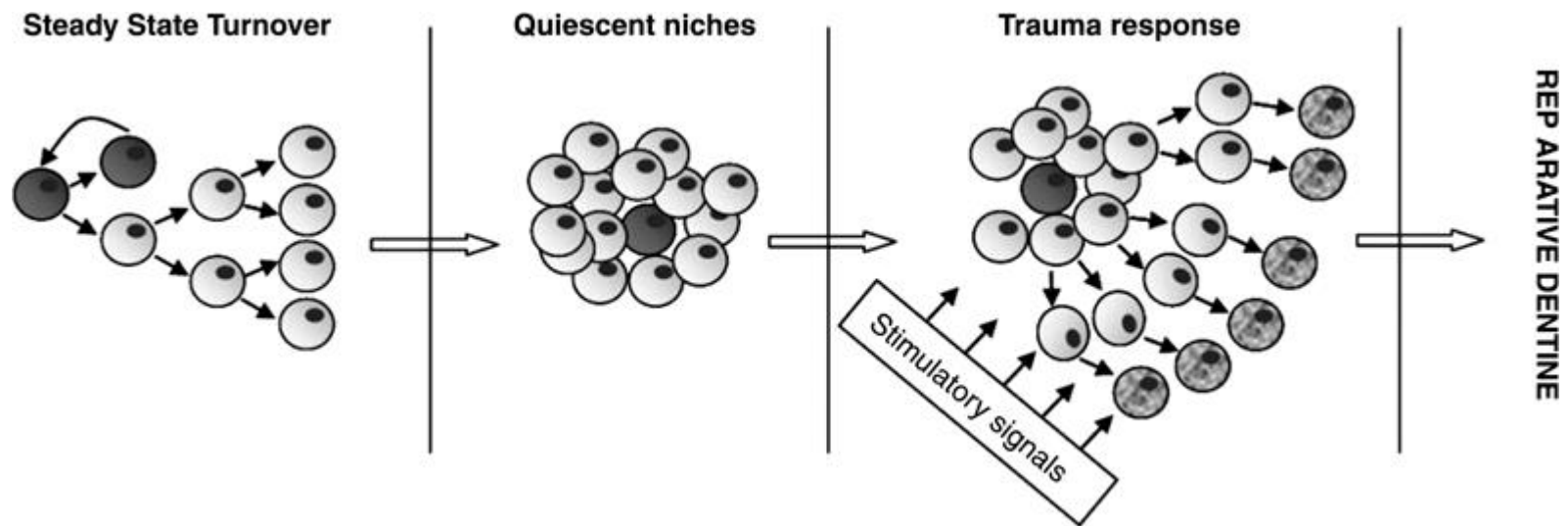
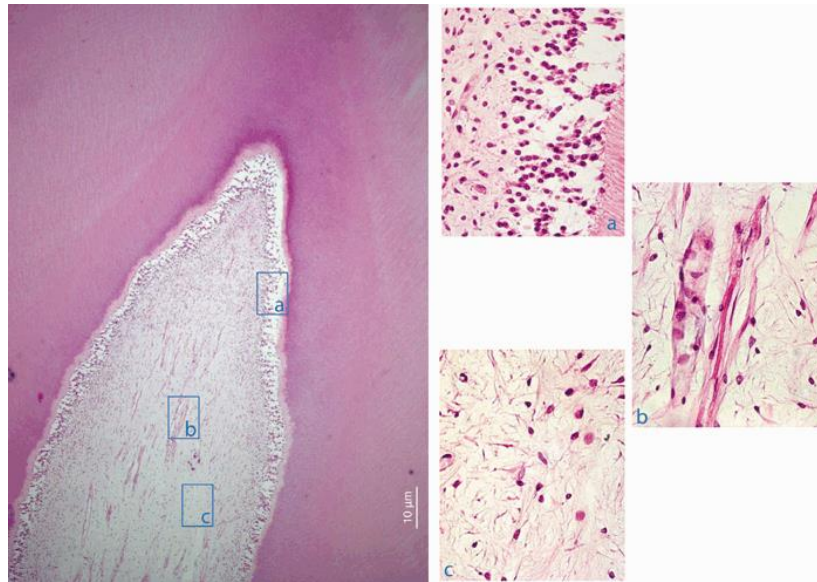


Pulp Dental Stem cells

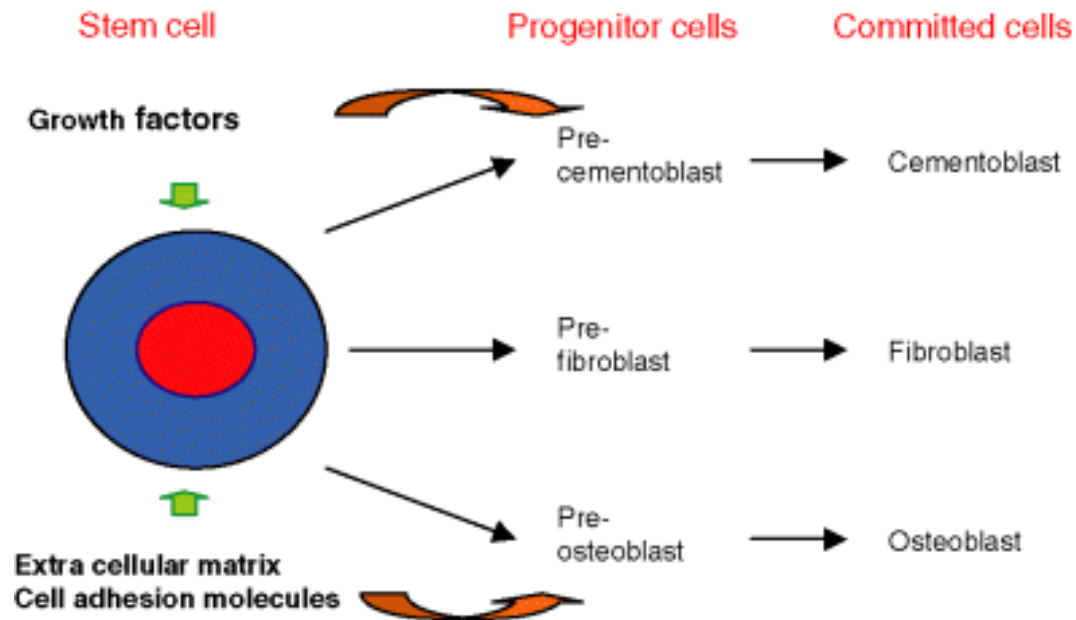


Pulp Dental Stem cells



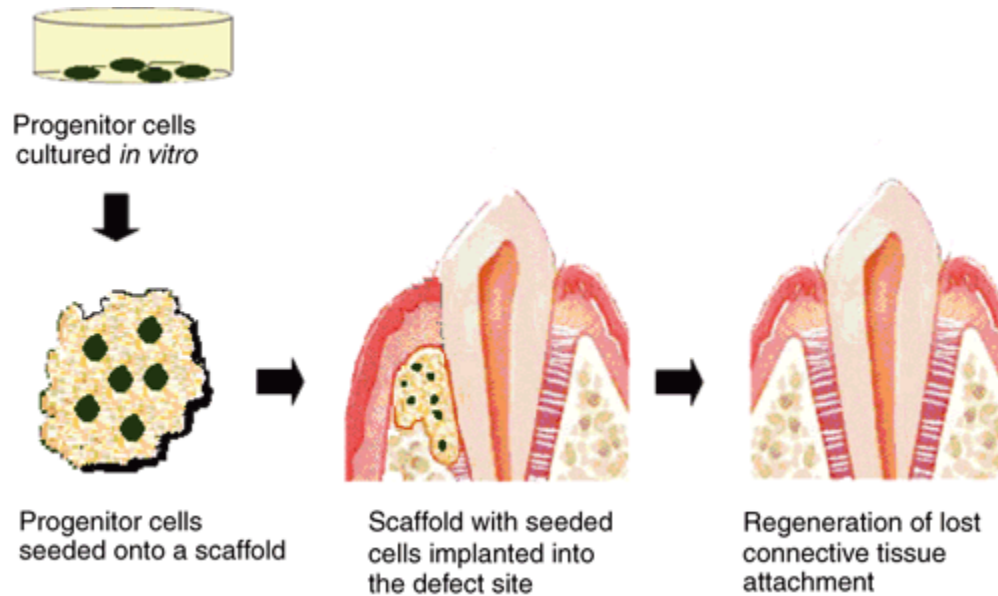
Several stem/progenitor cell niches may exist in the mature dental pulp. These include (a) undifferentiated mesenchymal cells (so-called subodontoblasts) residing in the cell-rich layer close to the existing post-mitotic odontoblasts; (b) a perivascular cell population associated with the pulpal vasculature; and (c) a Notch-2 positive cell population within the central pulpal stroma.

Pulp Dental Stem cells



Differentiation of adult mesenchymal stem cells and progenitor cells into periodontal cells.

Pulp Dental Stem cells



Pulp Dental Stem cells

Pulp Dental Stem cells

Pulp Dental Stem cells

Pulp Dental Stem cells

Pulp Dental Stem cells

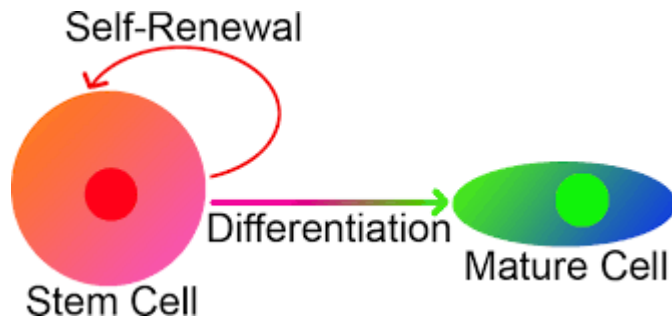
Pulp Dental Stem cells

کشت و تمایز سلول های بنیادی پالپی و غیر پالپی
برای درمان بیماری های دهان و دندان

ارایه دهنده: دکتر محمد حسین حدادی

ویژگی های سلول های بنیادی

1. خود تجدید پذیر
2. تمایز به رده های سلولی مختلف



جدا سازی سلول های شبه استرومال از کشت اولیه DPSCs پیوند داده شده یا کشت مجدد داده شده در موش های ایمنو کمپرومایز نشان از خود تجدید پذیری DPSCs دارد.

ویژگی های سلول های بنیادی

Neurovascularization

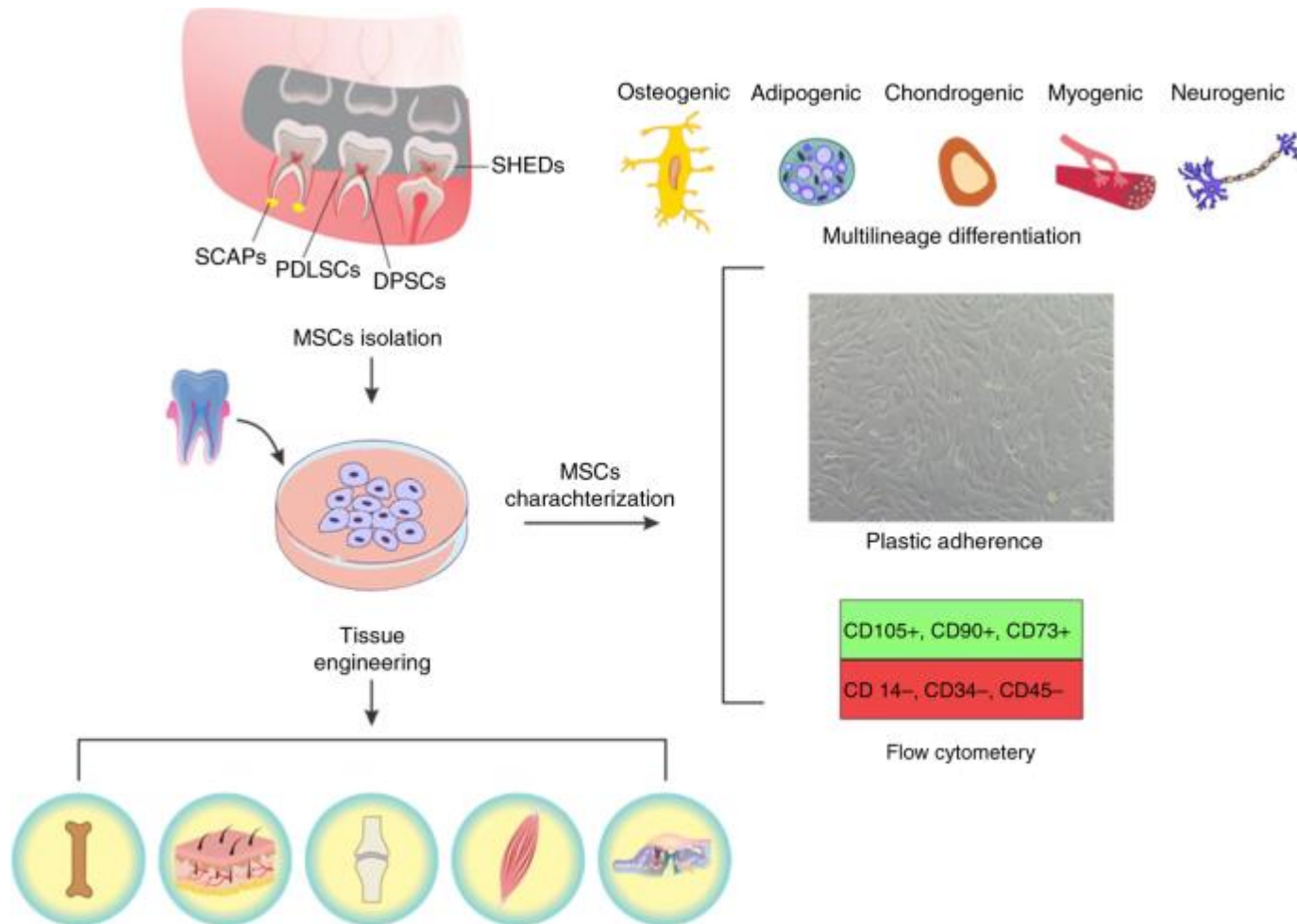
1. خود تجدید پذیر
2. تمایز به رده های سلولی مختلف

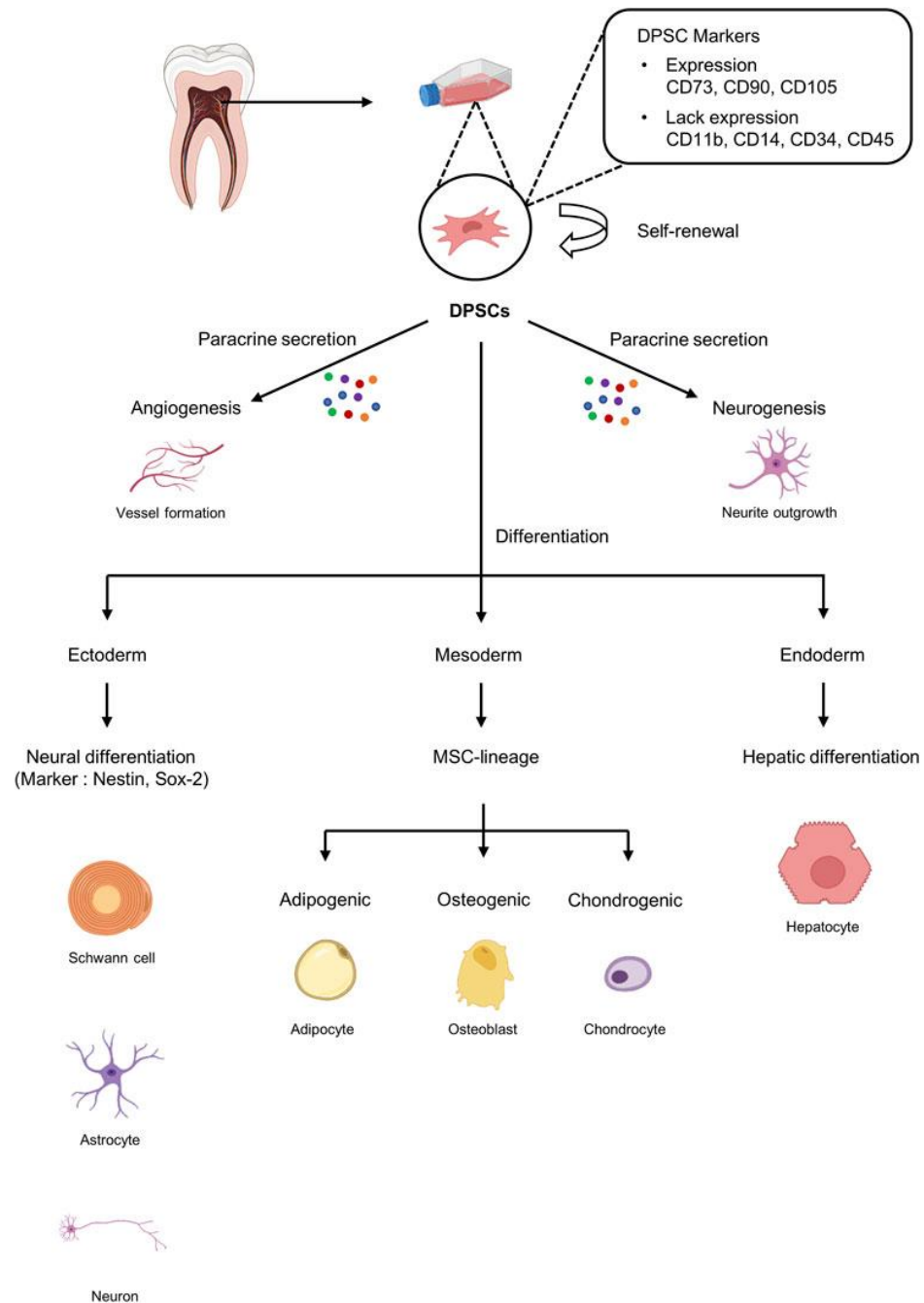
سلول های DPSCs به راحتی تحت محیط کشت اختصاصی به سلول های ادیپوز و نورونی تمایز می یابند.
دو مورد در حفظ پالپ دندان اهمیت ویژه ای دارند.

- 1- خون رسانی
- 2- عصب زایی

❖ راه حل کشت سلول های بنیادی

ویژگی های سلول های بنیادی



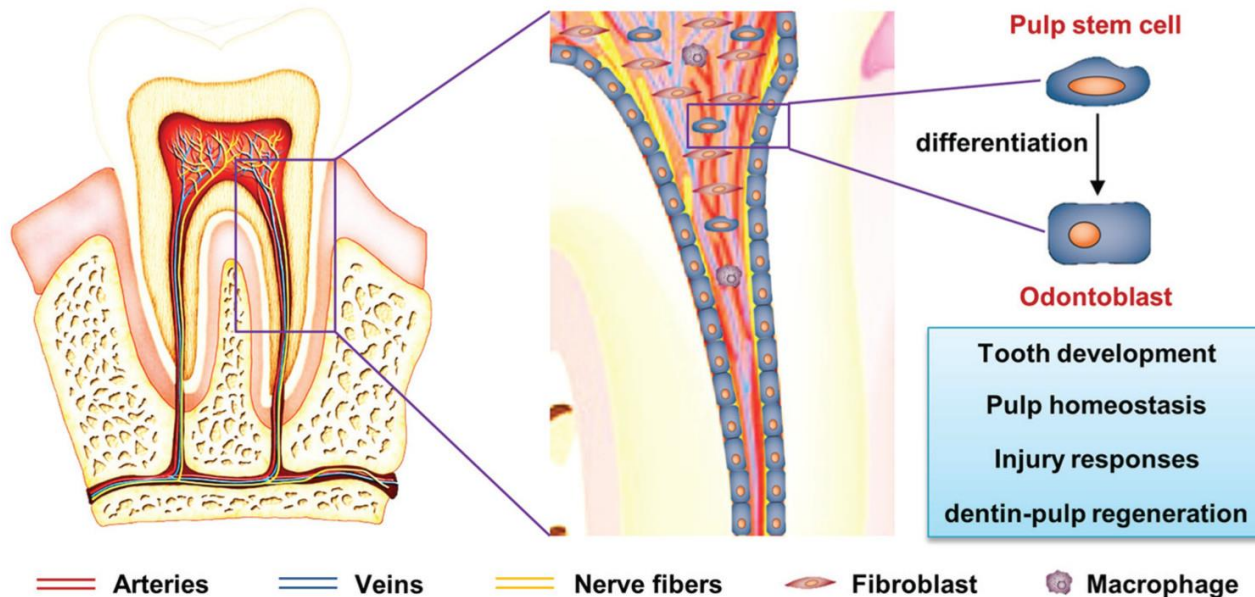


Mesenchymal Stem cells

1. Odontoblasts
2. Cementoblasts
3. Osteoblasts
4. Chondrocytes
5. Myocytes
6. Epithelial cells
7. Neural cells
8. Hepatocytes
9. Adipocytes.

سلول های DPSCs مانند BMSCs توانایی تمایز به انواع رده های سلولی مرتبط را دارند. امضا پی ژنتیک.

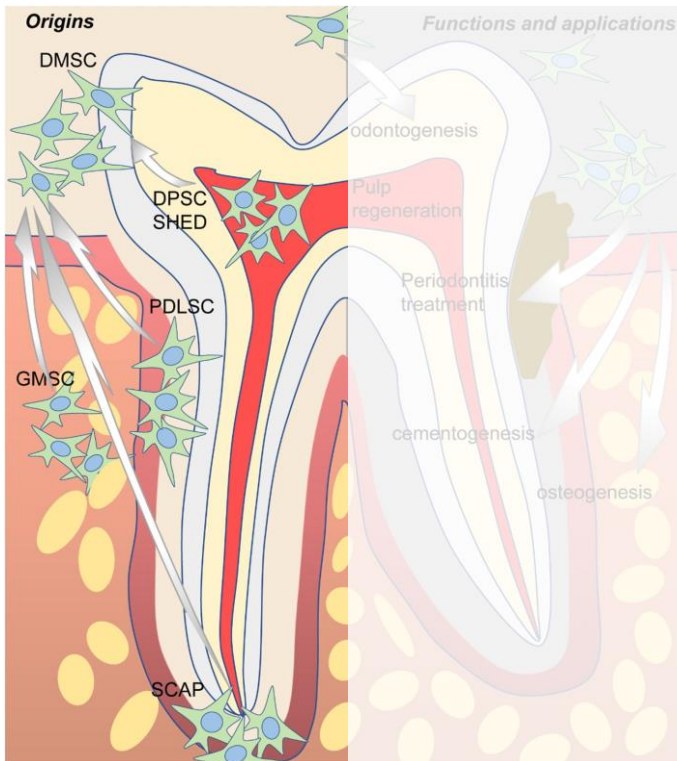
Physiological properties



Physiologic structure of dental pulp and the contributions of pulp stem cells. Pulp tissue is infiltrated by arterial and venous vascular networks and nerve fibers (left panel). This neurovascular bundle supports the activity of the surrounding pulp stem cells, which co-reside with fibroblasts and macrophages in the dental pulp (middle panel) and possess the capability of differentiation into odontoblasts lining the dentin wall. Accordingly, pulp stem cells putatively contribute to tooth development, pulp tissue homeostasis, injury responses, and dentin-pulp regeneration (right panel).

نوع و کاربرد سلول های بنیادی پالپی

Dental-Derived Mesenchymal Stem Cells



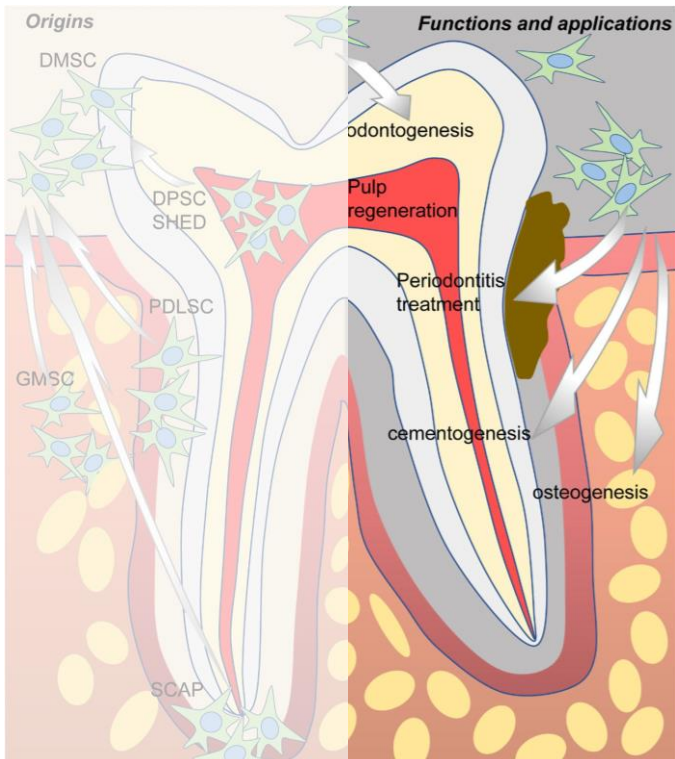
Origin

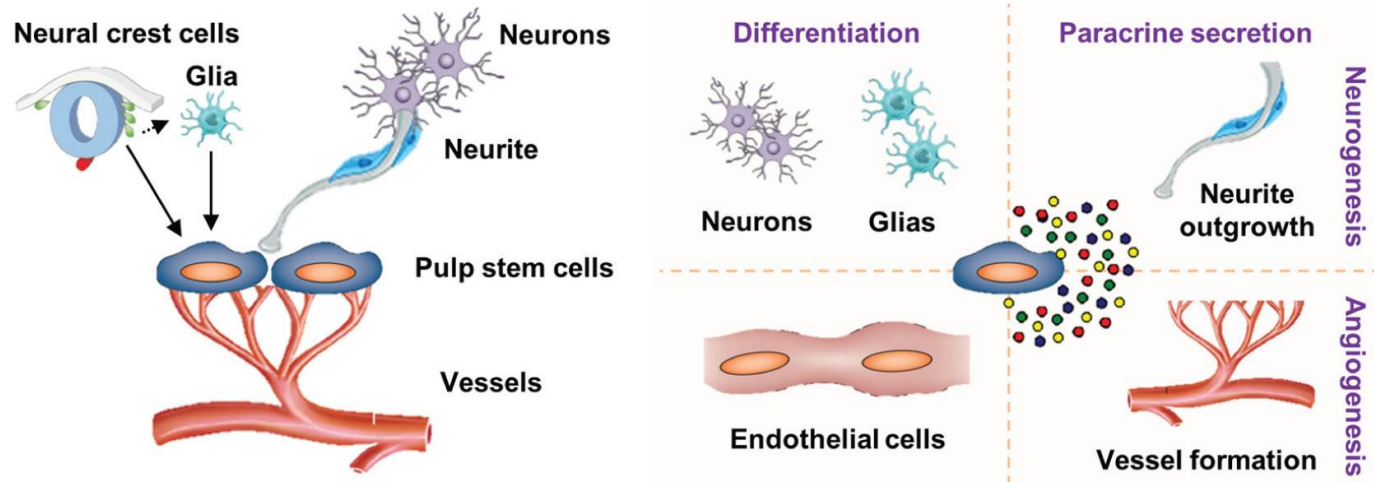
1. Dental pulp
2. Periodontal ligament
3. Dental follicle
4. Apical papilla
5. Gingiva

نوع و کاربرد سلول های بنیادی پالپی

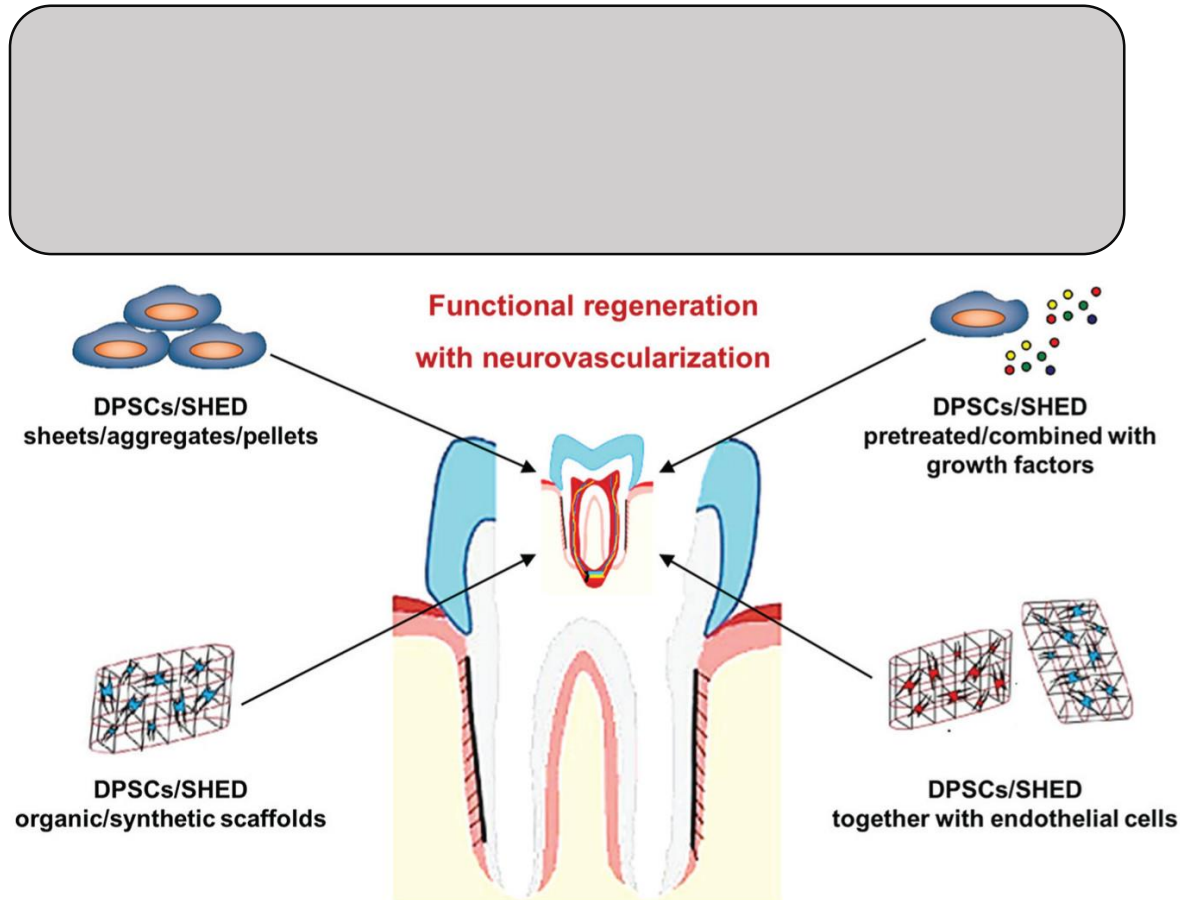
بنا به محیطی که سلول در آنجا کشت داده می شود و فاکتور های رشد انواع کاربرد ها را داریم

1. Odontogenesis
2. Pulp regeneration
3. Periodontitis treatment
4. Cementogenesis
5. Osteogenesis



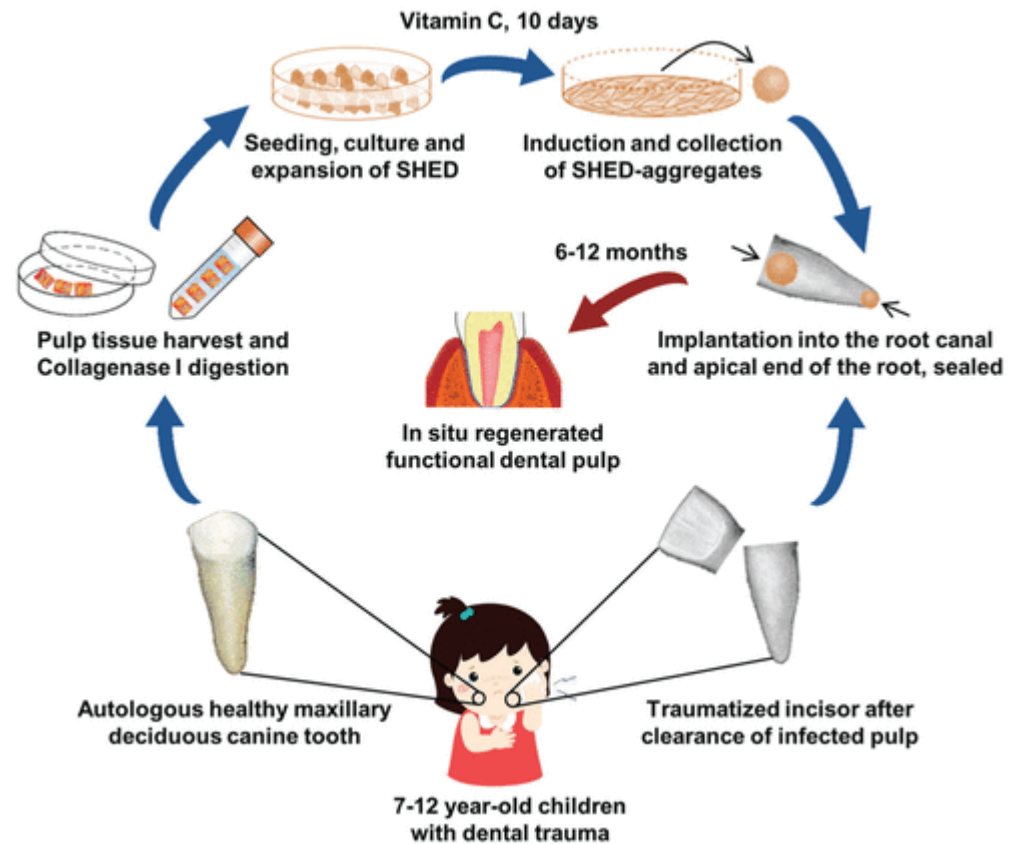


Pulp stem cells possess neurovascular properties based on their origin, niche location, and function. Pulp stem cells are recognized to derive from postmigratory craniofacial neural crest cells and/or peripheral nerve-associated glia (also neural crest-like cells). Furthermore, pulp stem cells were revealed to reside in a perivascular niche and be supported by the abundant neurites in the dental pulp (left panel). Regarding the function of pulp stem cells, for neurogenesis, they are able to differentiate into active neurons and glia and secrete neurotrophic factors that mediate neuroprotection and neurite outgrowth. For angiogenesis, they can also secrete a broad range of regulatory proteins for vessel formation and differentiate into endothelial cells to form capillary-like structures (right panel). These remarkable neurovascular properties make pulp stem cells the optimal population for vitalized functional pulp regeneration.

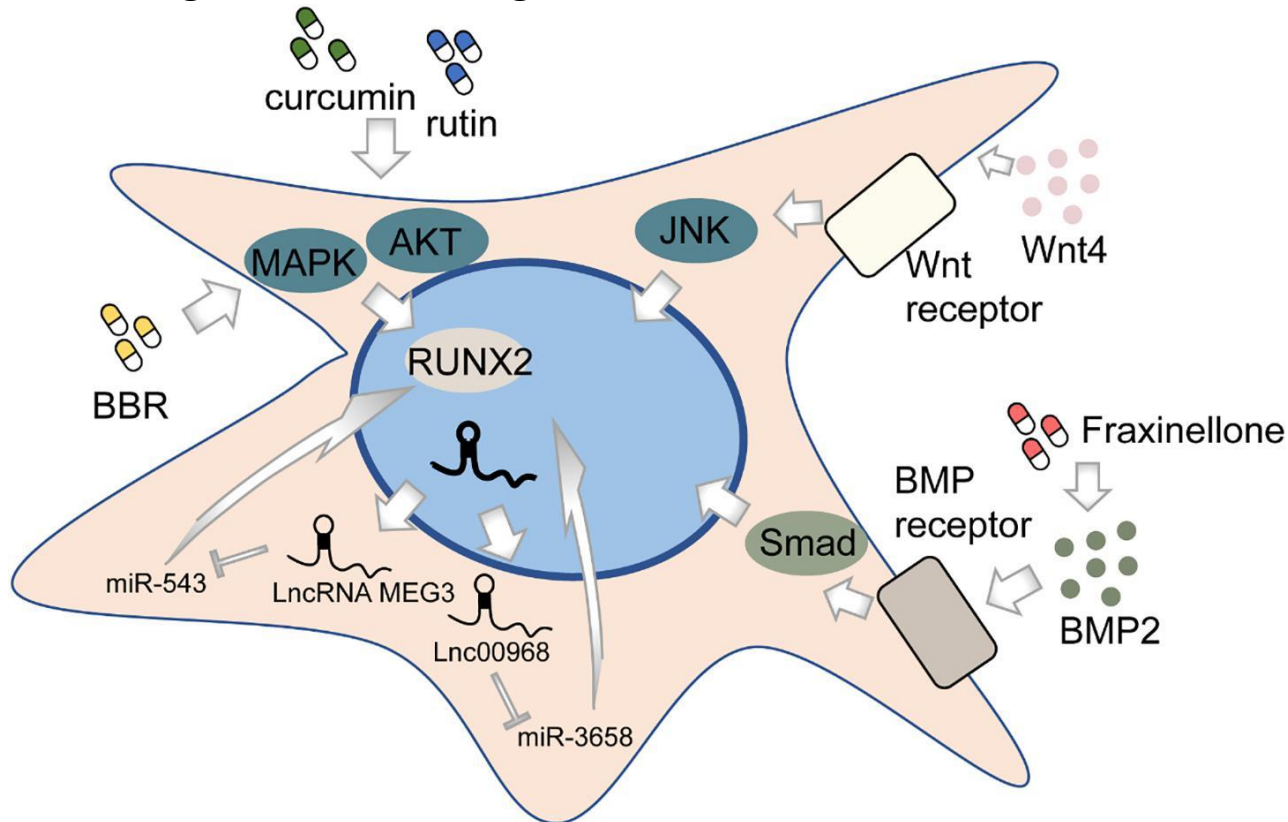


Current exogenous mesenchymal stem cell (MSC) transplantation strategies for pulp regeneration. For the 4 major application methods: (1) simple MSC transplantation with organic or synthetic scaffolds; (2) MSC co-transplantation with microvascular endothelial cells aiming to provide extra vascularization; (3) transplantation of MSCs pretreated or combined with growth factors; and (4) scaffold-free transplantation with self-organized MSC sheets, aggregates, or pellets. Human dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) are the most widely used MSC seed cells, while the ultimate goal is to achieve de novo neurovascularized functional pulp regeneration.

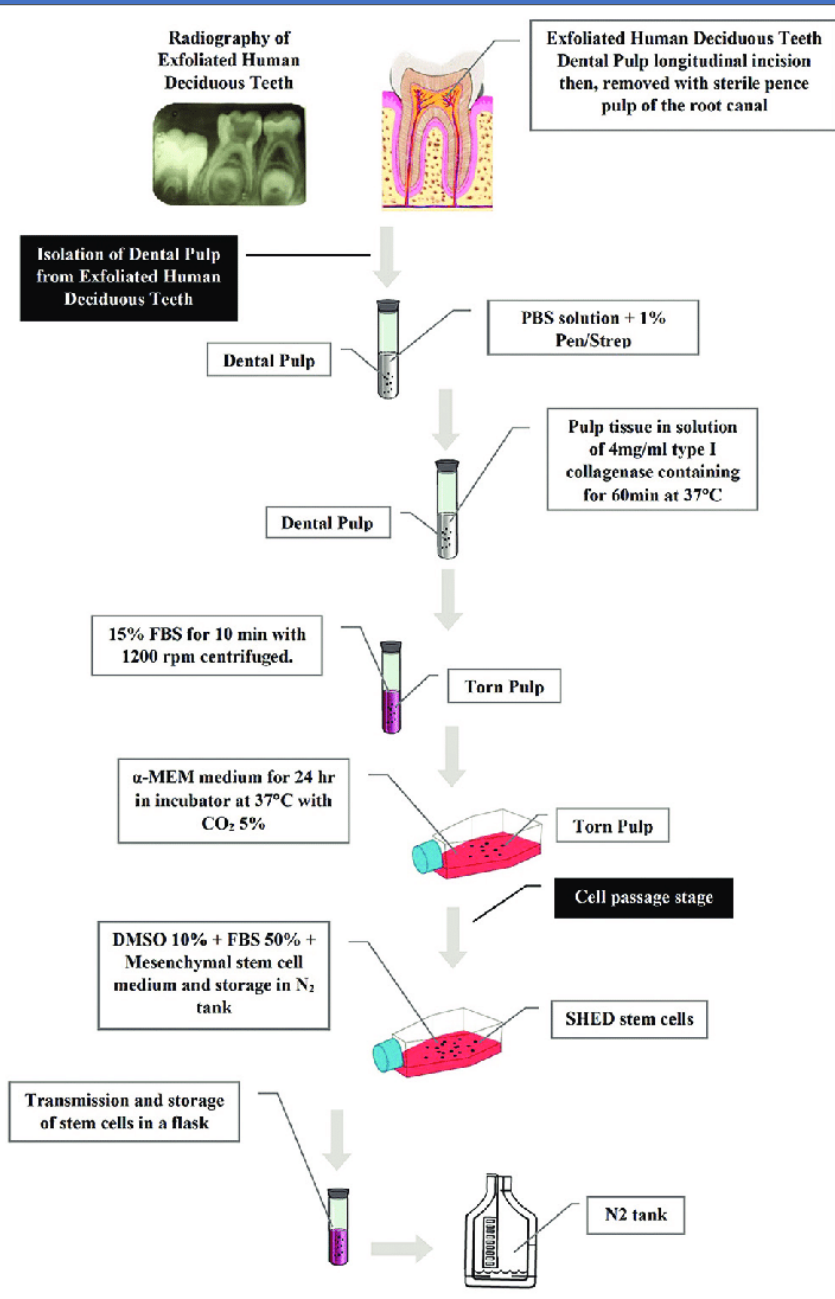
Methodology of using SHED aggregates for functional pulp regeneration in human. For 7- to 12-y-old children with dental trauma, pulp tissues can be harvested from autologous healthy maxillary deciduous teeth, and SHED can be isolated according to collagenase I digestion and colony expansion. After induction by vitamin C for 10 d, the second-passaged SHED will form aggregates, which are collected for implantation. Upon clearance of original infected pulp tissues, the traumatized incisors accept implantation of SHED aggregates from both the root canal and the apical end of the root. The access cavities are then sealed, and functional dental pulp will be regenerated in situ after 6 to 12 mo. SHED, stem cells from human exfoliated deciduous teeth.



The regulation of osteogenic differentiation of DMSCs.



periodontal ligament stem cells (PDLSCs)



Culture

Periodontal ligament (PDL) tissue collected from extracted adult and primary teeth is an easily accessible source of MSCs and such MSCs isolated from the PDL were found to have the ability to regenerate bone, cementum, and PDL upon in vivo transplantation.

Primary PDL cells (PDLCs) were isolated from the PDL of extracted adult third molars. PDLCs were cultured in a modified Eagle's medium (alpha-MEM) containing 20% fetal bovine serum (FBS), non-essential amino acids, 100 μmL^{-1} penicillin, and 100 μmL^{-1} streptomycin, in a humidified 5% CO_2 incubator at 37 $^{\circ}\text{C}$. Media was changed every 2 days, and cells were passaged at 80%–90% confluency. PDLCs used in this study were from passages 4–8.

Isolation and Medium

Table 1 Enzyme digestion protocols used for DPSCs isolation

Enzymatic cocktail	Conditions	Teeth	References
3 mg/ml collagenase I, 4 mg/ml dispase	1 h at 37°C	Third molars, molars	[7]
3 mg/ml collagenase I, 4 mg/ml dispase	1-2 h at 37°C	Mouse incisor	[27]
4 mg/ml collagenase I, 4 mg/ml dispase II	24 h at 4°C	Deciduous teeth	[28]
4 mg/ml collagenase I, 2 mg/ml dispase	1 h at 37°C	Incisors	[29]
0.2 mg/ml collagenase I, 2 mg/ml dispase	70 min 37°C	Third molars	[30]
1 mg/ml collagenase I, 2.4 mg/ml dispase	1 h at 37°C	Deciduous teeth, adult molars	[31]
0.3 mg/ml collagenase I, 0.1% dispase II	1 h at 37°C	Deciduous teeth, premolars	[10]
1-3 mg/ml collagenase/dispase	20 min at 37°C	NS	[32]
1 mg/ml collagenase/dispase	30 min at 37°C	Third molars	[26]
3 mg/ml collagenase type I	1 h at 37°C	Third molars	[33]
3 mg/ml collagenase type I	40 min at 37°C	Third molars	[34]
0.2% collagenase type II	30 min at 37°C	Third molars	[35]
0.2% collagenase type I	1 h at 37°C	Incisors, canines, molars, deciduous teeth	[36]
1 mg/ml collagenase I	30 min at 37°C	Third molars	[18]
Accutase solution	30 min at 37°C	Third molars	[18]
0.04 mg/ml Liberase (mix collagenase I and II)	NS	Third molars	[37]
0.2% trypsin pretreated explants	5 min at 37°C	Third molars	[17]

NS not specified

Table 2 Media and supplements commonly used for cell culture of DPSCs

Basal medium	Supplements	Serum	Tissue origin	Differentiation potential <i>in vitro</i>	References
α -MEM	Platelet lysate (1-10) used only for expansion	20% FBS	Third molars	Adipogenic, osteogenic	[53]
	L-glutamine, ascorbic acid-2-phosphate; 2% UCB-PRP plus 5% FBS only for expansion	15% FBS	Deciduous teeth, molars	Osteogenic	[31]
	GlutaMAX, L-ascorbate-2-phosphate	10% FBS	Mouse incisors	Neuronal	[27]
	L-glutamine, L-ascorbic acid-2-phosphate	15-20% FBS	Deciduous teeth, third molars	Osteogenic, adipogenic, odontogenic, hepatic, chondrogenic, endothelial, neuronal	[7, 54]
	L-glutamine	10% FBS	Third molars	Neuronal, myogenic, osteogenic, chondrogenic, adipogenic	[19, 55]
	GlutaMAX	10% FBS	Permanent teeth	Osteogenic, adipogenic, chondrogenic	[34]
	L-glutamine, ascorbic acid, bovine pituitary extract	10% FBS	Human/rat molars	Osteogenic (spontaneous)	[11]
	L-glutamine, ascorbic acid-2-phosphate, dexamethasone, EGF and PDGF-BB	2% FCS	Third molars	Osteogenic, chondrogenic, endothelial, myogenic, neuronal	[30]
	Dexamethasone, ITS, EGF and PDGF-BB	2% FCS	Premolar, third molars, deciduous teeth	NA	[45]
	Non-essential amino acids	20% FBS	Third molars	Odontogenic, chondrogenic	[56]
DMEM-KO	GlutaMAX	10% FBS	Permanent teeth	Osteogenic, adipogenic, chondrogenic	[34]
	GlutaMax, ascorbic acid, ITS	10% FBS or PL	Third molars	Osteogenic, adipogenic, chondrogenic	[57]
	SFM was used for expansion and supplemented with combinations of ITS-X, ETF, FGF-a, sodium pyruvate and ascorbic acid	10% FBS	Deciduous teeth, third molars	NA	[58]
DMEM	None	10-15% FBS	Third molars, premolars deciduous teeth	Hepatic, osteogenic, adipogenic, chondrogenic	[25, 28, 59]
	Nonessential Amino Acids	20% FBS	Molars	Osteogenic, adipogenic	[60]
	L-ascorbic acid-2-phosphate	20% FCS	Deciduous incisors	Adipogenic, chondrogenic, myogenic, osteogenic	[61]
	L-glutamine, nonessential amino acids, sodium pyruvate, β -mercaptoethanol	15% FBS	Molars	Osteogenic	[62]
	G-CSF was added to 2nd passaged to mobilize DPSCs	10% HS	Third molars	Osteogenic, odontogenic, neuronal, angiogenic	[63]
	L-glutamine, β -mercaptoethanol	10% FCS	Third molars	Osteogenic, chondrogenic, myogenic	[17]
	GlutaMAX	10% FBS	Permanent teeth	Osteogenic, adipogenic, chondrogenic	[34]
MegaCell DMEM DMEM/F12	GlutaMAX	15-20% HS or 10% FBS	Third molars	Osteogenic, chondrogenic, adipogenic	[64]
	L-glutamine, nonessential amino acids	15% FBS	Deciduous teeth	Myogenic, chondrogenic	[23]
	GlutaMAX, L-ascorbic acid-2-phosphate	10% FBS	Third molars	Osteogenic, adipogenic, chondrogenic	[65]
	Basal medium: Glucose and HEPES. After 12 h, medium was supplemented with N2, EGF, bFGF and heparin, FBS was not added	10% FBS	Third molars	Neuronal, osteogenic	[66]
	ITS, LA-BSA, dexamethasone, ascorbic acid-2-phosphate, PDGF-BB, EGF, LIF, chemically defined lipid concentrate, BSA, β -ME	2% FBS	Third molars	Osteogenic, endothelial, hepatocyte, neuronal	[33, 44]
	GlutaMAX	10% FBS	Permanent teeth	Chondrogenic, adipogenic, osteogenic,	[34]
	Medium is similar to DMEM and contains FBS and L-glutamine	FBS	Molars	Osteogenic, endothelial, neuronal, glial, chondrogenic, adipogenic	[67]

Isolation and Medium

Alpha-MEM and DMEM-KO were the most optimal culture media maintaining a higher proliferation rate, differentiation potential and lower levels of senescence, when compared with DMEM-LG and DMEM/F12.

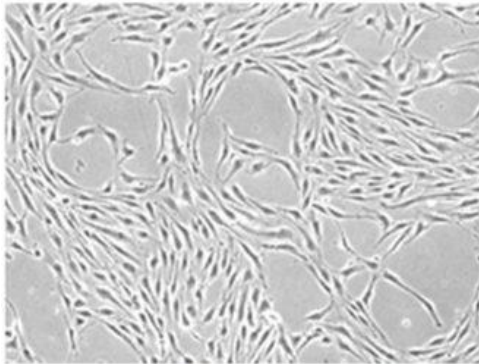
Alpha-MEM also enhanced the expression of osteogenic genes during differentiation at early and late DPSCs passages, whereas the other media showed a reduced level of the same genes.

Table 3 Growth factors supplemented to media culture of DPSCs

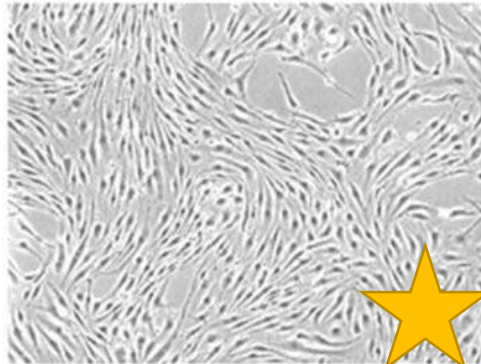
Growth factor(s)	Conditions	Results	References
EGF and PDGF-BB (concentration not specified)	α -MEM, 2% FBS, dexamethasone and ITS	Enhanced proliferation of DPSCs maintaining a stable karyotype beyond 65 population doublings without exhibiting signs of spontaneous differentiation	[45]
100 μ g/ml ETF	DMEM with 1% ITS-X	DPSCs and SHEDs exhibited higher survival and proliferation rates compared with other combinations of growth factors during expansion, although was not compared with FBS. Surface marker expression was comparable to FBS	[58]
10 ng/ml EGF, 10 ng/ml PDGF-BB	α -MEM, 2% FBS, L-glutamine, ascorbic acid-2-phosphate, dexamethasone	Enhanced initial cell adhesion of primary culture compared to serum free-media, but cell recovery was lower than FBS; DPSCs expressed several stem cell-associated markers similar to FBS medium, except CD146 and α -SMA	[30]
1000 units/ml LIF, 10 ng/ml EGF, 10 ng/ml PDGF-BB	60% DMEM low glucose, 40% MCDB-201, 2% FBS, ITS, LA-BSA, ascorbic acid-2-phosphate, BSA, β -ME, dexamethasone, chemically defined lipid concentrate	Allowed the isolation of a heterogeneous population containing DPPSCs and favored long-term expansion after 65 passages maintaining a stable karyotype	[33, 44]
10 ng/ml EGF, 25 ng/ml bFGF	DMEM/F12, glucose, Hepes, N2 supplement, heparin	This medium was used for culturing of adherent (ADH)-DPSCs and non-ADH DPSCs; comparison of medium without growth factors was not performed	[66]
EGF and IGF-1 (concentration not specified)	EBM2, 10% FBS	Isolation of a highly proliferative DPSCs population, although comparison of medium without growth factors was not performed	[35]
10 μ g/ml PDGF- $\beta\beta$, 100 μ g/ml EGF, 100 μ g/ml IGF-1 and 100 μ g/ml bFGF	F-12 Coon's and Ambesi's modified:Medium 199: CMRL166 with 1.25% HS	Enhanced proliferation when combined with low levels of HS displaying a similar expression of cell markers compared to FBS	[72]

Confluency

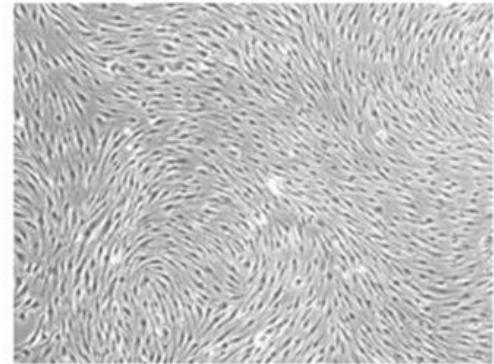
70%



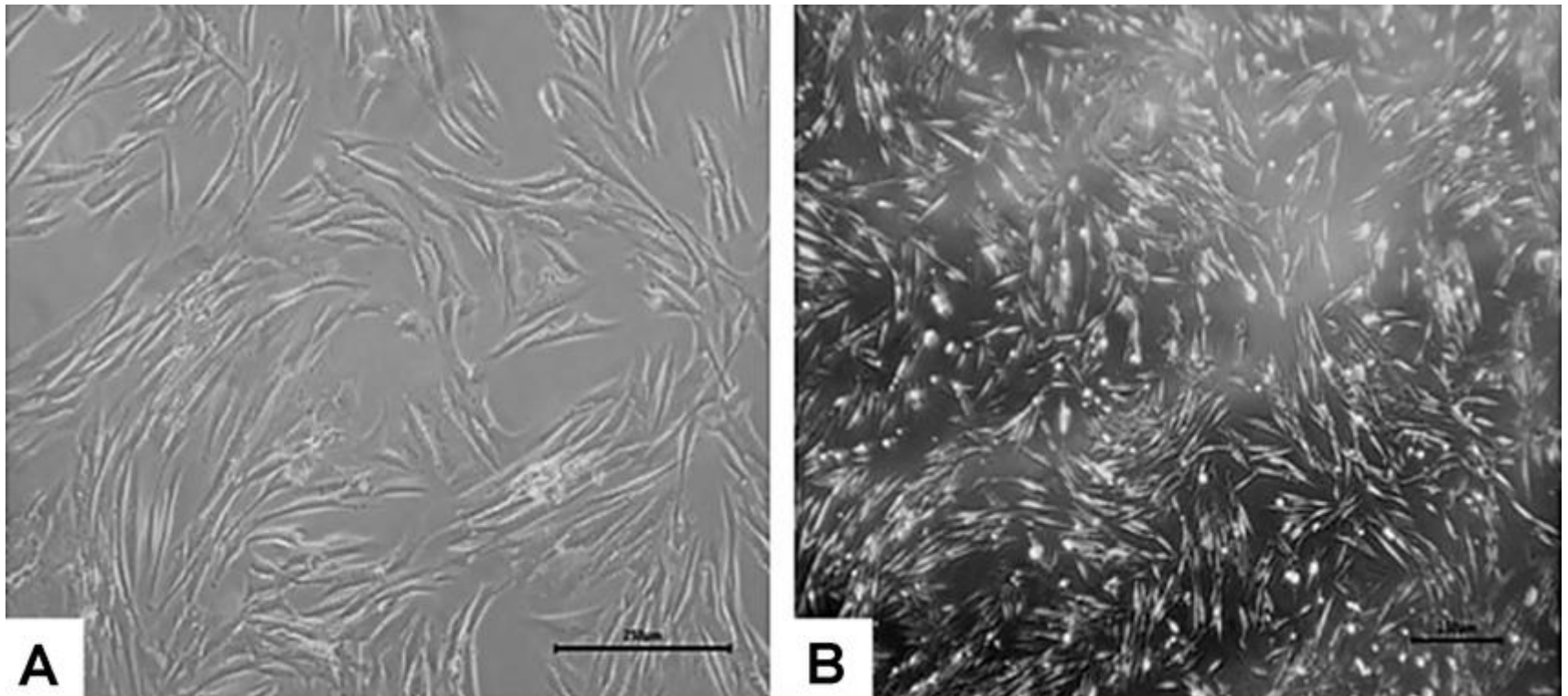
90%



100%



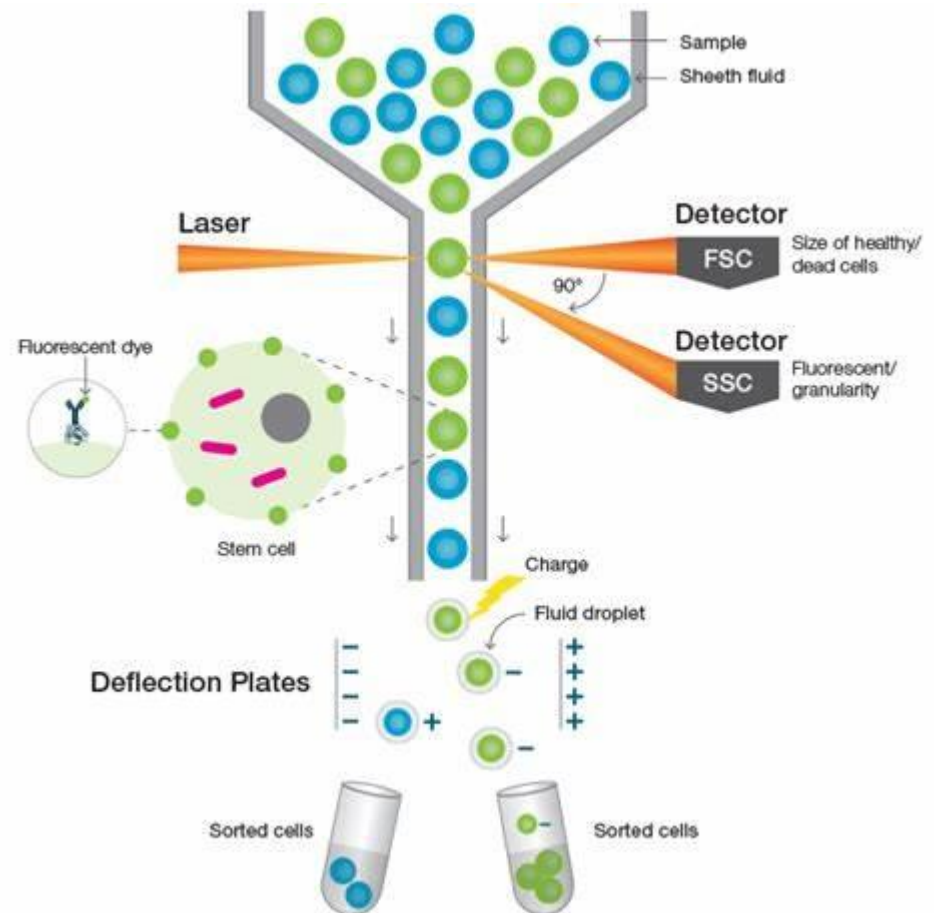
Cell culture



Cell culture. (A) Fibroblast-like morphology of Human Dental Pulp Cells after 10 days of cell culture (10X). (B) Confluence >80% of Human Dental Pulp Cells after 28 days of cell culture (4X).

Isolation

Fluorescence-activated cell sorting (FACS) is a technique to purify specific cell populations based on phenotypes detected by flow cytometry.



Isolation

Magnetic-activated cell sorting (MACS) is a method for separation of various cell populations depending on their surface antigens (CD molecules) invented by Miltenyi Biotec.

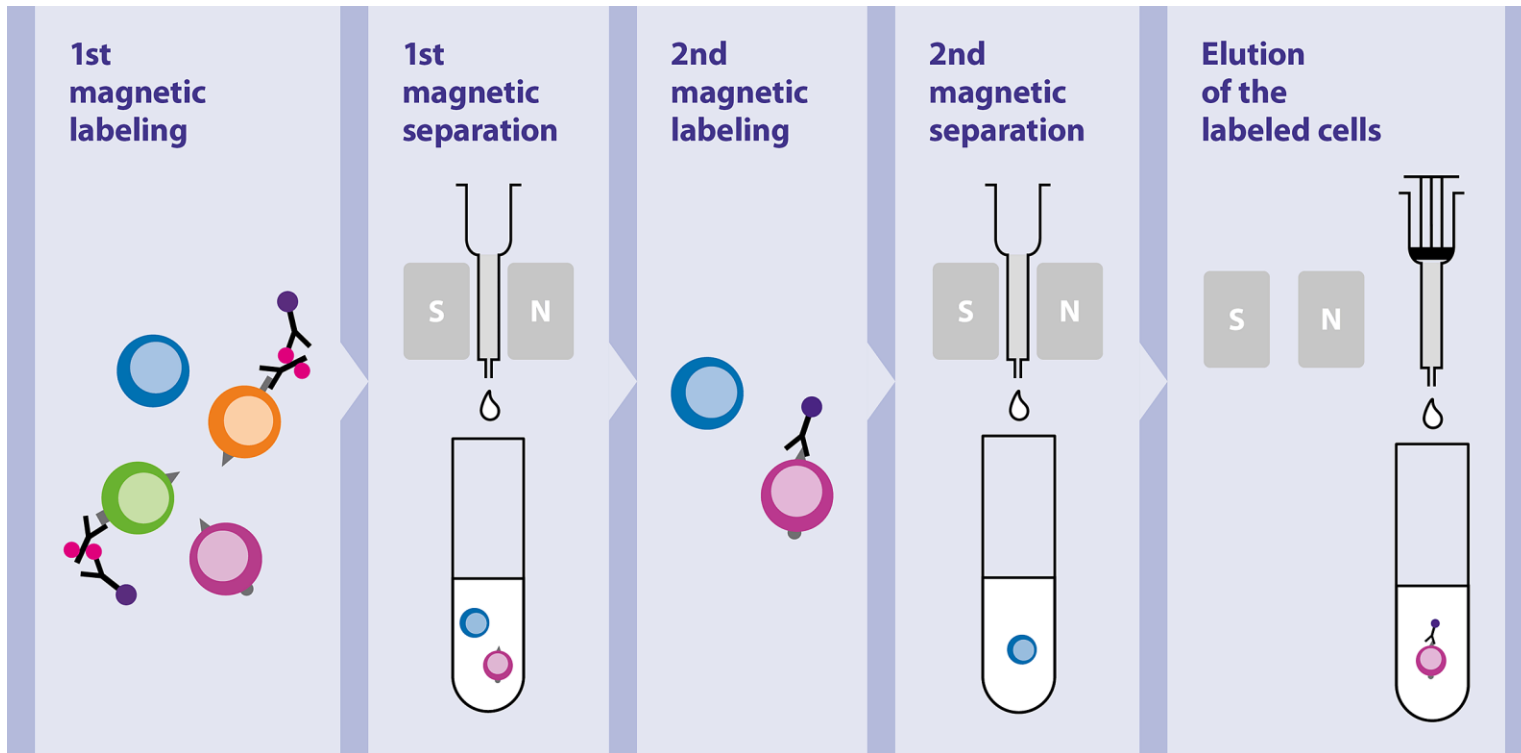
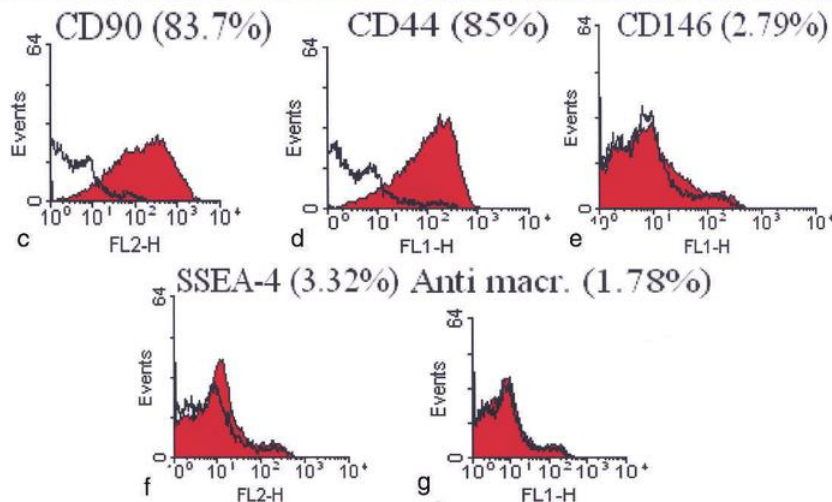
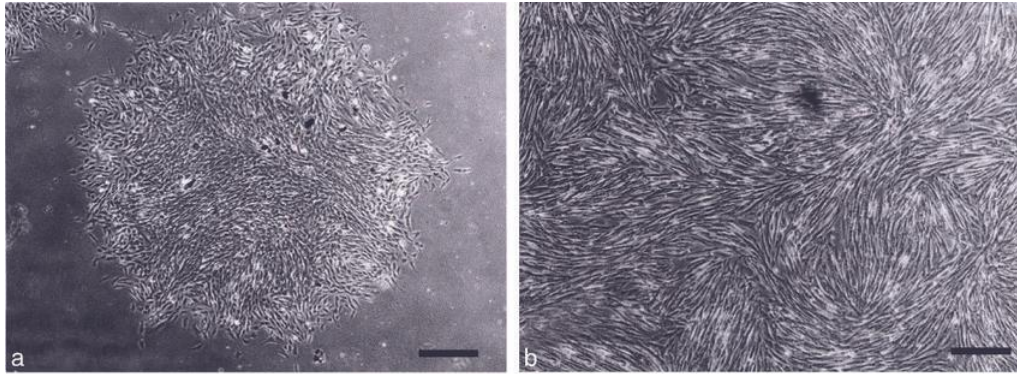


Table 4 Comparison of cell surface markers used for specific cell enrichment in DPSCs cultures

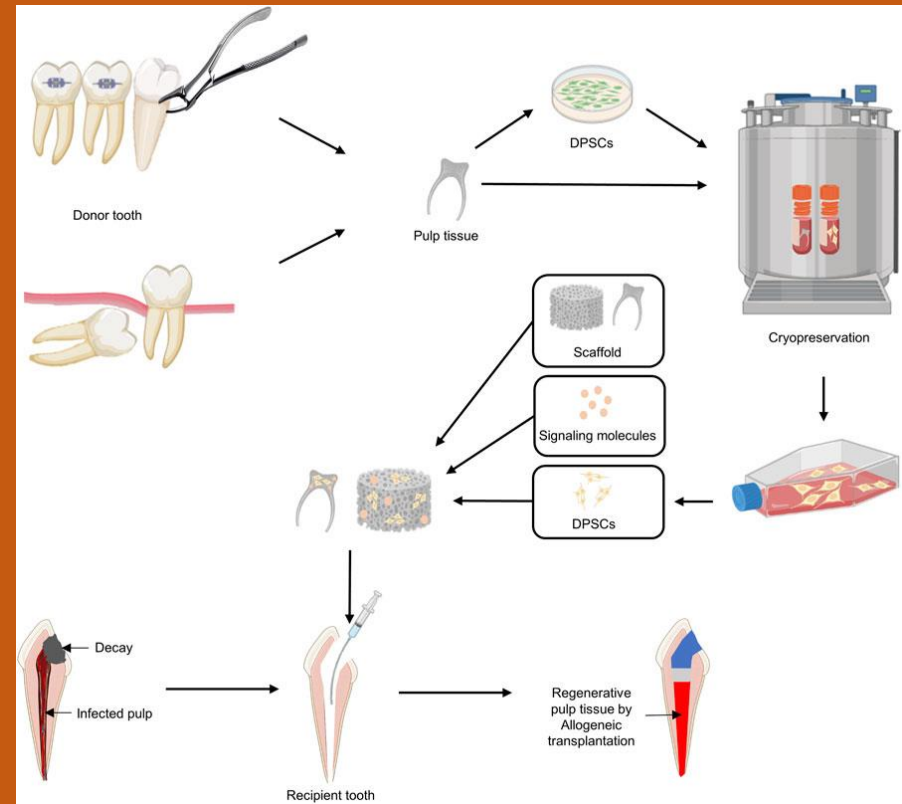
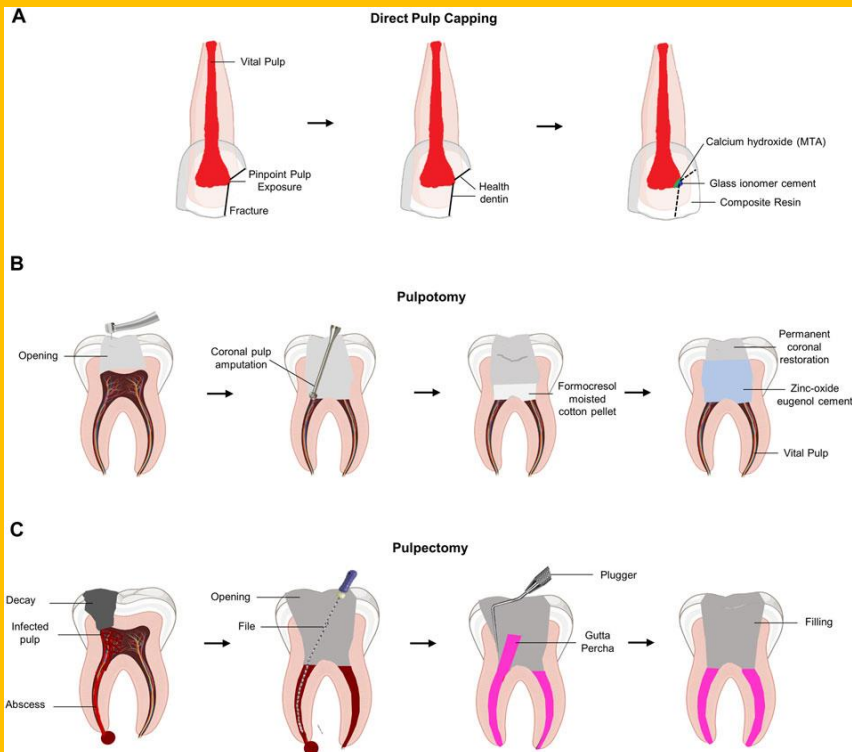
Cell surface marker(s)	Method	Enrichment	Tissue	Description	Ref.
STRO-1 ⁺ or CD146 ⁺ or 3G5 ⁺	MACS	After tissue dissociation	Third molars	Significant increase in the number of colony-forming cells when compared with unsorted cells	[100]
CD73 ⁻	MACS	Primary culture	Third molars	DPSCs expressed TERT, Oct3/4 and Sox2, and displayed a high proliferative potential unlike its CD73 ⁺ counterpart.	[44]
c-kit ⁺ /CD34 ⁺ /CD45 ⁻	FACS	Primary culture	Molars	Subpopulation highly clonogenic with the potential to differentiate into self-renewing osteoblast precursors	[99]
STRO-1 ⁺ /c-Kit ⁺ /CD34 ⁻	MACS	Primary culture	Third molars	Higher proliferation in long-term cultures, reduced senescence and apoptosis rates compared to STRO-1 ⁺ /c-Kit ⁺ /CD34 ⁺ cells; differentiation was similar for mesodermal lineages, but STRO-1 ⁺ /c-Kit ⁺ /CD34 ⁺ showed a greater commitment into neurogenic induction.	[55]
CD271 ⁺	FACS	Primary culture	Third molars	Higher differentiation potential, especially odontogenic, compared with STRO-1 ⁺ CD146 ⁺ or CD51 ⁺ /CD140 α ⁺ subpopulations isolated in the same study	[56]
CD271 ⁺	MACS	Primary culture	Permanent teeth	Cells expressed STRO1, vimentin, CD105 and Notch-2 and differentiated toward mesenchymal lineages	[101]
STRO1 ⁺	MACS	Primary culture	Human/rat molars	STRO1 ⁺ cells decreased proliferation and increased spontaneous differentiation during subsequent passages. Probably represent a heterogeneous subpopulation	[11]
CD117 ⁺	MACS	Primary culture	Deciduous teeth	Homogeneous subpopulation differentiated toward hepatocytes and pancreatic cells	[28]
CD117 ⁺	MACS	Primary culture	Molars	Homogeneous subpopulation was enhanced into osteogenic differentiation in novel conditioned medium	[62]
CD105 ⁺	FACS	Primary culture	Third molars	Subpopulations used for isolation of mobilized DPSCs (MDPSCs) by G-CSF. CD105 ⁺ cells showed greater neovascularization and pulp regeneration in models than DPSCs unsorted, but less than MDPSCs	[63]
SSEA4 ⁺	FACS	Primary culture	Third molars	Highly pluripotent stems cells with multilineage differentiation potential	[33]
CD117 ⁺ /CD34 ⁺ /STRO-1 ⁺ /flk1 ⁺	FACS	Primary culture	Adult teeth	Homogeneous subpopulation differentiated toward several lineages, including osteoblasts and endotheliocytes	[71]
CD31 ⁻ /CD146 ⁻	FACS	After tissue dissociation	Porcine tooth germ	Side population was sorted with CD31 ⁻ /CD146 ⁻ markers; selected cells showed differentiation to several lineages, but showed a high angiogenic potential	[102]
CXCR4 ⁺	MACS	Primary culture	Permanent teeth	STRO-1 and CD146 were higher in CXCR4 ⁺ cells than CXCR4 ⁻ or unsorted cells	[103]

CD271 is the same that LANGFR, low-affinity nerve growth factor receptor; CD117 is c-kit marker
G-CSF growth colony stimulating factor, *CXCR4* CXC chemokine receptor type 4, *NS* not specified

Cell culture and FC markers



Periodontal Ligament Stem Cells
cementoblast/osteoblast markers
suggesting the possibility of
periodontal tissue regeneration





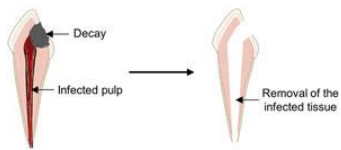
Neurogenic

In particular, human dental pulp cells express a neuronal phenotype and produce neurotrophic factors such as NGF, GDNF, BDNF, and bone morphogenetic protein (BMP)-2, suggesting that they may be potential candidates for cell-based therapy.

Angiogenic

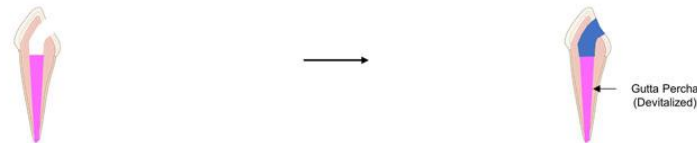
Human DPSCs induce angiogenesis and alleviate infarction in rats with acute myocardial infarction.

Revascularization was induced by transplantation of the CD31– CD146– SP of DPSCs isolated from porcine pulp tissue into the mouse hindlimb ischemia site.



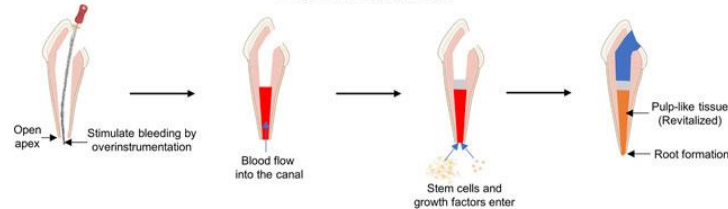
A

Classical Endodontic Treatment



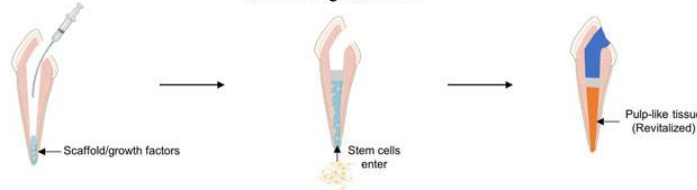
B

Pulp Revascularization



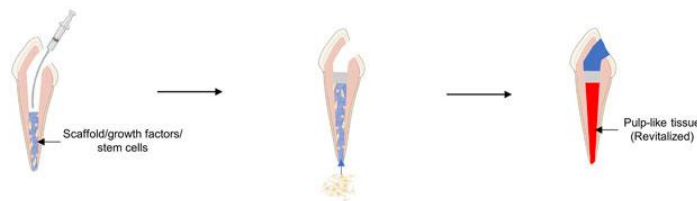
C

Cell Homing-based RET



D

Cell Transplantation-based RET



Permanent coronal restoration

MTA

Stem cells

Growth Factors

Scaffold

Sodium hypochlorite, a representative chemical disinfectant, is known to be cytotoxic at a concentration of 3% or more and interferes with stem cell adhesion

EDTA

Growth factors

Growth factors

bFGF and G-CSF application and reported that bFGF could be replaced with G-CSF.

PDGF (factors for cellular chemotaxis), NGF (for neural growth), VEGF (for angiogenesis), and BMP7 (for odontoblast differentiation and mineralization).

Allogeneic Transplantation

Dis-auto; wisdom teeth

Osteogenic potential decreases in aged human DPSCs.

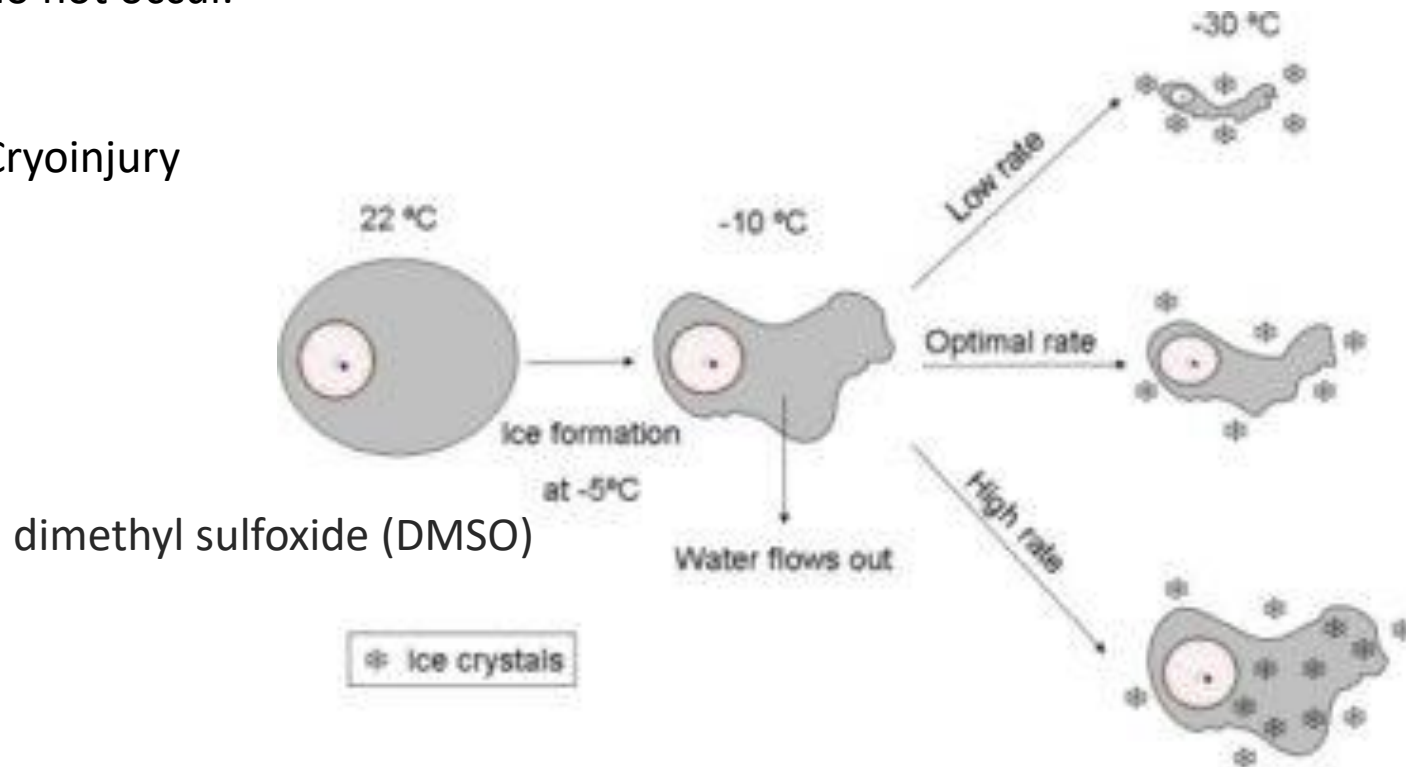
A concern with the use of allogeneic cells is that immune rejection may occur in the host due to a MHC mismatch. The reason that allogeneic MSCs can be applied despite these concerns is that MSCs themselves have low immunogenicity and immunosuppressive properties.

Rejection: addressing; Immunotolerance activity.

Cryopreservation

Cryopreservation is the process of maintaining cell viability by freezing and storing them at extremely low temperatures where biochemical reactions do not occur.

Cryoinjury





Adv.

DPSCs did not impair viability, proliferation, stemness, or differentiation capacity after cryopreservation at -80°C for 1 year.

Dis.

DPSCs can be cryopreserved only when an appropriate number is obtained by isolating and culturing DPSCs from pulp tissue after tooth extraction. This method takes a long time for cryopreservation, resulting in excessive labor and other costs, and there is even a risk of potential contamination by microorganisms.

Solution. Therefore, there are studies moving from cell-level cryopreservation to tissue-level cryopreservation.

Encapsulation