exhibited upregulated osteogenic and neuronal differentiation as well as angiogenesis[69].

Hypoxia promoted PDLSC clone formation and proliferation *via* the p38/MAPK and ERK/MAPK signaling pathways[70]. A study on PDLSCs under hypoxia showed enhanced osteogenic differentiation both *in vitro* and *in vivo*[71]. This effect could be the reason that hypoxia mediates the expression of RUNX2 in PDLSCs *via* HIF-1 α -induced VEGF[72]. However, hypoxia induced by CoCl₂ maintained the stemness of PDLSCs while inhibiting the osteoblastic differentiation of PDLSCs[49]. The secretome of hypoxia-preconditioned PDLSCs showed an augmented anti-inflammatory effect mediated by interleukin (IL)-37 expression[73]. After culture in 1% O₂ for 24 h, the proteomic profile of PDLSCs, mainly proteins related to energy metabolism, autophagy, and stimuli-responsive proteins, was changed[74].

These findings highlight the advantages of hypoxic pretreatment in dental MSC culture. However, to enable the practical use of hypoxia-treated dental MSCs, some issues should be considered. In experimental settings, a wide range of O₂ concentrations (from 5% to < 1%) were used in different studies, and each concentration may stimulate different properties of dental MSCs. This issue was indicated in adipose-derived MSCs (ASCs). Choi *et al*[75] reported that the differentiation potential of ASCs was improved significantly by 2%–5% O₂, while a lower O₂ level reduced the effect. Moreover, MSCs of different dental origins require distinct O₂ tensions to meet tissue-

specific demands. For instance, the O₂ concentration required by DPSCs for pulp regeneration could differ from that of PDLSCs for periodontal tissue regeneration. Therefore, the optimal O₂ level for maximizing the capacity of each type of dental MSCs should be determined before clinical application.

3D spheroid culture

In traditional two-dimensional (2D) culture, cells attach to a plastic surface and grow as a monolayer in a culture flask. However, this is a highly artificial environment that fails to recapitulate cell-to-cell or cell-to-ECM interactions, stimulating dedifferentiation capacity and decreasing the therapeutic performance of MSCs[76]. 3D spheroid culture techniques have been applied to overcome this issue. 3D spherical cell aggregates are multicellular structures that consist of cells, ECM, and paracrine factors, mimicking the spontaneous metabolic microenvironment in both nutrient and O₂ concentrations and providing superior *in vivo* models compared to monolayer culture systems[77]. Several studies have compared the therapeutic effects of 2D- and 3D-cultured MSCs and shown the improved regenerative capacity of spheroid-cultured MSCs[78]. It has been reported that spheroid culture potentiates the proliferative and differentiative capacity of MSCs[79]. MSCs cultured as spheroids showed altered adhesion molecule gene expression patterns and enhanced immunomodulatory capacity[80]. In addition, the augmented migration and homing efficiency of MSCs to the damaged site were found to promote engraftment after *in vivo* transplantation[81].

Based on these findings, the regenerative potential of 3D spheroid cultured dental MSCs was evaluated in various preclinical studies. Xiao *et al*[82] found that DPSCs were able to form large aggregates on Matrigel under osteogenic induction by undergoing a process of central cell death, cavitation, and spontaneous multilineage differentiation. Yamamoto *et al*[83] fabricated 3D DPSC aggregates through a low-attachment method. The DPSC spheroids exhibited improved odonto/osteoblastic differentiation ability *in vitro* that was mediated by integrin signaling. Lee *et al*[84] then demonstrated that 3D DPSC spheres exhibited enhanced odontogenic differentiation *in vivo*. In addition, the spheres were enriched in pluripotency transcription factors, such as Sox2, Lin28, Esrrb and Klf4. 2D- and 3D-cultured DPSCs were further compared by microarray analysis, and the expression of genes related to the ECM, cell differentiation, cell-to-cell/cell-to-matrix, and osteoblast differentiation was promoted [85]. Dissanayaka *et al*[86] fabricated microtissue spheroids by coculturing DPSCs and human umbilical vein endothelial cells in an agarose 3D Petri dish. Upon subcutaneous implantation *in vivo*, effective pulp-like tissue formation and capillary-like structures were successfully anastomosed with the host vasculature. PDLSC spheroids were also examined and showed enhanced osteogenic differentiation regulated by SFRP3-mediated ALP activation[87]. Collectively, DPSC or PDLSC spheroids could maintain their enhanced therapeutic functions both *in vitro* and *in vivo*.

Although there is a general consensus that 3D spheroid culture exhibits therapeutic advantages over monolayer culture, several technical points still need to be considered to optimize 3D culture methods. Currently, hanging drops and low attachment surfaces are the two primary methods of spheroid fabrication, but both methods are inefficient and have low yields in the laboratory. Automated 3D-bioreactor systems have been designed to minimize labor-intensive, time-consuming procedures and improve the yield of 3D-cultured MSCs[76,77,88]. The size and cell number of each spheroid and the culture period are other important factors to consider. Since the core area of spheroids is often hypoxic and lacks nutrients, excessively large spheroids could lead to cell death or dysfunction[89]. In addition, the culture duration (short-term or long-term) tends to affect the density of spheroids, which influences the cell gene expression profiles and other secretory factors[81]. Therefore, researchers need to determine an effective culture protocol to obtain a sufficient number of homogeneous spheroids with stable therapeutic efficacy.

Mechanical and physical stimuli

Dental MSCs are mechanosensitive cells that can recognize and transform mechanical changes into cellular responses[90,91]. Sübay *et al*[92] found that the application of orthodontic extrusive forces to teeth had no significant adverse effect on human pulp tissue. Since then, several studies have shown that mechanical stimuli such as low-intensity pulsed ultrasound (LIPUS), uniaxial mechanical stretch, cyclic mechanical tension and cyclic uniaxial compressive stress are able to induce the proliferation of dental MSCs. In addition, physical stimuli including surface topographies, dynamic hydrostatic pressure and pulsating fluid flow have been reported to promote the differentiation of dental MSCs[93].

The positive effects of mechanical and physical stimuli on the biological behavior of dental MSCs have been well described. Gao *et al*[94] showed that LIPUS, which is applied clinically to promote healing, can promote DPSC, PDLSC and BMMSC proliferation in an intensity- and cell-specific dependent manner *via* activation of distinct mitogen-activated protein kinase (MAPK) pathways. The author further demonstrated that Piezo1 and Piezo2, two mechanosensitive membrane ion channels, contributed to transducing ultrasound-associated mechanical signals and activating downstream MAPK signaling processes in dental MSCs[95]. Two other studies noted that uniaxial stretch increased the proliferation of DPSCs but inhibited their osteo/odontogenic differentiation potential, indicating that additional studies are required to clarify the intra- and intercellular mechanisms associated with mechanical stress[96,97]. To better understand the effect of mechanical stress on dental MSC differentiation, Han *et al*[98] observed increased proliferation and mRNA expression levels of osteogenic markers under cyclic mechanical tension. These findings suggest that the mechanical cyclic tension is a potent positive modulator of osteogenic differentiation in DPSCs. Yang *et al*[99] demonstrated that compressive stress can induce cell morphology changes and odontogenic differentiation in DPSCs. The study showed increased expression of ALP, DMP1, BMP2, DSPP and collagen (COL) type I under compressive stress, indicating that mechanical stimuli could initiate repair mechanisms in the dentin-pulp complex. It should be noted that Lee *et al*[100] showed that mechanical stimuli increased the release of proinflammatory cytokines and antioxidant defense enzymes. This study demonstrated that proinflammatory cytokines and reactive oxygen species produced in response to mechanical strain might play a key role in activating the early cellular signals involved in Nrf2-ARE-mediated gene transcription, providing a guidance for cellular protection or suppressing harmful side effects.

Cytokine and growth factor priming to enhance MSC functions

It is generally accepted that cytokine and growth factor priming may influence host tissues *via* paracrine effects. The interaction between growth factors and their receptors on cell surfaces activates downstream signal transduction for cell survival, migration and differentiation. Moreover, MSCs have inherent immunomodulatory characteristics to inhibit T cell and B cell activation and dendritic cell differentiation, impair the cytolytic potential of natural killer (NK) cells, and promote regulatory T cell (Treg) differentiation[101]. However, the immunomodulatory effect of MSCs is not always achieved but requires stimulation by inflammatory factors, such as interferon (IFN)- γ , tumor necrosis factor (TNF)- α and IL-1 β [102]. Therefore, priming MSCs with cytokines and growth factors in this context is suggested to be a supplemental molecular strategy to foster the therapeutic potential of MSCs and contribute to establishing a hospitable microenvironment for dental tissue repair.

Cytokine priming

Stromal cell-derived factor-1 priming: Stromal cell-derived factor (SDF)-1, also known as chemokine CXC ligand (CXCL)12, plays a major role in cell trafficking and homing. SDF-1 has been shown to bind to the G-protein coupled receptor CXC receptor (CXCR)4 to induce SDF-1/CXCR4 signaling[103]. SDF-1 pretreatment enhanced proliferation, migration and differentiation, and inhibited apoptosis in DPSCs and PDLSCs [104-107]. Stimulating CD105⁺ DPSCs with SDF-1 was shown to significantly improve the therapeutic effects in a canine pulpectomy model[108]. SDF-1-induced migration was reported to be mediated by the AKT and GSK3 β / β -catenin pathways[109]. Autophagy is also involved in SDF-1-mediated DPSC migration during pulp regeneration[110]. In periodontal regeneration, Liang *et al*[111] demonstrated that cotreatment with SDF-1/exendin-4 facilitated the proliferation, migration, and osteogenic differentiation of PDLSCs and promoted periodontal bone regeneration. Similar effects on periodontal regeneration were also shown in studies of SDF-1 cotransplantation with bFGF[112] or parathyroid hormone (PTH)[113].

Granulocyte-colony stimulating factor priming: Granulocyte-colony stimulating factor (G-CSF) is a cytokine that stimulates the bone marrow to produce and release neutrophils into the bloodstream. G-CSF is frequently used to mobilize hematopoietic stem cells from the bone marrow to the systemic circulation[114]. G-CSF has a migratory effect on DPSCs similar to that of SDF-1, suggesting a potential alternative to SDF-1. Stimulation of MSC migration by G-CSF was demonstrated to strictly depend on the expression of G-CSF receptor (G-CSFR)[114]. It was revealed that 44%–56% of G-CSF-mobilized DPSCs were G-CSFR-positive cells[115]. G-CSF-mobilized DPSCs showed higher regenerative potential than untreated DPSCs[116].

Proinflammatory cytokine priming

IFN- γ priming: IFN- γ is a well-known proinflammatory cytokine secreted by activated T and NK cells. IFN- γ stimulates 2,3-indolamine dioxygenase (IDO) expression in MSCs to enhance immunosuppressive properties[117]. Wada *et al*[118] revealed that DPSCs, PDLSCs and gingival mesenchymal stem cells have immunosuppressive properties that are mediated partly by IFN- γ produced by activated peripheral blood mononuclear cells (PBMCs). Another study further demonstrated that the immunosuppressive effect of DPSCs on PBMC proliferation and B cell immunoglobulin production was significantly enhanced by IFN- γ and mediated by TGF- β [119]. Sonoda *et al*[120] found that DPSCs isolated from diseased teeth with pulpitis had impaired immunosuppressive abilities, but these abilities could be restored by IFN- γ treatment. This study revealed that IFN- γ improved dentin formation and T cell suppression of pulpitis-derived DPSCs by enhancing telomerase activity. In addition, other studies reported that healthy DPSCs exposed to IFN- γ exhibited increased proliferation and migration but impaired odonto/osteogenic differentiation, which may be regulated by the nuclear factor (NF)- κ B and MAPK signaling pathways[121]. Increased release of CXCL6 and CXCL12 by IFN- γ -primed DPSCs may contribute to the homing of MSCs for pulp repair[122].

TNF- α priming: TNF- α is a pleiotropic cytokine produced predominantly by macrophages in response to bacterial endotoxin. TNF- α priming has a similar effect as that of IFN- γ priming and upregulates the expression of immunoregulatory factors, such as prostaglandin E2, IDO and hepatocyte growth factor (HGF)[123]. TNF signaling plays dual roles that are likely to be transduced through its two distinct receptors, TNFR1 and TNFR2. The interaction of TNF- α with TNFR1 mediates proinflammatory effects and cell death, while the interaction with TNFR2 mediates anti-inflammatory effects and cell survival. Recent studies have revealed that TNF-TNFR2 signaling but not TNF-TNFR1 signaling is a crucial mediator that regulates the regenerative and immunomodulatory effects of MSCs[124]. TNFR2 expression is correlated with NF- κ B, which could be a possible explanation for the effect of TNF priming. The researchers confirmed the results by investigating the role of TNFR2 in proangiogenic functions, the suppression of T cells, the induction of Tregs, and alternations in T cell cytokine secretion pattern. Moreover, it was also mentioned by the authors that TNFR2 expression was essential after TNF pretreatment[125,126]. Paula-Silva *et al*[127] showed that 10 ng/mL TNF- α stimulated the differentiation of DPSCs toward an odontoblastic phenotype *via* the p38 signaling pathway while downregulating matrix metalloproteinase (MMP)-1 expression. The miR-21/STAT3 and NF- κ B signaling pathways are reported to be involved during osteogenic differentiation[128,129]. Liu *et al*[130] revealed continuous transition in transcriptome changes during TNF- α mediated osteogenic differentiation. The TGF- β and PI3K/Akt pathways are sequentially activated. TNF- α (50 ng/mL) stimulated DPSC migration through upregulation of integrin α 6[131]. However, the osteogenic differentiation of DPSCs was suppressed by high dose TNF- α (50–100 ng/mL) by activating Wnt/ β -catenin signaling[132]. In addition to odontoblastic differentiation, TNF- α increased the angiogenic potential of cells in a coculture model of DPSCs and endothelial cells[133]. TNF- α in combination with lipopolysaccharide promoted angiogenesis *via* VEGF and sirtuin 1 signaling in DPSCs[134].

Growth factor priming

bFGF priming: bFGF, also known as FGF-2, is considered to be an important growth factor that assists tissue regeneration[135]. bFGF plays a potential role in the multipotent differentiation of DPSCs. Studies have reported that bFGF stimulates DPSC proliferation, angiogenesis, odontoblastic differentiation, and neuronal differentiation *in vitro* at concentrations of 20–50 ng/mL[136–138]. *In vivo* studies showed that bFGF at concentrations of 15 μ g/mL–5 mg/mL significantly promoted angiogenesis[139,140] and odontoblastic differentiation[141,142] for pulp regeneration. Gorin *et al*[143] found that bFGF-treated DPSCs increased angiogenesis through HGF and VEGF secretion. bFGF priming augmented proangiogenic properties compared to the effect of hypoxia. Sagomonyants *et al*[138] reported that bFGF-induced mineralization of DPSCs was mediated by activation of the FGFR/MEK/ERK1/2 and BMP/BMPR signaling pathways. Zhang *et al*[144] revealed that bFGF induced neural differentiation in DPSCs by upregulation of nestin, MAP-2, β 3-tubulin, glial intermediate filament protein (GFAP), and silent information regulator protein 1 expression through the ERK and AKT signaling pathways. bFGF assists DPSC differentiation by increasing the gene expression of MEPE, DSPP, DMP-1 and OCN[138]. bFGF is also involved in the production of hyaluronic acid (HA), which is a key component of the ECM. Tooth

development and odontoblastic differentiation require HA synthesis[145]. It is suggested that bFGF-primed DPSCs promote anti-inflammatory effects and odontoblastic differentiation through increased HA secretion[146,147]. Furthermore, bFGF-primed DPSCs exhibit altered expression of cytokines, including IL-6, IL-8, monocyte chemoattractant protein-1, macrophage inflammatory protein-1 α , and CC chemokine ligand 20[148]. The PKC/PI3K-AKT/MAPK signaling pathways have been shown to contribute to cytokine upregulation[148]. Consistent with pulp regeneration studies, bFGF priming for periodontal tissue regeneration has shown similar results, including enhanced PDLSC migration, proliferation, osteogenesis and neurogenesis *in vitro* and *in vivo*[149-152].

However, some studies showed that bFGF did not benefit cell differentiation[142, 153-155]. He *et al*[153] and Kim *et al*[142] showed that DPSCs preconditioned with 20 ng/mL bFGF exhibited increased cell proliferation, but inhibited cell differentiation. Likewise, Takeuchi *et al*[154] reported that bFGF promoted cell proliferation at a concentration of 50 ng/mL but inhibited cell differentiation at 100 ng/mL. Odontoblastic differentiation markers such as COLI[156], ALP activity[155], and calcium deposition were reported in some studies to have no changes[157]. Although there was a contradictory effect of bFGF on cell behaviors, most previous studies confirmed the potential effect of bFGF on cell proliferation. The effect of bFGF on cell differentiation was suggested to depend on the spatially and temporally controlled priming of MSCs by bFGF[158,159]. bFGF induced odontoblastic differentiation in dental pulp at the early stage (days 3-7)[138] but inhibited odontoblastic differentiation during late exposure (days 7-21)[158,159]. These findings suggested that the concentration of bFGF and the release duration should be accurately controlled.

Insulin-like growth factor-1 priming: Insulin-like growth factor (IGF)-1, a member of the insulin-like peptide family, has been shown to play an essential role in the growth and differentiation of various tissues, including teeth. Tooth germ explants treated with IGF-1 showed increased formation of dentin and enamel[160]. IGF-1 promoted DPSC proliferation and osteogenic differentiation by activating the mammalian target of rapamycin (mTOR) signaling pathway[161]. IGF-1 was also found to target EphrinB1 to regulate tertiary dentin formation[162]. Ma *et al*[163] further revealed the role of the IGF-1/IGF-1R/hsa-let-7c axis in regulating the committed differentiation of SCAPs. Yan *et al*[164] reported that IGF-1 rescued the adverse effects of high glucose concentration on DPSCs and protected against apoptosis. Regarding periodontal ligament regeneration, IGF-1 was shown to enhance the survival of PDLSCs[165]. Yu *et al*[166] described the beneficial effect of IGF-1 on PDLSC proliferation and osteogenesis *via* the ERK and JNK MAPK pathways.

Preconditioning with cytokines and growth factors is a promising way to improve the therapeutic efficacy of cells for dental tissue regeneration. However, these treatments synergistically or antagonistically influence MSC properties. Further and intensive studies need to be conducted to clarify the optimal concentration or combination of these factors and verify the detailed mechanisms according to their chemical characteristics.

Genetic modification of MSCs is an experimental technique that introduces exogenous DNA into MSCs to produce or overexpress specific factors[167]. This technique can enhance MSC survival and functions after transplantation, particularly in a hostile environment. To date, genes involved in survival, migration and regenerative properties have been mainly targeted in MSCs for dental tissue regeneration. Genetically modified dental MSCs were found to be more effective than wild-type cells. Here, we introduce several reports on enhancing the function and therapeutic effects of dental MSCs through gene modification.

Genetic modification to enhance retention and migration

It is generally accepted that transplanted MSCs are vulnerable to the harsh microenvironment *in vivo*; most cells can be cleared or become dysfunctional within a short time. This situation hinders the migration of transplanted MSCs to the target site to exert their effects. Therefore, enhancing cell retention and migration capabilities is crucial in improving the therapeutic efficacy of transplanted MSCs. The overexpression of genes related to apoptosis inhibition and self-renewal can be an effective method to achieve this goal. Factors secreted by genetically modified MSCs may exert therapeutic effects *via* paracrine actions.

One strategy to enhance the survivability of grafted DPSCs is to overexpress *Bcl-2*, an antiapoptotic gene that is important for maintaining cell viability. Several studies have demonstrated the effects of *Bcl-2* overexpression on proliferation, antiapoptosis, and osteo/odontogenic differentiation[168,169]. The forced expression of stemness-

rated genes such as *Sox2*, *Oct4* and *Nanog*, which contribute to the maintenance of pluripotency in embryonic stem cells, is reported to improve proliferation and prevent senescence in MSCs. Huang *et al*[170] showed that DPSCs overexpressing *Oct4* and *Nanog* exhibited enhanced proliferation, as well as osteogenic/chondrogenic/adipogenic differentiation. *Sox2*-overexpressing DPSCs showed beneficial effects on proliferation, migration and adhesion capability[171]. In general, MSCs transduced with pluripotent genes show remarkable benefits in their proliferation. However, conflicting results regarding differentiation potential and possible adverse effects such as tumor formation should be considered in the context of clinical applications[172, 173].

Forkhead box protein (Fox)O1 is a master regulator that mediates glucose metabolism, tumorigenesis, oxidative stress and bone formation[174]. Huang *et al*[175] investigated the role of FoxO1-transfected PDLSCs in regulating oxidative stress resistance and osteogenesis. The authors found that FoxO1 overexpression protected PDLSCs against oxidative damage and promoted ECM mineralization by increasing the expression of the osteogenic markers Runx-related transcription factor (Runx)2 and SP7 in an inflammatory environment. This study demonstrates the promising anti-inflammatory role of FoxO1 in periodontium regeneration for periodontitis treatment.

Genetic modification to modulate osteo/odontogenic differentiation

Lineage differentiation can be achieved *in vitro* by priming MSCs with extrinsic signaling molecules or by modifying culture conditions. Genetic modification of MSCs may be an alternative way to induce stable and effective lineage transdifferentiation [176]. BMP family members are regarded as crucial factors that initiate and maintain osteo/odontogenesis. Taşlı *et al*[177] carried out genetic modification of BMP2 and BMP7 in human tooth germ stem cells (hTGSCs). The researchers found that overexpression of BMP2 and BMP7 in hTGSCs led to enhanced expression of early markers of osteo/odontogenic differentiation, such as DSPP, OCN and COL1A. Yang *et al*[178-180] reported that DPSCs transfected with BMP2 showed increased expression of ALP, OCN, COL1A, BSP, DSPP and DMP1, indicating stimulation of osteo/odontogenic differentiation. *In vitro* tests showed that transfected DPSCs differentiated into odontoblast-like cells without osteogenic induction[180]. Zhang *et al*[181] investigated BMP2-transfected SCAPs. The modified SCAPs underwent cell differentiation toward the odontogenic lineage by upregulating the *ALP*, *OCN*, *DSPP* and *DMP1* genes. Another promising inducer of osteo/odontogenesis is growth/differentiation factor (GDF)11, also known as BMP11. Nakashima *et al*[182] reported that DPSCs overexpressing GDF11 exhibited induced expression of dentin sialoprotein and the formation of large amounts of reparative dentin in canine teeth. In addition, the authors demonstrated that a GDF11-transfected cell mass stimulated reparative dentin formation on the amputated pulp[183]. These results revealed the feasibility of using BMPs in gene-modified MSC applications for endodontic regeneration.

Runx2 is a crucial factor for bone formation and tooth development. Pan *et al*[184] demonstrated that Runx2-overexpressing DFCS upregulated osteoblast/cementoblast-related genes and enhanced osteogenic differentiation *in vitro*. The authors also investigated the effects of mutant Runx2 without the VWRPY motif, which is responsible for suppressing transcriptional activation by Runx2. Overexpression of mutant Runx2 compared with full-length Runx2 led to higher expression levels of OPN, COL1 and CP23 in DFCS.

CONCLUSION

MSCs are increasingly being investigated as promising cell materials for tissue regeneration therapies due to their multilineage differentiation capabilities. MSCs derived from different dental tissues can be used as alternatives to bone marrow- and adipose tissue-derived MSCs. Numerous studies have demonstrated the high therapeutic potential of dental MSCs in various diseases, such as pulpitis, periapical, coronary artery, and neurodegenerative diseases.

Prior to clinical application, many efforts are still needed to focus on innovative strategies to maximize the therapeutic potential of MSCs. As summarized in this review, state-of-the-art technologies, including advanced culture systems, priming with cytokines and growth factors, and genetic editing, combined with understanding the therapeutic mechanism of dental MSCs, would significantly improve efficacy of MSC treatment in dental tissue regeneration. Overall, modified MSCs exhibit better therapeutic effects with high specificity on targets than ordinary cells. More studies

should be carried out to explore the therapeutic impact of the combined application of different modification or priming techniques to ensure improved outcomes and novel discoveries and further enhance therapeutic goals.

Researchers should also consider that MSC priming approaches must meet proper criteria and specific quality for clinical applications. The multilineage differentiation potential of MSCs is the required minimal criterion since it is a liability for many clinical applications. However, if MSCs are applied to treat neurodegenerative disorders, such as Parkinson's disease, then their osteogenic and adipogenic potentials are hazards. Thus, to utilize MSC products for specific applications, it is sometimes possible to lose certain MSC-defining characteristics through appropriate priming approaches[185]. Rather than focusing on the minimal criteria, as primed MSCs get closer to clinical application, they should meet the criteria that correlate with the safety and efficacy of the MSC product for treating specific diseases.

However, in preclinical trials, priming approaches for MSCs still have many limitations, including high costs, immunogenicity, donor-to-donor variability, variable source-dependent effects, and lack of good manufacturing practice (GMP) grade certification for clinical applications. Further studies are currently needed to evaluate the long-term *in vivo* tumorigenic potential of primed MSCs and the efficacy of each priming method for different clinical applications. Primed MSCs should meet qualified cell therapy standards and allow for GMP grade production while not compromise the quality attributes of the cells or be overly expensive. Ultimately, in conjunction with rigorous preclinical and clinical trials, primed MSCs can have enormous potential for wider applications in clinical settings in the future.

REFERENCES

- 1 **Klimczak A**, Kozłowska U. Mesenchymal Stromal Cells and Tissue-Specific Progenitor Cells: Their Role in Tissue Homeostasis. *Stem Cells Int* 2016; **2016**: 4285215 [PMID: 26823669 DOI: 10.1155/2016/4285215]
- 2 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]
- 3 **Witt R**, Weigand A, Boos AM, Cai A, Dippold D, Boccaccini AR, Schubert DW, Hardt M, Lange C, Arkudas A, Horch RE, Beier JP. Mesenchymal stem cells and myoblast differentiation under HGF and IGF-1 stimulation for 3D skeletal muscle tissue engineering. *BMC Cell Biol* 2017; **18**: 15 [PMID: 28245809 DOI: 10.1186/s12860-017-0131-2]
- 4 **Anghileri E**, Marconi S, Pignatelli A, Cifelli P, Galié M, Sbarbati A, Krampera M, Belluzzi O, Bonetti B. Neuronal differentiation potential of human adipose-derived mesenchymal stem cells. *Stem Cells Dev* 2008; **17**: 909-916 [PMID: 18564036 DOI: 10.1089/scd.2007.0197]
- 5 **Oswald J**, Boxberger S, Jørgensen B, Feldmann S, Ehninger G, Bornhäuser M, Werner C. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells* 2004; **22**: 377-384 [PMID: 15153614 DOI: 10.1634/stemcells.22-3-377]
- 6 **Afshari A**, Shamdani S, Uzan G, Naserian S, Azarpira N. Different approaches for transformation of mesenchymal stem cells into hepatocyte-like cells. *Stem Cell Res Ther* 2020; **11**: 54 [PMID: 32033595 DOI: 10.1186/s13287-020-1555-8]
- 7 **Khosravi M**, Azarpira N, Shamdani S, Hojjat-Assari S, Naserian S, Karimi MH. Differentiation of umbilical cord derived mesenchymal stem cells to hepatocyte cells by transfection of miR-106a, miR-574-3p, and miR-451. *Gene* 2018; **667**: 1-9 [PMID: 29763649 DOI: 10.1016/j.gene.2018.05.028]
- 8 **Galipeau J**, Sensébé L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell Stem Cell* 2018; **22**: 824-833 [PMID: 29859173 DOI: 10.1016/j.stem.2018.05.004]
- 9 **Gronthos S**, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 2000; **97**: 13625-13630 [PMID: 11087820 DOI: 10.1073/pnas.240309797]
- 10 **Miura M**, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A* 2003; **100**: 5807-5812 [PMID: 12716973 DOI: 10.1073/pnas.0937635100]
- 11 **Seo BM**, Miura M, Gronthos S, Bartold PM, Batouli S, Brahimi J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; **364**: 149-155 [PMID: 15246727 DOI: 10.1016/S0140-6736(04)16627-0]
- 12 **Morsczeck C**, Moehl C, Götz W, Heredia A, Schäffer TE, Eckstein N, Sippel C, Hoffmann KH. In vitro differentiation of human dental follicle cells with dexamethasone and insulin. *Cell Biol Int* 2005; **29**: 567-575 [PMID: 15951208 DOI: 10.1016/j.cellbi.2005.03.020]
- 13 **Sonoyama W**, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT. Characterization of the

- apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* 2008; **34**: 166-171 [PMID: [18215674](#) DOI: [10.1016/j.joen.2007.11.021](#)]
- 14 **Noronha NC**, Mizukami A, Calíari-Oliveira C, Cominal JG, Rocha JLM, Covas DT, Swiech K, Malmegrim KCR. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. *Stem Cell Res Ther* 2019; **10**: 131 [PMID: [31046833](#) DOI: [10.1186/s13287-019-1224-y](#)]
- 15 **Nava MM**, Raimondi MT, Pietrabissa R. Controlling self-renewal and differentiation of stem cells via mechanical cues. *J Biomed Biotechnol* 2012; **2012**: 797410 [PMID: [23091358](#) DOI: [10.1155/2012/797410](#)]
- 16 **Mas-Bargues C**, Sanz-Ros J, Román-Domínguez A, Inglés M, Gimeno-Mallench L, El Alami M, Viña-Almunia J, Gambini J, Viña J, Borrás C. Relevance of Oxygen Concentration in Stem Cell Culture for Regenerative Medicine. *Int J Mol Sci* 2019; **20** [PMID: [30857245](#) DOI: [10.3390/ijms20051195](#)]
- 17 **Hu C**, Li L. Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. *J Cell Mol Med* 2018; **22**: 1428-1442 [PMID: [29392844](#) DOI: [10.1111/jcmm.13492](#)]
- 18 **Saparov A**, Ogay V, Nurgozhin T, Jumabay M, Chen WC. Preconditioning of Human Mesenchymal Stem Cells to Enhance Their Regulation of the Immune Response. *Stem Cells Int* 2016; **2016**: 3924858 [PMID: [27822228](#) DOI: [10.1155/2016/3924858](#)]
- 19 **Ocansey DKW**, Pei B, Yan Y, Qian H, Zhang X, Xu W, Mao F. Improved therapeutics of modified mesenchymal stem cells: an update. *J Transl Med* 2020; **18**: 42 [PMID: [32000804](#) DOI: [10.1186/s12967-020-02234-x](#)]
- 20 **Seo Y**, Shin TH, Kim HS. Current Strategies to Enhance Adipose Stem Cell Function: An Update. *Int J Mol Sci* 2019; **20** [PMID: [31387282](#) DOI: [10.3390/ijms20153827](#)]
- 21 **Gronthos S**, Brahimi J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S. Stem cell properties of human dental pulp stem cells. *J Dent Res* 2002; **81**: 531-535 [PMID: [12147742](#) DOI: [10.1177/154405910208100806](#)]
- 22 **Wang X**, Sha XJ, Li GH, Yang FS, Ji K, Wen LY, Liu SY, Chen L, Ding Y, Xuan K. Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. *Arch Oral Biol* 2012; **57**: 1231-1240 [PMID: [22455989](#) DOI: [10.1016/j.archoralbio.2012.02.014](#)]
- 23 **Wang J**, Wang X, Sun Z, Yang H, Shi S, Wang S. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. *Stem Cells Dev* 2010; **19**: 1375-1383 [PMID: [20131979](#) DOI: [10.1089/scd.2009.0258](#)]
- 24 **Morsczeck C**, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, Hoffmann KH. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol* 2005; **24**: 155-165 [PMID: [15890265](#) DOI: [10.1016/j.matbio.2004.12.004](#)]
- 25 **Nakashima M**, Iohara K, Murakami M, Nakamura H, Sato Y, Aiji Y, Matsushita K. Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. *Stem Cell Res Ther* 2017; **8**: 61 [PMID: [28279187](#) DOI: [10.1186/s13287-017-0506-5](#)]
- 26 **Xuan K**, Li B, Guo H, Sun W, Kou X, He X, Zhang Y, Sun J, Liu A, Liao L, Liu S, Liu W, Hu C, Shi S, Jin Y. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. *Sci Transl Med* 2018; **10** [PMID: [30135248](#) DOI: [10.1126/scitranslmed.aaf3227](#)]
- 27 **Brizuela C**, Meza G, Urrejola D, Quezada MA, Concha G, Ramírez V, Angelopoulos I, Cadiz MI, Tapia-Limonchi R, Khoury M. Cell-Based Regenerative Endodontics for Treatment of Periapical Lesions: A Randomized, Controlled Phase I/II Clinical Trial. *J Dent Res* 2020; **99**: 523-529 [PMID: [32202965](#) DOI: [10.1177/0022034520913242](#)]
- 28 **Zheng C**, Chen J, Liu S, Jin Y. Stem cell-based bone and dental regeneration: a view of microenvironmental modulation. *Int J Oral Sci* 2019; **11**: 23 [PMID: [31423011](#) DOI: [10.1038/s41368-019-0060-3](#)]
- 29 **Yamada Y**, Nakamura-Yamada S, Kusano K, Baba S. Clinical Potential and Current Progress of Dental Pulp Stem Cells for Various Systemic Diseases in Regenerative Medicine: A Concise Review. *Int J Mol Sci* 2019; **20** [PMID: [30845639](#) DOI: [10.3390/ijms20051132](#)]
- 30 **Chalisserry EP**, Nam SY, Park SH, Anil S. Therapeutic potential of dental stem cells. *J Tissue Eng* 2017; **8**: 2041731417702531 [PMID: [28616151](#) DOI: [10.1177/2041731417702531](#)]
- 31 **Bakopoulou A**, Georgopoulou A, Grivas I, Bekiari C, Prymak O, Loza K, Epple M, Papadopoulos GC, Koidis P, Chatzinikolaidou M. Dental pulp stem cells in chitosan/gelatin scaffolds for enhanced orofacial bone regeneration. *Dent Mater* 2019; **35**: 310-327 [PMID: [30527589](#) DOI: [10.1016/j.dental.2018.11.025](#)]
- 32 **Moshaverinia A**, Xu X, Chen C, Akiyama K, Snead ML, Shi S. Dental mesenchymal stem cells encapsulated in an alginate hydrogel co-delivery microencapsulation system for cartilage regeneration. *Acta Biomater* 2013; **9**: 9343-9350 [PMID: [23891740](#) DOI: [10.1016/j.actbio.2013.07.023](#)]
- 33 **Martínez-Sarrà E**, Montori S, Gil-Recio C, Núñez-Toldrà R, Costamagna D, Rotini A, Atari M, Luttun A, Sampaioles M. Human dental pulp pluripotent-like stem cells promote wound healing and muscle regeneration. *Stem Cell Res Ther* 2017; **8**: 175 [PMID: [28750661](#) DOI: [10.1186/s13287-017-0621-3](#)]
- 34 **Pereira LV**, Bento RF, Cruz DB, Marchi C, Salomone R, Oiticica J, Costa MP, Haddad LA, Mingroni-Netto RC, Costa HJZR. Stem Cells from Human Exfoliated Deciduous Teeth (SHED) Differentiate in vivo and Promote Facial Nerve Regeneration. *Cell Transplant* 2019; **28**: 55-64 [PMID: [30380914](#) DOI: [10.1177/0963689718809090](#)]
- 35 **Inoue T**, Sugiyama M, Hattori H, Wakita H, Wakabayashi T, Ueda M. Stem cells from human

- exfoliated deciduous tooth-derived conditioned medium enhance recovery of focal cerebral ischemia in rats. *Tissue Eng Part A* 2013; **19**: 24-29 [PMID: 22839964 DOI: 10.1089/ten.TEA.2011.0385]
- 36 **Hirata M**, Ishigami M, Matsushita Y, Ito T, Hattori H, Hibi H, Goto H, Ueda M, Yamamoto A. Multifaceted Therapeutic Benefits of Factors Derived From Dental Pulp Stem Cells for Mouse Liver Fibrosis. *Stem Cells Transl Med* 2016; **5**: 1416-1424 [PMID: 27280796 DOI: 10.5966/sctm.2015-0353]
 - 37 **Omi M**, Hata M, Nakamura N, Miyabe M, Kobayashi Y, Kamiya H, Nakamura J, Ozawa S, Tanaka Y, Takebe J, Matsubara T, Naruse K. Transplantation of dental pulp stem cells suppressed inflammation in sciatic nerves by promoting macrophage polarization towards anti-inflammation phenotypes and ameliorated diabetic polyneuropathy. *J Diabetes Invest* 2016; **7**: 485-496 [PMID: 27181261 DOI: 10.1111/jdi.12452]
 - 38 **Ishikawa J**, Takahashi N, Matsumoto T, Yoshioka Y, Yamamoto N, Nishikawa M, Hibi H, Ishigro N, Ueda M, Furukawa K, Yamamoto A. Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental rheumatoid arthritis. *Bone* 2016; **83**: 210-219 [PMID: 26603475 DOI: 10.1016/j.bone.2015.11.012]
 - 39 **Mita T**, Furukawa-Hibi Y, Takeuchi H, Hattori H, Yamada K, Hibi H, Ueda M, Yamamoto A. Conditioned medium from the stem cells of human dental pulp improves cognitive function in a mouse model of Alzheimer's disease. *Behav Brain Res* 2015; **293**: 189-197 [PMID: 26210934 DOI: 10.1016/j.bbr.2015.07.043]
 - 40 **Brahimi-Horn MC**, Pouyssegur J. Oxygen, a source of life and stress. *FEBS Lett* 2007; **581**: 3582-3591 [PMID: 17586500 DOI: 10.1016/j.febslet.2007.06.018]
 - 41 **Shi R**, Yang H, Lin X, Cao Y, Zhang C, Fan Z, Hou B. Analysis of the characteristics and expression profiles of coding and noncoding RNAs of human dental pulp stem cells in hypoxic conditions. *Stem Cell Res Ther* 2019; **10**: 89 [PMID: 30867055 DOI: 10.1186/s13287-019-1192-2]
 - 42 **Buizer AT**, Bulstra SK, Veldhuizen AG, Kuijter R. The balance between proliferation and transcription of angiogenic factors of mesenchymal stem cells in hypoxia. *Connect Tissue Res* 2018; **59**: 12-20 [PMID: 28165799 DOI: 10.1080/03008207.2017.1289189]
 - 43 **Krinner A**, Zscharnack M, Bader A, Drasdo D, Galle J. Impact of oxygen environment on mesenchymal stem cell expansion and chondrogenic differentiation. *Cell Prolif* 2009; **42**: 471-484 [PMID: 19594581 DOI: 10.1111/j.1365-2184.2009.00621.x]
 - 44 **Sung HJ**, Ma W, Wang PY, Hynes J, O'Riordan TC, Combs CA, McCoy JP Jr, Bunz F, Kang JG, Hwang PM. Mitochondrial respiration protects against oxygen-associated DNA damage. *Nat Commun* 2010; **1**: 5 [PMID: 20975668 DOI: 10.1038/ncomms1003]
 - 45 **Bétous R**, Renoud ML, Hoede C, Gonzalez I, Jones N, Longy M, Sensebé L, Cazaux C, Hoffmann JS. Human Adipose-Derived Stem Cells Expanded Under Ambient Oxygen Concentration Accumulate Oxidative DNA Lesions and Experience Procarcinogenic DNA Replication Stress. *Stem Cells Transl Med* 2017; **6**: 68-76 [PMID: 28170194 DOI: 10.5966/sctm.2015-0401]
 - 46 **Seno K**, Tanikawa N, Takahashi H, Ohkuchi A, Suzuki H, Matsubara S, Iwata H, Kuwayama T, Shirasuna K. Oxygen concentration modulates cellular senescence and autophagy in human trophoblast cells. *Am J Reprod Immunol* 2018; **79**: e12826 [PMID: 29446169 DOI: 10.1111/aji.12826]
 - 47 **Rosová I**, Dao M, Capoccia B, Link D, Nolta JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. *Stem Cells* 2008; **26**: 2173-2182 [PMID: 18511601 DOI: 10.1634/stemcells.2007-1104]
 - 48 **Pavlacký J**, Polak J. Technical Feasibility and Physiological Relevance of Hypoxic Cell Culture Models. *Front Endocrinol (Lausanne)* 2020; **11**: 57 [PMID: 32153502 DOI: 10.3389/fendo.2020.00057]
 - 49 **Osathanon T**, Vivatbutsiri P, Sukarawan W, Sriarj W, Pavasant P, Soompon S. Cobalt chloride supplementation induces stem-cell marker expression and inhibits osteoblastic differentiation in human periodontal ligament cells. *Arch Oral Biol* 2015; **60**: 29-36 [PMID: 25244616 DOI: 10.1016/j.archoralbio.2014.08.018]
 - 50 **Laksana K**, Soompon S, Pavasant P, Sriarj W. Cobalt Chloride Enhances the Stemness of Human Dental Pulp Cells. *J Endod* 2017; **43**: 760-765 [PMID: 28343926 DOI: 10.1016/j.joen.2017.01.005]
 - 51 **Jiang L**, Peng WW, Li LF, Du R, Wu TT, Zhou ZJ, Zhao JJ, Yang Y, Qu DL, Zhu YQ. Effects of deferoxamine on the repair ability of dental pulp cells in vitro. *J Endod* 2014; **40**: 1100-1104 [PMID: 25069915 DOI: 10.1016/j.joen.2013.12.016]
 - 52 **Yeh TL**, Leissing TM, Abboud MI, Thinnies CC, Atasoylu O, Holt-Martyn JP, Zhang D, Tumber A, Lippl K, Lohans CT, Leung IKH, Morcrette H, Clifton IJ, Claridge TDW, Kawamura A, Flashman E, Lu X, Ratcliffe PJ, Chowdhury R, Pugh CW, Schofield CJ. Molecular and cellular mechanisms of HIF prolyl hydroxylase inhibitors in clinical trials. *Chem Sci* 2017; **8**: 7651-7668 [PMID: 29435217 DOI: 10.1039/c7sc02103h]
 - 53 **Liu L**, Gao J, Yuan Y, Chang Q, Liao Y, Lu F. Hypoxia preconditioned human adipose derived mesenchymal stem cells enhance angiogenic potential via secretion of increased VEGF and bFGF. *Cell Biol Int* 2013; **37**: 551-560 [PMID: 23505143 DOI: 10.1002/cbin.10097]
 - 54 **Beegle J**, Lakatos K, Kalomoiris S, Stewart H, Isseroff RR, Nolta JA, Fierro FA. Hypoxic preconditioning of mesenchymal stromal cells induces metabolic changes, enhances survival, and promotes cell retention in vivo. *Stem Cells* 2015; **33**: 1818-1828 [PMID: 25702874 DOI: 10.1002/stem.1976]
 - 55 **Feng Y**, Zhu M, Dangelmajer S, Lee YM, Wijesekera O, Castellanos CX, Denduluri A, Chaichana

- KL, Li Q, Zhang H, Levchenko A, Guerrero-Cazares H, Quiñones-Hinojosa A. Hypoxia-cultured human adipose-derived mesenchymal stem cells are non-oncogenic and have enhanced viability, motility, and tropism to brain cancer. *Cell Death Dis* 2014; **5**: e1567 [PMID: [25501828](#) DOI: [10.1038/cddis.2014.521](#)]
- 56 Estrada JC, Albo C, Benguría A, Dopazo A, López-Romero P, Carrera-Quintanar L, Roche E, Clemente EP, Enriquez JA, Bernad A, Samper E. Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis. *Cell Death Differ* 2012; **19**: 743-755 [PMID: [22139129](#) DOI: [10.1038/cdd.2011.172](#)]
- 57 Colombo JS, Jia S, D'Souza RN. Modeling Hypoxia Induced Factors to Treat Pulpal Inflammation and Drive Regeneration. *J Endod* 2020; **46**: S19-S25 [PMID: [32950190](#) DOI: [10.1016/j.joen.2020.06.039](#)]
- 58 Ahmed NE, Murakami M, Kaneko S, Nakashima M. The effects of hypoxia on the stemness properties of human dental pulp stem cells (DPSCs). *Sci Rep* 2016; **6**: 35476 [PMID: [27739509](#) DOI: [10.1038/srep35476](#)]
- 59 Kanafi MM, Ramesh A, Gupta PK, Bhonde RR. Influence of hypoxia, high glucose, and low serum on the growth kinetics of mesenchymal stem cells from deciduous and permanent teeth. *Cells Tissues Organs* 2013; **198**: 198-208 [PMID: [24192068](#) DOI: [10.1159/000354901](#)]
- 60 Bakopoulou A, Kritis A, Andreadis D, Papachristou E, Leyhausen G, Koidis P, Geurtsen W, Tsiftoglou A. Angiogenic Potential and Secretome of Human Apical Papilla Mesenchymal Stem Cells in Various Stress Microenvironments. *Stem Cells Dev* 2015; **24**: 2496-2512 [PMID: [26203919](#) DOI: [10.1089/scd.2015.0197](#)]
- 61 Wu Y, Yang Y, Yang P, Gu Y, Zhao Z, Tan L, Zhao L, Tang T, Li Y. The osteogenic differentiation of PDLSCs is mediated through MEK/ERK and p38 MAPK signalling under hypoxia. *Arch Oral Biol* 2013; **58**: 1357-1368 [PMID: [23806288](#) DOI: [10.1016/j.archoralbio.2013.03.011](#)]
- 62 Yuan C, Wang P, Zhu L, Dissanayaka WL, Green DW, Tong EH, Jin L, Zhang C. Coculture of stem cells from apical papilla and human umbilical vein endothelial cell under hypoxia increases the formation of three-dimensional vessel-like structures in vitro. *Tissue Eng Part A* 2015; **21**: 1163-1172 [PMID: [25380198](#) DOI: [10.1089/ten.TEA.2014.0058](#)]
- 63 Aranha AM, Zhang Z, Neiva KG, Costa CA, Hebling J, Nör JE. Hypoxia enhances the angiogenic potential of human dental pulp cells. *J Endod* 2010; **36**: 1633-1637 [PMID: [20850667](#) DOI: [10.1016/j.joen.2010.05.013](#)]
- 64 Müller HD, Cvikl B, Janjić K, Nürnberger S, Moritz A, Gruber R, Agis H. Effects of Prolyl Hydroxylase Inhibitor L-mimosine on Dental Pulp in the Presence of Advanced Glycation End Products. *J Endod* 2015; **41**: 1852-1861 [PMID: [26395911](#) DOI: [10.1016/j.joen.2015.08.002](#)]
- 65 Müller HD, Cvikl B, Gruber R, Watzek G, Agis H. Prolyl hydroxylase inhibitors increase the production of vascular endothelial growth factor in dental pulp-derived cells. *J Endod* 2012; **38**: 1498-1503 [PMID: [23063224](#) DOI: [10.1016/j.joen.2012.08.003](#)]
- 66 Sakdee JB, White RR, Pagonis TC, Hauschka PV. Hypoxia-amplified proliferation of human dental pulp cells. *J Endod* 2009; **35**: 818-823 [PMID: [19482178](#) DOI: [10.1016/j.joen.2009.03.001](#)]
- 67 Fukuyama Y, Ohta K, Okoshi R, Suehara M, Kizaki H, Nakagawa K. Hypoxia induces expression and activation of AMPK in rat dental pulp cells. *J Dent Res* 2007; **86**: 903-907 [PMID: [17720864](#) DOI: [10.1177/154405910708600919](#)]
- 68 Li L, Zhu YQ, Jiang L, Peng W, Ritchie HH. Hypoxia promotes mineralization of human dental pulp cells. *J Endod* 2011; **37**: 799-802 [PMID: [21787492](#) DOI: [10.1016/j.joen.2011.02.028](#)]
- 69 Vanacker J, Viswanath A, De Berdt P, Everard A, Cani PD, Bouzin C, Feron O, Diogenes A, Leprince JG, des Rieux A. Hypoxia modulates the differentiation potential of stem cells of the apical papilla. *J Endod* 2014; **40**: 1410-1418 [PMID: [25146023](#) DOI: [10.1016/j.joen.2014.04.008](#)]
- 70 He Y, Jian CX, Zhang HY, Zhou Y, Wu X, Zhang G, Tan YH. Hypoxia enhances periodontal ligament stem cell proliferation via the MAPK signaling pathway. *Genet Mol Res* 2016; **15** [PMID: [27886339](#) DOI: [10.4238/gmr15048965](#)]
- 71 Yu Y, Bi CS, Wu RX, Yin Y, Zhang XY, Lan PH, Chen FM. Effects of short-term inflammatory and/or hypoxic pretreatments on periodontal ligament stem cells: in vitro and in vivo studies. *Cell Tissue Res* 2016; **366**: 311-328 [PMID: [27301447](#) DOI: [10.1007/s00441-016-2437-3](#)]
- 72 Xu Q, Liu Z, Guo L, Liu R, Li R, Chu X, Yang J, Luo J, Chen F, Deng M. Hypoxia Mediates Runt-Related Transcription Factor 2 Expression via Induction of Vascular Endothelial Growth Factor in Periodontal Ligament Stem Cells. *Mol Cells* 2019; **42**: 763-772 [PMID: [31659886](#) DOI: [10.14348/molcells.2019.0023](#)]
- 73 Giacoppo S, Thangavelu SR, Diomedea F, Bramanti P, Conti P, Trubiani O, Mazzon E. Anti-inflammatory effects of hypoxia-preconditioned human periodontal ligament cell secretome in an experimental model of multiple sclerosis: a key role of IL-37. *FASEB J* 2017; **31**: 5592-5608 [PMID: [28842429](#) DOI: [10.1096/fj.201700524R](#)]
- 74 Li Q, Luo T, Lu W, Yi X, Zhao Z, Liu J. Proteomic analysis of human periodontal ligament cells under hypoxia. *Proteome Sci* 2019; **17**: 3 [PMID: [31496921](#) DOI: [10.1186/s12953-019-0151-2](#)]
- 75 Choi JR, Yong KW, Wan Safwani WKZ. Effect of hypoxia on human adipose-derived mesenchymal stem cells and its potential clinical applications. *Cell Mol Life Sci* 2017; **74**: 2587-2600 [PMID: [28224204](#) DOI: [10.1007/s00018-017-2484-2](#)]
- 76 Laschke MW, Menger MD. Life is 3D: Boosting Spheroid Function for Tissue Engineering. *Trends Biotechnol* 2017; **35**: 133-144 [PMID: [27634310](#) DOI: [10.1016/j.tibtech.2016.08.004](#)]
- 77 Duval K, Grover H, Han LH, Mou Y, Pegoraro AF, Fredberg J, Chen Z. Modeling Physiological

- Events in 2D vs. 3D Cell Culture. *Physiology (Bethesda)* 2017; **32**: 266-277 [PMID: [28615311](#) DOI: [10.1152/physiol.00036.2016](#)]
- 78 **Frith JE**, Thomson B, Genever PG. Dynamic three-dimensional culture methods enhance mesenchymal stem cell properties and increase therapeutic potential. *Tissue Eng Part C Methods* 2010; **16**: 735-749 [PMID: [19811095](#) DOI: [10.1089/ten.TEC.2009.0432](#)]
 - 79 **Cheng NC**, Wang S, Young TH. The influence of spheroid formation of human adipose-derived stem cells on chitosan films on stemness and differentiation capabilities. *Biomaterials* 2012; **33**: 1748-1758 [PMID: [22153870](#) DOI: [10.1016/j.biomaterials.2011.11.049](#)]
 - 80 **Bartosh TJ**, Ylöstalo JH, Mohammadipoor A, Bazhanov N, Coble K, Claypool K, Lee RH, Choi H, Prockop DJ. Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. *Proc Natl Acad Sci U S A* 2010; **107**: 13724-13729 [PMID: [20643923](#) DOI: [10.1073/pnas.1008117107](#)]
 - 81 **Cheng NC**, Chen SY, Li JR, Young TH. Short-term spheroid formation enhances the regenerative capacity of adipose-derived stem cells by promoting stemness, angiogenesis, and chemotaxis. *Stem Cells Transl Med* 2013; **2**: 584-594 [PMID: [23847001](#) DOI: [10.5966/sctm.2013-0007](#)]
 - 82 **Xiao L**, Kumazawa Y, Okamura H. Cell death, cavitation and spontaneous multi-differentiation of dental pulp stem cells-derived spheroids in vitro: a journey to survival and organogenesis. *Biol Cell* 2014; **106**: 405-419 [PMID: [25175801](#) DOI: [10.1111/boc.201400024](#)]
 - 83 **Yamamoto M**, Kawashima N, Takashino N, Koizumi Y, Takimoto K, Suzuki N, Saito M, Suda H. Three-dimensional spheroid culture promotes odonto/osteoblastic differentiation of dental pulp cells. *Arch Oral Biol* 2014; **59**: 310-317 [PMID: [24581854](#) DOI: [10.1016/j.archoralbio.2013.12.006](#)]
 - 84 **Lee SH**, Inaba A, Mohindroo N, Ganesh D, Martin CE, Chugal N, Kim RH, Kang MK, Park NH, Shin KH. Three-dimensional Sphere-forming Cells Are Unique Multipotent Cell Population in Dental Pulp Cells. *J Endod* 2017; **43**: 1302-1308 [PMID: [28606672](#) DOI: [10.1016/j.joen.2017.03.016](#)]
 - 85 **Zhang S**, Buttler-Buecher P, Denecke B, Arana-Chavez VE, Apel C. A comprehensive analysis of human dental pulp cell spheroids in a three-dimensional pellet culture system. *Arch Oral Biol* 2018; **91**: 1-8 [PMID: [29621667](#) DOI: [10.1016/j.archoralbio.2018.02.008](#)]
 - 86 **Dissanayaka WL**, Zhu L, Hargreaves KM, Jin L, Zhang C. Scaffold-free Prevascularized Microtissue Spheroids for Pulp Regeneration. *J Dent Res* 2014; **93**: 1296-1303 [PMID: [25201919](#) DOI: [10.1177/0022034514550040](#)]
 - 87 **Moritani Y**, Usui M, Sano K, Nakazawa K, Hanatani T, Nakatomi M, Iwata T, Sato T, Ariyoshi W, Nishihara T, Nakashima K. Spheroid culture enhances osteogenic potential of periodontal ligament mesenchymal stem cells. *J Periodontol Res* 2018; **53**: 870-882 [PMID: [29900548](#) DOI: [10.1111/jre.12577](#)]
 - 88 **Zhang S**, Liu P, Chen L, Wang Y, Wang Z, Zhang B. The effects of spheroid formation of adipose-derived stem cells in a microgravity bioreactor on stemness properties and therapeutic potential. *Biomaterials* 2015; **41**: 15-25 [PMID: [25522961](#) DOI: [10.1016/j.biomaterials.2014.11.019](#)]
 - 89 **Cesarz Z**, Tamama K. Spheroid Culture of Mesenchymal Stem Cells. *Stem Cells Int* 2016; **2016**: 9176357 [PMID: [26649054](#) DOI: [10.1155/2016/9176357](#)]
 - 90 **Kraft DC**, Bindslev DA, Melsen B, Abdallah BM, Kassem M, Klein-Nulend J. Mechanosensitivity of dental pulp stem cells is related to their osteogenic maturity. *Eur J Oral Sci* 2010; **118**: 29-38 [PMID: [20156262](#) DOI: [10.1111/j.1600-0722.2009.00709.x](#)]
 - 91 **Huang H**, Yang R, Zhou YH. Mechanobiology of Periodontal Ligament Stem Cells in Orthodontic Tooth Movement. *Stem Cells Int* 2018; **2018**: 6531216 [PMID: [30305820](#) DOI: [10.1155/2018/6531216](#)]
 - 92 **Sübay RK**, Kaya H, Tarim B, Sübay A, Cox CF. Response of human pulpal tissue to orthodontic extrusive applications. *J Endod* 2001; **27**: 508-511 [PMID: [11501587](#) DOI: [10.1097/00004770-200108000-00003](#)]
 - 93 **Marrelli M**, Codispoti B, Shelton RM, Scheven BA, Cooper PR, Tatullo M, Paduano F. Dental Pulp Stem Cell Mechanoresponsiveness: Effects of Mechanical Stimuli on Dental Pulp Stem Cell Behavior. *Front Physiol* 2018; **9**: 1685 [PMID: [30534086](#) DOI: [10.3389/fphys.2018.01685](#)]
 - 94 **Gao Q**, Walmsley AD, Cooper PR, Scheven BA. Ultrasound Stimulation of Different Dental Stem Cell Populations: Role of Mitogen-activated Protein Kinase Signaling. *J Endod* 2016; **42**: 425-431 [PMID: [26830427](#) DOI: [10.1016/j.joen.2015.12.019](#)]
 - 95 **Gao Q**, Cooper PR, Walmsley AD, Scheven BA. Role of Piezo Channels in Ultrasound-stimulated Dental Stem Cells. *J Endod* 2017; **43**: 1130-1136 [PMID: [28527849](#) DOI: [10.1016/j.joen.2017.02.022](#)]
 - 96 **Cai X**, Zhang Y, Yang X, Grottkau BE, Lin Y. Uniaxial cyclic tensile stretch inhibits osteogenic and odontogenic differentiation of human dental pulp stem cells. *J Tissue Eng Regen Med* 2011; **5**: 347-353 [PMID: [20827678](#) DOI: [10.1002/term.319](#)]
 - 97 **Hata M**, Naruse K, Ozawa S, Kobayashi Y, Nakamura N, Kojima N, Omi M, Katanosaka Y, Nishikawa T, Tanaka Y, Matsubara T. Mechanical stretch increases the proliferation while inhibiting the osteogenic differentiation in dental pulp stem cells. *Tissue Eng Part A* 2013; **19**: 625-633 [PMID: [23153222](#) DOI: [10.1089/ten.tea.2012.0099](#)]
 - 98 **Han MJ**, Seo YK, Yoon HH, Song KY, Park JK. Effect of mechanical tension on the human dental pulp cells. *Biotechnol Bioprocess Eng* 2008; **13**: 410-417 [DOI: [10.1007/s12257-008-0146-9](#)]
 - 99 **Yang H**, Shu YX, Wang LY, Zou WL, Guo LY, Shao MY, Gao QH, Hu T. Effect of cyclic uniaxial compressive stress on human dental pulp cells in vitro. *Connect Tissue Res* 2018; **59**: 255-262

- [PMID: 28816569 DOI: 10.1080/03008207.2017.1367773]
- 100 **Lee SK**, Min KS, Kim Y, Jeong GS, Lee SH, Lee HJ, Lee SI, Kim YS, Lee YM, Park SJ, Seo SW, Lee SK, Kim EC. Mechanical stress activates proinflammatory cytokines and antioxidant defense enzymes in human dental pulp cells. *J Endod* 2008; **34**: 1364-1369 [PMID: 18928848 DOI: 10.1016/j.joen.2008.08.024]
 - 101 **Song N**, Scholtemeijer M, Shah K. Mesenchymal Stem Cell Immunomodulation: Mechanisms and Therapeutic Potential. *Trends Pharmacol Sci* 2020; **41**: 653-664 [PMID: 32709406 DOI: 10.1016/j.tips.2020.06.009]
 - 102 **Ren G**, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008; **2**: 141-150 [PMID: 18371435 DOI: 10.1016/j.stem.2007.11.014]
 - 103 **Cencioni C**, Capogrossi MC, Napolitano M. The SDF-1/CXCR4 axis in stem cell preconditioning. *Cardiovasc Res* 2012; **94**: 400-407 [PMID: 22451511 DOI: 10.1093/cvr/cvs132]
 - 104 **Kim DS**, Kang SI, Lee SY, Noh KT, Kim EC. Involvement of SDF-1 and monocyte chemoattractant protein-1 in hydrogen peroxide-induced extracellular matrix degradation in human dental pulp cells. *Int Endod J* 2014; **47**: 298-308 [PMID: 23815460 DOI: 10.1111/iej.12147]
 - 105 **Feng Y**, Fu X, Lou X, Fu B. Stromal cell-derived factor 1 protects human periodontal ligament stem cells against hydrogen peroxide-induced apoptosis. *Mol Med Rep* 2017; **16**: 5001-5006 [PMID: 28791359 DOI: 10.3892/mmr.2017.7192]
 - 106 **Kim DS**, Kim YS, Bae WJ, Lee HJ, Chang SW, Kim WS, Kim EC. The role of SDF-1 and CXCR4 on odontoblastic differentiation in human dental pulp cells. *Int Endod J* 2014; **47**: 534-541 [PMID: 24033610 DOI: 10.1111/iej.12182]
 - 107 **Du L**, Yang P, Ge S. Stromal cell-derived factor-1 significantly induces proliferation, migration, and collagen type I expression in a human periodontal ligament stem cell subpopulation. *J Periodontol* 2012; **83**: 379-388 [PMID: 21749168 DOI: 10.1902/jop.2011.110201]
 - 108 **Iohara K**, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, Matsushita K, Nakamura H, Nakashima M. Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. *Tissue Eng Part A* 2011; **17**: 1911-1920 [PMID: 21417716 DOI: 10.1089/ten.TEA.2010.0615]
 - 109 **Li M**, Sun X, Ma L, Jin L, Zhang W, Xiao M, Yu Q. SDF-1/CXCR4 axis induces human dental pulp stem cell migration through FAK/PI3K/Akt and GSK3 β /catenin pathways. *Sci Rep* 2017; **7**: 40161 [PMID: 28067275 DOI: 10.1038/srep40161]
 - 110 **Yang JW**, Zhang YF, Wan CY, Sun ZY, Nie S, Jian SJ, Zhang L, Song GT, Chen Z. Autophagy in SDF-1 α -mediated DPSC migration and pulp regeneration. *Biomaterials* 2015; **44**: 11-23 [PMID: 25617122 DOI: 10.1016/j.biomaterials.2014.12.006]
 - 111 **Liang Q**, Du L, Zhang R, Kang W, Ge S. Stromal cell-derived factor-1/Exendin-4 cotherapy facilitates the proliferation, migration and osteogenic differentiation of human periodontal ligament stem cells in vitro and promotes periodontal bone regeneration in vivo. *Cell Prolif* 2021; **54**: e12997 [PMID: 33511708 DOI: 10.1111/cpr.12997]
 - 112 **Xu M**, Wei X, Fang J, Xiao L. Combination of SDF-1 and bFGF promotes bone marrow stem cell-mediated periodontal ligament regeneration. *Biosci Rep* 2019; **39** [PMID: 31789340 DOI: 10.1042/BSR20190785]
 - 113 **Du L**, Feng R, Ge S. PTH/SDF-1 α cotherapy promotes proliferation, migration and osteogenic differentiation of human periodontal ligament stem cells. *Cell Prolif* 2016; **49**: 599-608 [PMID: 27523567 DOI: 10.1111/cpr.12286]
 - 114 **Ponte AL**, Ribeiro-Fleury T, Chabot V, Gouilleux F, Langonné A, Hérault O, Charbord P, Domenech J. Granulocyte-colony-stimulating factor stimulation of bone marrow mesenchymal stromal cells promotes CD34+ cell migration via a matrix metalloproteinase-2-dependent mechanism. *Stem Cells Dev* 2012; **21**: 3162-3172 [PMID: 22651889 DOI: 10.1089/scd.2012.0048]
 - 115 **Horibe H**, Murakami M, Iohara K, Hayashi Y, Takeuchi N, Takei Y, Kurita K, Nakashima M. Isolation of a stable subpopulation of mobilized dental pulp stem cells (MDPSCs) with high proliferation, migration, and regeneration potential is independent of age. *PLoS One* 2014; **9**: e98553 [PMID: 24870376 DOI: 10.1371/journal.pone.0098553]
 - 116 **Murakami M**, Horibe H, Iohara K, Hayashi Y, Osako Y, Takei Y, Nakata K, Motoyama N, Kurita K, Nakashima M. The use of granulocyte-colony stimulating factor induced mobilization for isolation of dental pulp stem cells with high regenerative potential. *Biomaterials* 2013; **34**: 9036-9047 [PMID: 23988014 DOI: 10.1016/j.biomaterials.2013.08.011]
 - 117 **Chinnadurai R**, Copland IB, Patel SR, Galipeau J. IDO-independent suppression of T cell effector function by IFN- γ -licensed human mesenchymal stromal cells. *J Immunol* 2014; **192**: 1491-1501 [PMID: 24403533 DOI: 10.4049/jimmunol.1301828]
 - 118 **Wada N**, Menicanin D, Shi S, Bartold PM, Gronthos S. Immunomodulatory properties of human periodontal ligament stem cells. *J Cell Physiol* 2009; **219**: 667-676 [PMID: 19160415 DOI: 10.1002/jcp.21710]
 - 119 **Kwack KH**, Lee JM, Park SH, Lee HW. Human Dental Pulp Stem Cells Suppress Alloantigen-induced Immunity by Stimulating T Cells to Release Transforming Growth Factor Beta. *J Endod* 2017; **43**: 100-108 [PMID: 27871783 DOI: 10.1016/j.joen.2016.09.005]
 - 120 **Sonoda S**, Yamaza H, Ma L, Tanaka Y, Tomoda E, Aijima R, Nonaka K, Kukita T, Shi S, Nishimura F, Yamaza T. Interferon-gamma improves impaired dentinogenic and immunosuppressive functions of irreversible pulpitis-derived human dental pulp stem cells. *Sci Rep*

- 2016; **6**: 19286 [PMID: [26775677](#) DOI: [10.1038/srep19286](#)]
- 121 **He X**, Jiang W, Luo Z, Qu T, Wang Z, Liu N, Zhang Y, Cooper PR, He W. IFN- γ regulates human dental pulp stem cells behavior via NF- κ B and MAPK signaling. *Sci Rep* 2017; **7**: 40681 [PMID: [28098169](#) DOI: [10.1038/srep40681](#)]
- 122 **Strojny C**, Boyle M, Bartholomew A, Sundivakkam P, Alapati S. Interferon Gamma-treated Dental Pulp Stem Cells Promote Human Mesenchymal Stem Cell Migration In Vitro. *J Endod* 2015; **41**: 1259-1264 [PMID: [26051078](#) DOI: [10.1016/j.joen.2015.02.018](#)]
- 123 **Prasanna SJ**, Gopalakrishnan D, Shankar SR, Vasandan AB. Pro-inflammatory cytokines, IFN γ and TNF α , influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. *PLoS One* 2010; **5**: e9016 [PMID: [20126406](#) DOI: [10.1371/journal.pone.0009016](#)]
- 124 **Beldi G**, Khosravi M, Abdelgawad ME, Salomon BL, Uzan G, Haouas H, Naserian S. TNF α /TNFR2 signaling pathway: an active immune checkpoint for mesenchymal stem cell immunoregulatory function. *Stem Cell Res Ther* 2020; **11**: 281 [PMID: [32669116](#) DOI: [10.1186/s13287-020-01740-5](#)]
- 125 **Beldi G**, Bahiraii S, Lezin C, Nouri Barkestani M, Abdelgawad ME, Uzan G, Naserian S. TNFR2 Is a Crucial Hub Controlling Mesenchymal Stem Cell Biological and Functional Properties. *Front Cell Dev Biol* 2020; **8**: 596831 [PMID: [33344453](#) DOI: [10.3389/fcell.2020.596831](#)]
- 126 **Naserian S**, Shamdani S, Arouche N, Uzan G. Regulatory T cell induction by mesenchymal stem cells depends on the expression of TNFR2 by T cells. *Stem Cell Res Ther* 2020; **11**: 534 [PMID: [33303019](#) DOI: [10.1186/s13287-020-02057-z](#)]
- 127 **Paula-Silva FW**, Ghosh A, Silva LA, Kapila YL. TNF- α promotes an odontoblastic phenotype in dental pulp cells. *J Dent Res* 2009; **88**: 339-344 [PMID: [19407154](#) DOI: [10.1177/0022034509334070](#)]
- 128 **Feng X**, Feng G, Xing J, Shen B, Li L, Tan W, Xu Y, Liu S, Liu H, Jiang J, Wu H, Tao T, Gu Z. TNF- α triggers osteogenic differentiation of human dental pulp stem cells via the NF- κ B signalling pathway. *Cell Biol Int* 2013; **37**: 1267-1275 [PMID: [23765556](#) DOI: [10.1002/cbin.10141](#)]
- 129 **Xu K**, Xiao J, Zheng K, Feng X, Zhang J, Song D, Wang C, Shen X, Zhao X, Wei C, Huang D, Feng G. MiR-21/STAT3 Signal Is Involved in Odontoblast Differentiation of Human Dental Pulp Stem Cells Mediated by TNF- α . *Cell Reprogram* 2018; **20**: 107-116 [PMID: [29620442](#) DOI: [10.1089/cell.2017.0042](#)]
- 130 **Liu YK**, Zhou ZY, Liu F. Transcriptome changes during TNF- α promoted osteogenic differentiation of dental pulp stem cells (DPSCs). *Biochem Biophys Res Commun* 2016; **476**: 426-430 [PMID: [27237976](#) DOI: [10.1016/j.bbrc.2016.05.137](#)]
- 131 **Shi L**, Fu S, Fahim S, Pan S, Lina H, Mu X, Niu Y. TNF- α stimulation increases dental pulp stem cell migration in vitro through integrin α -6 subunit upregulation. *Arch Oral Biol* 2017; **75**: 48-54 [PMID: [28043012](#) DOI: [10.1016/j.archoralbio.2016.12.005](#)]
- 132 **Qin Z**, Fang Z, Zhao L, Chen J, Li Y, Liu G. High dose of TNF- α suppressed osteogenic differentiation of human dental pulp stem cells by activating the Wnt/ β -catenin signaling. *J Mol Histol* 2015; **46**: 409-420 [PMID: [26115593](#) DOI: [10.1007/s10735-015-9630-7](#)]
- 133 **An L**, Shen S, Wang L, Li Y, Fahim S, Niu Y, Pan S. TNF- α increases angiogenic potential in a co-culture system of dental pulp cells and endothelial cells. *Braz Oral Res* 2019; **33**: e059 [PMID: [31664357](#) DOI: [10.1590/1807-3107bor-2019.vol33.0059](#)]
- 134 **Shin MR**, Kang SK, Kim YS, Lee SY, Hong SC, Kim EC. TNF- α and LPS activate angiogenesis via VEGF and SIRT1 signalling in human dental pulp cells. *Int Endod J* 2015; **48**: 705-716 [PMID: [25311745](#) DOI: [10.1111/iej.12396](#)]
- 135 **Yun YR**, Won JE, Jeon E, Lee S, Kang W, Jo H, Jang JH, Shin US, Kim HW. Fibroblast growth factors: biology, function, and application for tissue regeneration. *J Tissue Eng* 2010; **2010**: 218142 [PMID: [21350642](#) DOI: [10.4061/2010/218142](#)]
- 136 **Mullane EM**, Dong Z, Sedgley CM, Hu JC, Botero TM, Holland GR, Nör JE. Effects of VEGF and FGF2 on the revascularization of severed human dental pulps. *J Dent Res* 2008; **87**: 1144-1148 [PMID: [19029083](#) DOI: [10.1177/154405910808701204](#)]
- 137 **Mathieu S**, Jeanneau C, Sheibat-Othman N, Kalaji N, Fessi H, About I. Usefulness of controlled release of growth factors in investigating the early events of dentin-pulp regeneration. *J Endod* 2013; **39**: 228-235 [PMID: [23321236](#) DOI: [10.1016/j.joen.2012.11.007](#)]
- 138 **Sagomonyants K**, Kalajzic I, Maye P, Mina M. Enhanced Dentinogenesis of Pulp Progenitors by Early Exposure to FGF2. *J Dent Res* 2015; **94**: 1582-1590 [PMID: [26276371](#) DOI: [10.1177/0022034515599768](#)]
- 139 **Kikuchi N**, Kitamura C, Morotomi T, Inuyama Y, Ishimatsu H, Tabata Y, Nishihara T, Terashita M. Formation of dentin-like particles in dentin defects above exposed pulp by controlled release of fibroblast growth factor 2 from gelatin hydrogels. *J Endod* 2007; **33**: 1198-1202 [PMID: [17889689](#) DOI: [10.1016/j.joen.2007.07.025](#)]
- 140 **Yang JW**, Zhang YF, Sun ZY, Song GT, Chen Z. Dental pulp tissue engineering with bFGF-incorporated silk fibroin scaffolds. *J Biomater Appl* 2015; **30**: 221-229 [PMID: [25791684](#) DOI: [10.1177/0885328215577296](#)]
- 141 **Ishimatsu H**, Kitamura C, Morotomi T, Tabata Y, Nishihara T, Chen KK, Terashita M. Formation of dentinal bridge on surface of regenerated dental pulp in dentin defects by controlled release of fibroblast growth factor-2 from gelatin hydrogels. *J Endod* 2009; **35**: 858-865 [PMID: [19482186](#) DOI: [10.1016/j.joen.2009.03.049](#)]

- 142 **Kim J**, Park JC, Kim SH, Im GI, Kim BS, Lee JB, Choi EY, Song JS, Cho KS, Kim CS. Treatment of FGF-2 on stem cells from inflamed dental pulp tissue from human deciduous teeth. *Oral Dis* 2014; **20**: 191-204 [PMID: [23496287](#) DOI: [10.1111/odi.12089](#)]
- 143 **Gorin C**, Rochefort GY, Bascetin R, Ying H, Lesieur J, Sadoine J, Beckouche N, Berndt S, Novais A, Lesage M, Hosten B, Vercellino L, Merlet P, Le-Denmat D, Marchiol C, Letourneur D, Nicoletti A, Vital SO, Poliard A, Salmon B, Muller L, Chaussain C, Germain S. Priming Dental Pulp Stem Cells With Fibroblast Growth Factor-2 Increases Angiogenesis of Implanted Tissue-Engineered Constructs Through Hepatocyte Growth Factor and Vascular Endothelial Growth Factor Secretion. *Stem Cells Transl Med* 2016; **5**: 392-404 [PMID: [26798059](#) DOI: [10.5966/sctm.2015-0166](#)]
- 144 **Zhang J**, Lian M, Cao P, Bao G, Xu G, Sun Y, Wang L, Chen J, Wang Y, Feng G, Cui Z. Effects of Nerve Growth Factor and Basic Fibroblast Growth Factor Promote Human Dental Pulp Stem Cells to Neural Differentiation. *Neurochem Res* 2017; **42**: 1015-1025 [PMID: [28005222](#) DOI: [10.1007/s11064-016-2134-3](#)]
- 145 **Umemura N**, Ohkoshi E, Tajima M, Kikuchi H, Katayama T, Sakagami H. Hyaluronan induces odontoblastic differentiation of dental pulp stem cells via CD44. *Stem Cell Res Ther* 2016; **7**: 135 [PMID: [27651223](#) DOI: [10.1186/s13287-016-0399-8](#)]
- 146 **Shimabukuro Y**, Ueda M, Ichikawa T, Terashi Y, Yamada S, Kusumoto Y, Takedachi M, Terakura M, Kohya A, Hashikawa T, Murakami S. Fibroblast growth factor-2 stimulates hyaluronan production by human dental pulp cells. *J Endod* 2005; **31**: 805-808 [PMID: [16249723](#) DOI: [10.1097/01.don.0000158242.44155.49](#)]
- 147 **Sasaki R**, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, Ogiuchi H. Neurosphere generation from dental pulp of adult rat incisor. *Eur J Neurosci* 2008; **27**: 538-548 [PMID: [18279307](#) DOI: [10.1111/j.1460-9568.2008.06026.x](#)]
- 148 **Kim YS**, Min KS, Jeong DH, Jang JH, Kim HW, Kim EC. Effects of fibroblast growth factor-2 on the expression and regulation of chemokines in human dental pulp cells. *J Endod* 2010; **36**: 1824-1830 [PMID: [20951295](#) DOI: [10.1016/j.joen.2010.08.020](#)]
- 149 **Fortino VR**, Chen RS, Pelaez D, Cheung HS. Neurogenesis of neural crest-derived periodontal ligament stem cells by EGF and bFGF. *J Cell Physiol* 2014; **229**: 479-488 [PMID: [24105823](#) DOI: [10.1002/jcp.24468](#)]
- 150 **Murakami S**, Takayama S, Kitamura M, Shimabukuro Y, Yanagi K, Ikezawa K, Saho T, Nozaki T, Okada H. Recombinant human basic fibroblast growth factor (bFGF) stimulates periodontal regeneration in class II furcation defects created in beagle dogs. *J Periodontol Res* 2003; **38**: 97-103 [PMID: [12558943](#) DOI: [10.1034/j.1600-0765.2003.00640.x](#)]
- 151 **Takayama S**, Murakami S, Shimabukuro Y, Kitamura M, Okada H. Periodontal regeneration by FGF-2 (bFGF) in primate models. *J Dent Res* 2001; **80**: 2075-2079 [PMID: [11808765](#) DOI: [10.1177/00220345010800121001](#)]
- 152 **Kang W**, Liang Q, Du L, Shang L, Wang T, Ge S. Sequential application of bFGF and BMP-2 facilitates osteogenic differentiation of human periodontal ligament stem cells. *J Periodontol Res* 2019; **54**: 424-434 [PMID: [30851068](#) DOI: [10.1111/jre.12644](#)]
- 153 **He H**, Yu J, Liu Y, Lu S, Liu H, Shi J, Jin Y. Effects of FGF2 and TGFbeta1 on the differentiation of human dental pulp stem cells in vitro. *Cell Biol Int* 2008; **32**: 827-834 [PMID: [18442933](#) DOI: [10.1016/j.cellbi.2008.03.013](#)]
- 154 **Takeuchi N**, Hayashi Y, Murakami M, Alvarez FJ, Horibe H, Iohara K, Nakata K, Nakamura H, Nakashima M. Similar in vitro effects and pulp regeneration in ectopic tooth transplantation by basic fibroblast growth factor and granulocyte-colony stimulating factor. *Oral Dis* 2015; **21**: 113-122 [PMID: [24495211](#) DOI: [10.1111/odi.12227](#)]
- 155 **Chang YC**, Chang MC, Chen YJ, Liou JU, Chang HH, Huang WL, Liao WC, Chan CP, Jeng PY, Jeng JH. Basic Fibroblast Growth Factor Regulates Gene and Protein Expression Related to Proliferation, Differentiation, and Matrix Production of Human Dental Pulp Cells. *J Endod* 2017; **43**: 936-942 [PMID: [28416318](#) DOI: [10.1016/j.joen.2017.01.024](#)]
- 156 **Sagomonyants K**, Mina M. Stage-specific effects of fibroblast growth factor 2 on the differentiation of dental pulp cells. *Cells Tissues Organs* 2014; **199**: 311-328 [PMID: [25823776](#) DOI: [10.1159/000371343](#)]
- 157 **Shimabukuro Y**, Ueda M, Ozasa M, Anzai J, Takedachi M, Yanagita M, Ito M, Hashikawa T, Yamada S, Murakami S. Fibroblast growth factor-2 regulates the cell function of human dental pulp cells. *J Endod* 2009; **35**: 1529-1535 [PMID: [19840642](#) DOI: [10.1016/j.joen.2009.08.010](#)]
- 158 **Sagomonyants K**, Mina M. Biphasic effects of FGF2 on odontoblast differentiation involve changes in the BMP and Wnt signaling pathways. *Connect Tissue Res* 2014; **55** Suppl 1: 53-56 [PMID: [25158181](#) DOI: [10.3109/03008207.2014.923867](#)]
- 159 **Sagomonyants K**, Kalajic I, Maye P, Mina M. FGF Signaling Prevents the Terminal Differentiation of Odontoblasts. *J Dent Res* 2017; **96**: 663-670 [PMID: [28170285](#) DOI: [10.1177/0022034517691732](#)]
- 160 **Catón J**, Bringas P Jr, Zeichner-David M. IGFs increase enamel formation by inducing expression of enamel mineralizing specific genes. *Arch Oral Biol* 2005; **50**: 123-129 [PMID: [15721138](#) DOI: [10.1016/j.archoralbio.2004.11.012](#)]
- 161 **Feng X**, Huang D, Lu X, Feng G, Xing J, Lu J, Xu K, Xia W, Meng Y, Tao T, Li L, Gu Z. Insulin-like growth factor 1 can promote proliferation and osteogenic differentiation of human dental pulp stem cells via mTOR pathway. *Dev Growth Differ* 2014; **56**: 615-624 [PMID: [25388971](#) DOI: [10.1111/dgd.12179](#)]

- 162 **Matsumura S**, Quispe-Salcedo A, Schiller CM, Shin JS, Locke BM, Yakar S, Shimizu E. IGF-1 Mediates EphrinB1 Activation in Regulating Tertiary Dentin Formation. *J Dent Res* 2017; **96**: 1153-1161 [PMID: 28489485 DOI: 10.1177/0022034517708572]
- 163 **Ma S**, Liu G, Jin L, Pang X, Wang Y, Wang Z, Yu Y, Yu J. IGF-1/IGF-1R/hsa-let-7c axis regulates the committed differentiation of stem cells from apical papilla. *Sci Rep* 2016; **6**: 36922 [PMID: 27833148 DOI: 10.1038/srep36922]
- 164 **Yan L**, Sun S, Qu L. Insulin-like growth factor-1 promotes the proliferation and odontoblastic differentiation of human dental pulp cells under high glucose conditions. *Int J Mol Med* 2017; **40**: 1253-1260 [PMID: 28902344 DOI: 10.3892/ijmm.2017.3117]
- 165 **Han X**, Amar S. IGF-1 signaling enhances cell survival in periodontal ligament fibroblasts vs. gingival fibroblasts. *J Dent Res* 2003; **82**: 454-459 [PMID: 12766198 DOI: 10.1177/154405910308200610]
- 166 **Yu Y**, Mu J, Fan Z, Lei G, Yan M, Wang S, Tang C, Wang Z, Yu J, Zhang G. Insulin-like growth factor 1 enhances the proliferation and osteogenic differentiation of human periodontal ligament stem cells via ERK and JNK MAPK pathways. *Histochem Cell Biol* 2012; **137**: 513-525 [PMID: 22227802 DOI: 10.1007/s00418-011-0908-x]
- 167 **Wei W**, Huang Y, Li D, Gou HF, Wang W. Improved therapeutic potential of MSCs by genetic modification. *Gene Ther* 2018; **25**: 538-547 [PMID: 30254305 DOI: 10.1038/s41434-018-0041-8]
- 168 **Dissanayaka WL**, Han Y, Zhang L, Zou T, Zhang C. Bcl-2 Overexpression and Hypoxia Synergistically Enhance Angiogenic Properties of Dental Pulp Stem Cells. *Int J Mol Sci* 2020; **21** [PMID: 32859045 DOI: 10.3390/ijms21176159]
- 169 **Heng BC**, Ye X, Liu Y, Dissanayaka WL, Cheung GS, Zhang C. Effects of Recombinant Overexpression of Bcl2 on the Proliferation, Apoptosis, and Osteogenic/Odontogenic Differentiation Potential of Dental Pulp Stem Cells. *J Endod* 2016; **42**: 575-583 [PMID: 26898562 DOI: 10.1016/j.joen.2016.01.013]
- 170 **Huang CE**, Hu FW, Yu CH, Tsai LL, Lee TH, Chou MY, Yu CC. Concurrent expression of Oct4 and Nanog maintains mesenchymal stem-like property of human dental pulp cells. *Int J Mol Sci* 2014; **15**: 18623-18639 [PMID: 25322154 DOI: 10.3390/ijms151018623]
- 171 **Liu P**, Cai J, Dong D, Chen Y, Liu X, Wang Y, Zhou Y. Effects of SOX2 on Proliferation, Migration and Adhesion of Human Dental Pulp Stem Cells. *PLoS One* 2015; **10**: e0141346 [PMID: 26496354 DOI: 10.1371/journal.pone.0141346]
- 172 **Schönitzer V**, Wirtz R, Ulrich V, Berger T, Karl A, Mutschler W, Schieker M, Böcker W. Sox2 is a potent inhibitor of osteogenic and adipogenic differentiation in human mesenchymal stem cells. *Cell Reprogram* 2014; **16**: 355-365 [PMID: 25127284 DOI: 10.1089/cell.2014.0006]
- 173 **Park YS**, Nemeño JG, Choi NY, Lee JI, Ko K, Choi SC, Kim WS, Han DW, Tapia N. Ectopic overexpression of Nanog induces tumorigenesis in non-tumorous fibroblasts. *Biol Chem* 2016; **397**: 249-255 [PMID: 26733157 DOI: 10.1515/hsz-2015-0255]
- 174 **Rached MT**, Kode A, Xu L, Yoshikawa Y, Paik JH, Depinho RA, Kousteni S. FoxO1 is a positive regulator of bone formation by favoring protein synthesis and resistance to oxidative stress in osteoblasts. *Cell Metab* 2010; **11**: 147-160 [PMID: 20142102 DOI: 10.1016/j.cmet.2010.01.001]
- 175 **Huang X**, Chen H, Xie Y, Cao Z, Lin X, Wang Y. FoxO1 Overexpression Ameliorates TNF- α -Induced Oxidative Damage and Promotes Osteogenesis of Human Periodontal Ligament Stem Cells via Antioxidant Defense Activation. *Stem Cells Int* 2019; **2019**: 2120453 [PMID: 31781234 DOI: 10.1155/2019/2120453]
- 176 **Barzilay R**, Melamed E, Offen D. Introducing transcription factors to multipotent mesenchymal stem cells: making transdifferentiation possible. *Stem Cells* 2009; **27**: 2509-2515 [PMID: 19591229 DOI: 10.1002/stem.172]
- 177 **Taşlı PN**, Aydın S, Yalvaç ME, Sahin F. Bmp 2 and bmp 7 induce odonto- and osteogenesis of human tooth germ stem cells. *Appl Biochem Biotechnol* 2014; **172**: 3016-3025 [PMID: 24477555 DOI: 10.1007/s12010-013-0706-0]
- 178 **Yang X**, van der Kraan PM, Bian Z, Fan M, Walboomers XF, Jansen JA. Mineralized tissue formation by BMP2-transfected pulp stem cells. *J Dent Res* 2009; **88**: 1020-1025 [PMID: 19828890 DOI: 10.1177/0022034509346258]
- 179 **Yang X**, Walboomers XF, van den Dolder J, Yang F, Bian Z, Fan M, Jansen JA. Non-viral bone morphogenetic protein 2 transfection of rat dental pulp stem cells using calcium phosphate nanoparticles as carriers. *Tissue Eng Part A* 2008; **14**: 71-81 [PMID: 18333806 DOI: 10.1089/ten.a.2007.0102]
- 180 **Yang X**, van der Kraan PM, van den Dolder J, Walboomers XF, Bian Z, Fan M, Jansen JA. STRO-1 selected rat dental pulp stem cells transfected with adenoviral-mediated human bone morphogenetic protein 2 gene show enhanced odontogenic differentiation. *Tissue Eng* 2007; **13**: 2803-2812 [PMID: 17824831 DOI: 10.1089/ten.2006.0439]
- 181 **Zhang W**, Zhang X, Ling J, Liu W, Ma J, Zheng J. Proliferation and odontogenic differentiation of BMP2 genetransfected stem cells from human tooth apical papilla: an in vitro study. *Int J Mol Med* 2014; **34**: 1004-1012 [PMID: 25070743 DOI: 10.3892/ijmm.2014.1862]
- 182 **Nakashima M**, Tachibana K, Iohara K, Ito M, Ishikawa M, Akamine A. Induction of reparative dentin formation by ultrasound-mediated gene delivery of growth/differentiation factor 11. *Hum Gene Ther* 2003; **14**: 591-597 [PMID: 12718768 DOI: 10.1089/104303403764539369]
- 183 **Nakashima M**, Iohara K, Ishikawa M, Ito M, Tomokiyo A, Tanaka T, Akamine A. Stimulation of reparative dentin formation by ex vivo gene therapy using dental pulp stem cells electrotransfected

- with growth/differentiation factor 11 (Gdf11). *Hum Gene Ther* 2004; **15**: 1045-1053 [PMID: 15610605 DOI: 10.1089/hum.2004.15.1045]
- 184 **Pan K**, Sun Q, Zhang J, Ge S, Li S, Zhao Y, Yang P. Multilineage differentiation of dental follicle cells and the roles of Runx2 over-expression in enhancing osteoblast/cementoblast-related gene expression in dental follicle cells. *Cell Prolif* 2010; **43**: 219-228 [PMID: 20546240 DOI: 10.1111/j.1365-2184.2010.00670.x]
- 185 **Yin JQ**, Zhu J, Ankrum JA. Manufacturing of primed mesenchymal stromal cells for therapy. *Nat Biomed Eng* 2019; **3**: 90-104 [PMID: 30944433 DOI: 10.1038/s41551-018-0325-8]



Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

Telephone: +1-925-3991568

E-mail: bpgoffice@wjgnet.com

Help Desk: <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

