Application of dental stem cells in three-dimensional tissue regeneration

Hui-Yi Hsiao, Chung-Yi Nien, Hsiang-Hsi Hong, Ming-Huei Cheng, Tzung-Hai Yen

ORCID number: Hui-Yi Hsiao 0000-0003-3170-2112; Chung-Yi Nien 0000-0003-0462-725X; Hsiang-Hsi Hong 0000-0002-6688-4931; Ming-Huei Cheng 0000-0002-4237-2959; Tzung-Hai Yen 0000-0002-0907-1505.

Author contributions: Hsiao HY contributed data collection and manuscript writing; Nien CY, Hong HH and Cheng MH contributed data analysis; Yen TH contributed study design and supervision.

Conflict-of-interest statement: Authors have no conflict of interest for this manuscript.

Supported by Chang Gung Memorial Hospital, Linkou, Taiwan, No. CORPG3K0021 and No. CORPG3K0191.

Country/Territory of origin: Taiwan

Specialty type: Cell and tissue engineering

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, C
Grade D (Fair): 0
Grade E (Poor): E

Hui-Yi Hsiao, Ming-Huei Cheng, Tzung-Hai Yen, Center for Tissue Engineering, Chang Gung Memorial Hospital, Linkou Branch, Taoyuan 333, Taiwan
Chung-Yi Nien, Department of Life Sciences, National Central University, Zhongli, Taoyuan 320, Taiwan
Hsiang-Hsi Hong, Department of Periodontics, Chang Gung Memorial Hospital, Linkou Branch, Taoyuan 333, Taiwan
Ming-Huei Cheng, Division of Reconstructive Microsurgery, Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Linkou Branch, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan
Tzung-Hai Yen, Department of Nephrology, Clinical Poison Center, Chang Gung Memorial Hospital, Linkou Branch, Taoyuan 333, Taiwan
Tzung-Hai Yen, School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan

Corresponding author: Tzung-Hai Yen, MD, PhD, Doctor, Professor, Department of Nephrology, Clinical Poison Center, Chang Gung Memorial Hospital, Linkou Branch, No. 5 Fu-Hsing Street, Kweishan, Taoyuan 333, Taiwan. m19570@adm.cgmh.org.tw

Abstract

Dental stem cells can differentiate into different types of cells. Dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligament stem cells, stem cells from apical papilla, and dental follicle progenitor cells are five different types of dental stem cells that have been identified during different stages of tooth development. The availability of dental stem cells from discarded or removed teeth makes them promising candidates for tissue engineering. In recent years, three-dimensional (3D) tissue scaffolds have been used to reconstruct and restore different anatomical defects. With rapid advances in 3D tissue engineering, dental stem cells have been used in the regeneration of 3D engineered tissue. This review presents an overview of different types of dental stem cells used in 3D tissue regeneration, which are currently the most common type of stem cells used to treat human tissue conditions.

Key Words: Dental stem cells; Dental pulp stem cells; Stem cells from human exfoliated deciduous teeth; Periodontal ligament stem cells; Stem cells from apical papilla; Dental
Even though DPSCs exhibit features similar to those of BMSCs, their characteristics of proliferation rate, multilineage potential, and immunomodulatory properties identified in 2000. DPSCs located in the soft connective tissue inside the dental crown were first identified in 2000 (Figure 1)[8]. DPSCs exhibit MSC-like properties, including a high proliferation rate, multilineage potential, and immunomodulatory properties[8,9]. Even though DPSCs exhibit features similar to those of BMSCs, their characteristics of
## Table 1 List of dental stem cells used for three-dimensional tissue regeneration

| Dental stem cells | Biomaterials | Addition of materials/growth factors/cells | Type of tissue regeneration | Ref. |
|-------------------|--------------|------------------------------------------|----------------------------|------|
| **DPSCs**         |              |                                          |                            |      |
| DPSCs             | CaP porous granules, NF-gelatin/MgP | No                                       | Odontogenic differentiation | Nam et al[24], and Qu et al [30] |
|                   | SS-PLA-b-PLYS, pNIPAAm, NF-PLLA | No                                       | Pulp-dentin regeneration   | Inoh et al[16], Itoh et al [17], and Soares et al[18] |
|                   | Coll/HAP/PLCL, ABM/ABM-P, 15, PVA/PU | No                                       | Bone tissue                | Mohanam et al[33], Cooke et al[59], and Akkouch et al[21] |
|                   | OMMT/PVA     | No                                       | Neuro-like cells           | Ghasemi Hamidabadi et al [47] |
|                   | Matrigel     | No                                       | Endotheliocytes and pericytes | Luzuriaga et al[51] |
|                   | Collagen gel | SDF1, bFGF, BMP7                          | Dental pulp tissue         | Suzuki et al[23] |
| DPSCs with growth factors | Ti6Al4V            | Poly-L-lys coating                       | Osteoblastic differentiation | Galli et al[32] |
|                   | Porous silk fibroin | bFGF                                      | Dental pulp tissue         | Yang et al[26] |
|                   | PCL           | VEGF, BMP2                               | Vascularized bone tissue   | Park et al[39] |
|                   | HP hydrogel   | bFGF                                      | Spinal cord                | Luo et al[48] |
| DPSCs with other cells | Matrigel and collagen gel | Human normal oral epithelial cells        | Epithelium invagination-like structure | Xiao and Tsutsui[35] |
|                   | PCL/PLDLA     | Endothelial cells                        | Vascularized bone tissue   | Jin and Kim[36] |
|                   | PLLA/PLGA     | Human neonatal dermal fibroblasts         | Spinal cord                | Guo et al[50] |
| DPSCs in 3D printed scaffolds | HA/TCP          | Apical papilla (SCAP)                     | Pulp-dentin regeneration   | Hilkens et al[40] |
|                   | PCL           | Platelet-rich plasma                      | Calvaria bone              | Li et al[27] |
|                   | Alg-Gel       |                                          | Bone                       | Yu et al[38] |
|                   | PLAS          |                                          | Neural differentiation     | Hsiao et al[42] |
|                   | AMP/ECM       |                                          | Craniomaxillofacial bone   | Dubey et al[41] |
| **SHED**         |              |                                          |                            |      |
| SHED with growth factors | No            | EGF, FGF                                 | Spinal cord                | Feng et al[58] |
|                   | No            | SHED-conditioned medium                  | Sciatic nerve              | Sugimura-Wakayama et al [59] |
| SHED in 3D formed scaffolds | Polylactoglycolide, SHED aggregated hemisphere | Bone tissue                | Laino et al[59], and Vakhrushev et al[57] |
| **PDLSCs**   |              |                                          |                            |      |
| PDLSCs           | Hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) | Periodontal tissue       | Kim et al[66] |
|                   | GelMA/PEG    | Periodontal tissue                       | Ma et al[80] |
| PDLSCs with growth factors | PLGA           | CTGF, BMP-7, BMP-2                       | Periodontal tissue         | Cho et al[73] |
|                   | Platelet-rich fibrin | Aspirin                                   | Periodontal tissue         | Du et al[76] |
| PDLSCs with other cells | Collagen/Chitosan | Somatic MSCs and DPSCs                   | Odontogenic differentiation | Ravindran et al[79] |
|                   | No            | HUVECs                                    | Periodontal tissue         | Kramer[77] |
|                   | PLGA-PEG-PLGA thermal hydrogel | PDLSCs overexpressing PDGF-BB | Alveolar bone tissue       | Pan et al[78] |
DFFCs: Dental follicle progenitor cells; SCAP: Stem cells from apical papilla; PDLSCs: Periodontal ligament stem cells; SHED: Human exfoliated deciduous teeth; DFPCs: Dental pulp stem cells; SDF1: Stromal-derived factor-1; bFGF: Basic fibroblast growth factor; BMP-7: Bone morphogenetic protein-7; Ti6Al4V: Titanium-6-aluminum-4-vanadium; Poly-L-lys: Poly-L-lysine; CaP: Calcium phosphate; OECs: Human normal oral epithelial cells; PLLC: Collagen (ColI)/hydroxyapatite (HA)/poly(l-lactide-co-caprolactone); NF-gelatin/MgP: Gelatin/magnesium phosphate; VEGF: Vascular endothelial growth factor; BMP-2: Morphogenetic protein-2; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; PCL: Polycaprolactone; NF-SMS: Nanofibrous spongy microspheres; SS-PLLA-b-PDLS: Star-shaped poly(l-lactic acid)-block-poly(l-lysine); PLDLA: Poly-L/D-lactide; NF-MS: Nanofibrous poly(l-lactic acid) scaffolds; Alg-Gel: Alginate/gelatin hydrogel; 3DP-PLASs: Polylactic acid scaffolds; ABM: Bone mineral; BMP-P-15: Biomimetic collagen peptide; PVA: Polyvinyl alcohol; PU: Polyurethane; AMPs: Amorphous magnesium phosphates; ECM: Extracellular matrix; Coll-nano-HA/OPS: Collagen-nanohydroxyapatite/phosphoserine.

Figure 1 Schematic illustration of dental stem cells in three-dimensional tissue regeneration. A: Five different types of dental stem cells are harvested during different tooth developmental stages; B: Dental stem cells are incorporated with various forms of three-dimensional (3D) biomaterials (microspheres, hydrogels, or 3D printed scaffolds) to generate 3D engineered tissue; C: Dental stem cells are induced to differentiate into different types of tissue, such as teeth, neurons, bone, blood vessels and cartilage.

causing little morbidity at the donor site, a higher proliferation rate, and multipotency make DPSCs better stem cell sources for tissue regeneration[10]. DPSCs cocultured with apical bud cells (ABCs) exhibited more active odontogenic differentiation ability than BMSCs cocultured with ABCs[11]. The neural differentiation of IMR-32 cells was significantly enhanced when treated with secretomes derived from DPSCs compared to BMSCs[12]. The assessment of neurogenic potential on the secretome of DPSCs and BMSCs indicated that DPSCs presented better potential for neural differentiation[12]. Most DPSC studies have focused on dental pulp and bone tissue regeneration.
Compared to bone marrow stem cells, DPSCs have a higher proliferation rate and better osteogenic capacity when seeded in a scaffold of bone mineral (ABM) coated with a biomimetic collagen peptide (ABM-P-15), even generating a more organized collagenous matrix 8 wk after in vivo implantation[13]. Moreover, different gene expression patterns have been found in the transcriptome profiles of DPSCs compared to those of bone marrow stem cells, indicating unique gene expression patterns within DPSCs[14].

**Application in 3D tissue regeneration**

In addition to conventional tissue regeneration approaches with cells loaded on two-dimensional scaffolds, DPSCs have been cultured on 3D biomaterials for the development of tissue constructs. A bioink containing human DPSCs and fibrinogen incorporated with polycaprolactone (PCL) was designed for the production of dentin pulp complex structures[15]. Nanofibrous spongy microspheres made from star-shaped poly(l-lactic acid)-block-poly(l-lysine) (SS-PLLA-b-PLYS) were seeded with DPSCs for dental pulp tissue regeneration[16]. Poly-N-isopropyl acrylamide (pNIPAAm) gel containing DPSCs was made in a rod shape to fill in the root canal for pulp tissue regeneration[17]. Simvastatin and nanofibrous poly(l-lactic acid) (NF-PLLA) scaffolds[18] and a mixture of polyvinyl alcohol (PVA) and polyurethane (PU) [19] were combined with DPSCs to investigate the potential of tissue regeneration. Self-assembling peptides, with structures similar to the extracellular matrix (ECM), are among the smart materials used for 3D culture[20]. A 3D scaffold composed of collagen (Coll), hydroxyapatite (HA), and poly(L-lactide-co-caprolactone) (PLCL) increased the adhesion and viability of DPSCs and enhanced bone regeneration compared to a PLCL-only scaffold[21]. DPSCs grown in a peptide-based scaffold presented RGD- and vascular endothelial growth factor (VEGF)-mimetic peptide epitopes and exhibited better survival and angiogenic and odontogenic differentiation [22].

With increased knowledge of the function of growth factors, an increasing number of studies have introduced growth factors into different types of tissue regeneration. In 2011, human DPSCs were placed on the surface of 3D collagen cylinders and cultured with the addition of stromal-derived factor-1α, basic fibroblast growth factor (bFGF), and bone morphogenetic protein-7 (BMP-7) for dental pulp regeneration[23]. Seeding DPSCs on 3D calcium phosphate (CaP) porous granules promoted odontogenic differentiation by increasing the gene expression of dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP1)[24]. Porous silk fibroin scaffolds fabricated with bFGF, which has been reported to facilitate pulp regeneration[25], were used to fill the root canal space for tooth repair[26]. Platelet-rich plasma (PRP) containing various growth factors along with DPSCs was added to 3D printed PCL mesh for bone regeneration in a rat calvaria defect model[27].

In addition to growth factors, metal ions have also been confirmed to contribute to cell differentiation[28]. Magnesium (Mg) is involved in the process of biomineralization during bone and tooth development[29]. Qu et al[30] incorporated Mg into nanofibrous gelatin biomaterials to develop 3D gelatin/Mg phosphate (NF-gelatin/MgP) scaffolds seeded with DPSCs, and odontogenic proliferation and differentiation were enhanced. The materials used for dental implants, such as titanium-6-aluminum-4-vanadium (Ti6Al4V), are also used as 3D scaffolds for tissue regeneration. Their properties of low corrosion and smooth metal surfaces prevent stem cells from colonizing this biomaterial[31]. Coatings of poly-L-lysine (poly-L-lys), which carries positive charges, induced focal adhesion kinase activation and increased the osteoblastic differentiation of hDPSCs[32]. A coculture system not only provides intercellular factors but also enables communication between two types of cells, which is critical for the development and arrangement of the ECM[33,34]. DPSCs cocultured with human normal oral epithelial cells harvested from gingival tissue were inoculated into 3D Matrigel to form an epithelium invagination-like structure, a key feature of early tooth development[35]. Poly-L/D-lactide (PCL/PLDLA) porous microspheres were loaded with DPSCs and human endothelial cells to promote osteogenesis and angiogenesis for vascularized bone tissue regeneration[36].

3D printing techniques can print cells, growth factors, or biomaterials in the desired location to achieve more complicated multicell tissue structures[37]. In contrast to cultures in 2D alginate/gelatin hydrogel (Alg-Gel) scaffolds, 3D Alg-Gel scaffolds can be printed in a seven-layer coin shape and loaded with DPSCs. These DPSC-loaded 3D printed scaffolds achieved higher cell proliferation, odontoblastic differentiation, and bone mineralization, suggesting that a 3D environment is more suitable for cell proliferation and differentiation[38]. In addition, Park et al[39] designed the printing of DSPCs with VEGF in the central zone and bone morphogenetic protein-2 (BMP-2) in...
the peripheral area of the 3D-printed construct to fabricate vascularized bone structures. A cone-shaped scaffold was printed with hydroxyapatite/tricalcium phosphate (HA/TCP) powder that was polymerized by an ultraviolet (UV) photoinitiator. DPSCs and SCAP were mixed with collagen gel and loaded into the 3D printed HA/TCP scaffold for dental pulp regeneration[40]. 3D PCL mesh supplemented with PRP containing various growth factors along with DPSCs was custom printed to fit rat calvarial defects for bone regeneration[27]. PRP containing various growth factors, along with DPSCs, was added to 3D printed PCL mesh for bone regeneration in a rat calvarial defect model[27]. A novel DPSC-loaded bioink containing a mixture of amorphous Mg phosphates and ECM increased the bone density during craniomaxillofacial bone regeneration[41]. With the 3D printing technique, the shape, pore size, and gap size can be precisely controlled to study their microenvironmental effects on cell proliferation and differentiation. Polylactic acid scaffolds (PLASs) were printed in different gap sizes, and it was discovered that smaller gaps in 3D PLASs presented with different cellular orientations[42].

In addition to their osteogenic and odontoblastic potential, the chondrogenic potential of DPSCs has been investigated. Zhang et al[43] successfully induced DPSCs to undergo a chondrogenic differentiation process, and their synthesis of sulfated glycosaminoglycans was confirmed. DPSCs formed into 3D pellets were subjected to chondrogenic potential investigation, resulting in the enrichment of collagen I deposition. The content of glycosaminoglycan or collagen type II was not enhanced even with the addition of chondroinductive growth factors, suggesting that the chondrogenic lineage of DPSCs favors differentiation into fibrous cartilage rather than hyaline cartilage[44]. DPSCs, derived from cranial neurons, can differentiate into neuron-like cells for axon regeneration and are potential cell sources for neuron regeneration[45,46]. DPSCs were seeded within chitosan-intercalated montmorillonite/poly(vinyl alcohol) (OMMT/PVA) nanofibrous mesh, and they differentiated into neuron-like cells[47]. A thermosensitive heparin-poloxamer hydrogel with DPSCs and bFGF enhanced motor and sensory functional recovery after spinal cord injury repair[48]. Chitosan scaffolds have been demonstrated to enhance neuronal cell survival and differentiation. Zheng et al[49] incorporated bFGF into chitosan scaffolds and found that it promoted DPSC differentiation into neuronal cells but did not affect cell survival. Human adipose microvascular endothelial cells were cosedeed in a PLLA/poly(lactic-co-glycolic acids) (PLGA) scaffold with DPSCs to fabricate a prevascularized scaffold, which promoted revascularization, axon regeneration, myelin deposition, and sensory recovery in a rat complete spinal cord transection model[50]. Moreover, DPSCs seeded in Matrigel were able to differentiate into endothelialocytes and pericytes in serum-free culture media and secrete VEGF[51].

SHED

SHED cells, first isolated in 2003, present with positive expression of embryonic stem cell markers, such as OCT4 and NANOG, stage-specific embryonic antigens (SSEA-3 and SSEA-4), and mesenchymal stem cell markers (STRO-1 and CD146)[52-54]. Compared to DPSCs, SHEDs showed higher levels of osteocalcin expression and alkaline phosphatase activity[55]. SHEDs were confirmed to be more immature than DPSCs, allowing them to be “osteoblast-like’’ and “odontoblast-like’’, expressing osteocalcin and RUNX-2 markers[55]. Moreover, when SHEDs were cultured in medium with dexamethasone, they differentiated into adipocytes. After in vitro culturing for 2 wk in osteogenic medium, extracellular mineralized matrix started to be secreted by the SHEDs. This multilineage potential makes SHEDs alternative sources of dental stem cells[56].

Applications in 3D tissue regeneration

SHEDs cultured in vitro for 7 d were found to aggregate together, and they started to form a 3D ossification hemisphere after 36 d[56]. This mineral matrix was identified by alizarin red staining within the self-formed 3D woven bone tissue. SHEDs can be applied in a 3D polylactoglycolide scaffold fabricated by a surface-selective laser sintering device. The expression of osteocalcin was elevated in SHED-loaded polylactoglycolide scaffolds, suggesting that SHEDs are promising cell sources for scaffold populations in tissue bone engineering[57]. In addition to bone regeneration, SHEDs may be a source of neurons. When they were incubated in neuradifferentiation medium supplemented with epidermal growth factor (EGF) and fibroblast growth factor (FGF), SHEDs showed increased expression of neuron markers, such as βIII-
tubulin, microtubule-associated protein 2, tyrosine hydroxylase, and Nestin\cite{38}. These results confirmed the neurogenic potential of SHEDs. In spinal cord injury, a supply of SHEDs rescues hindlimb locomotor function\cite{32}. Furthermore, SHED-conditioned medium was demonstrated to regenerate peripheral nerves in sciatic nerve defects in a rat model. The rat static nerve defects at the mid-thigh level were covered with silicon conduits containing SHED-conditioned medium and resulted in an increase in Schwann cells, axon density and the number of regenerated myelinated fibers\cite{39}. Injection of SHEDs into the brain at the site of perinatal hypoxia-ischemia (HI) injury improved the survival rate of HI-injured mice through inhibition of the expression of proinflammatory cytokines\cite{60}. Although SHEDs have multidifferentiation potency and fewer limitations in terms of ethical concerns in their clinical application, only a few studies have investigated the application of SHEDs in 3D-printed scaffolds for tissue regeneration. It is possible that the collection, treatment methods, and storage of harvested SHEDs have not been standardized or popularized.

PDLSCs

Periodontitis is a very common oral disease resulting in periodontal tissue destruction and, more seriously, tooth loss\cite{61}. Many periodontal regeneration treatments have been performed to restore the damaged periodontium. PDLSCs were isolated from mature periodontal ligaments and found to express the stem cell markers CD105, CD90, and CD73\cite{62-64}. Seo et al\cite{63} successfully isolated PDLSCs from human third molars, and the expression of the stem cell markers STRO-1 and CD146/MUC18 was found in PDLSCs. In addition to the expression of stem cell markers, the osteogenic and adipogenic potential of PDLSCs was also identified\cite{65}, which makes PDLSCs alternative cell sources for tissue regeneration. The regeneration steps of periodontal tissue were demonstrated by PDLSCs incorporated with hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) as carriers\cite{66}. First, the proliferation of PDLSCs was increased, and collagen matrices were formed. Subsequently, the collagen fibers started to assemble, and cemental-like tissue was observed. Mineralization was present in the cemental-like tissue, and along with the presence of Sharpey’s fibers, mature collagen fibers were present. Later, the maturation of cemental-like tissue was identified by the expression of cemental tissue genes, such as α-smooth muscle actin antibody, collagen type XII (ColXII), osteoblast specific factor-2/periostin, and aspirin/PLAP-1\cite{67}.

Application in 3D tissue regeneration

A 3D collagen scaffold was fabricated with precise control of the pore size, pore wall alignment, and percolation diameter to investigate the effect of the scaffold structure on periodontal tissue regeneration. The results suggested that a larger percolation diameter increased PDLSC cell elongation and directionality, whereas the pore size influenced cell invasion and cell distribution\cite{68}. In addition to the manipulation of the scaffold structure, the addition of growth factors also promoted the capacity of tissue regeneration. During cemental tissue formation, connective tissue growth factor (CTGF) was found to promote the differentiation of periodontal ligament fibroblasts during the process of osteogenesis\cite{69}. BMP-7, expressed in the cementum, alveolar bone, and periodontal ligament, induces cementogenic differentiation by acting as a progenitor for cementoblasts\cite{70,71}. The expression of BMP-2, localized only in alveolar bone, was also involved in cementogenic differentiation by increasing the expression of cementum attachment protein (CAP)\cite{72}. Since CTGF, BMP-7, and BMP-2 are beneficial for periodontal ligament formation, Cho et al\cite{73} compared the effect of these three growth factors by incorporating them into 3D printed PLGA microspheres, and the results indicated that BMP-7 triggered thicker cementum-like layers, better integration with the dentin surface and higher expression of cementum protein 1\cite{73}. In addition to supplying growth factors to promote tissue regeneration, inhibition of inflammatory reactions can also improve tissue formation. For instance, Liu et al\cite{74} demonstrated that reductions in tumor necrosis factor-alpha and interferon-gamma levels by the introduction of BMSCs enhanced bone regeneration. Cao et al\cite{75} demonstrated that aspirin promoted BMSC-based calvarial bone regeneration. Thus, platelet-rich fibrin-containing PDLSCs were treated with aspirin, a non-steroidal anti-inflammatory drug, which increased periodontal bone formation\cite{76}.

Instead of providing a direct supply of factors that are required for tissue regeneration, human umbilical vein endothelial cells (HUVECs) were cocultured with PDLSCs to form 3D cell sheet constructs, which were wrapped around human tooth
roots for implantation into the subcutaneous layer of mice. The HUVEC and PDLSC coculture group exhibited the thickest PDL ligament-like arrangement compared to the PDLSC-only group, suggesting that HUVECs contributed to the remodeling of the periodontal compartment[77]. Another strategy for improving the supply of vasculature to bone regeneration is the introduction of genetically modified PDLSCs. A lentiviral construct containing platelet-derived growth factor BB (PDGF-BB), an angiogenic gene, was introduced into PDLSCs to overexpress PDGF-BB. A PLGA-PEG-PLGA thermostable hydrogel seeded with PDLSCs overexpressing PDGF-BB promoted bone formation in alveolar bone defects[78]. To investigate the possibility of incorporating somatic MSCs in tissue regeneration, a mixture of PDLSCs, somatic MSCs, and DPSCs was cocultured within 3D collagen/chitosan scaffolds for odontogenic differentiation[79]. The results indicated that many growth factors, transcription factors, and signaling molecules involved in odontogenic differentiation were significantly promoted in the group mixed with somatic MSCs. In addition to the application of periodontal tissue regeneration, 3D PDLSC-loaded constructs were applied to study the effect of the growth microenvironment on PDLSC differentiation. PDLSCs were seeded in a customized 3D cell-laden hydrogel array with a gradient of gelatin methacrylate (GelMA) and poly(ethylene glycol) (PEG) dimethacrylate compositions to study the response of PDLSCs to ECM[80]. The higher the ratio of PEG was, the better the performance of the PDLSCs in cell proliferation and cell spreading, indicating that the composition of the ECM influenced the behavior of the PDLSCs.

**SCAP**

SCAP is only present at the tip of the developing tooth root before the tooth erupts. Although SCAP shares some similar characteristics with DPSCs, there are still some differences between these two types of stem cells[8]. In contrast to DPSCs, which are the sources of replacement odontoblasts, SCAP is the primary source of odontoblasts involved in the formation of root dentin[81]. Comparing their *in vitro* osteo/odontogenic differentiation potential with DPSCs, SCAP presents stem cell markers (STRO-1, CD146, and CD34) similar to those of DPSCs but with a significantly higher proliferation rate and mineralization potential during dental formation[82]. Other MSC markers, CD73, CD90, and CD105, were also identified in SCAP[40]. Liu et al[83] found that CD24 was exclusively expressed in SCAP, not in DPSCs. SCAP are comparatively easy to isolate from the tips of developing roots. They are digested with a cocktail of collagenase to isolate single-cell suspensions, which are grown under routine cell culture conditions[84].

**Application in 3D tissue regeneration**

In addition to using residual dental pulp in dentin regeneration, SCAP with osteogenic potential obtained from dental roots have been applied for dentin regeneration[85]. Injectable PLLA nanofibrous microspheres (NF-MS) with the ability to controllably release BMP-2 were encapsulated in SCAP for dentin regeneration[86]. More mineralization and osteodontin formation were observed in NF-MS with controlled BMP-2 release microspheres, suggesting their potential for dental tissue repair. In addition to BMP-2 release, SCAP cotreated with stromal cell-derived factor-1α, which is able to promote odontoblast differentiation of dental pulp cells, were shown to undergo odontogenic differentiation-related gene and protein expression[87]. PDGF-BB is known to promote angiogenesis during tissue regeneration[88,89]. The addition of PDGF-BB promoted the proliferation of SCAP and improved new bone formation and mineralization in a rat calvaria defect model[90].

The growth factor TGBβ3 was shown to be involved in tissue regeneration[91]. Somoza et al[92] observed that TGBβ3 secretion by SCAP was elevated when they were grown in a 3D microenvironment regardless of the materials used for the scaffold. Thus, SCAP were applied and incorporated into a 3D scaffold for tissue regeneration. Considering the secretion properties of SCAP, Na et al[93] developed a 3D scaffold-free stem-cell sheet-derived pellet (CSDP) by culturing a large amount of SCAP on a culture dish to form a cell sheet that enriched the secreted ECM. CSDP exhibited the odontogenic/osteogenic potential to form dental pulp-like and dentine-like tissue after implantation into the subcutaneous layer in immunodeficient mice. Dental ECM was reported to enhance cell proliferation and mineralization[94]. A novel SCAP-loaded bioink was developed by applying dental ECM to printable alginate to form dentin-derived bioink, in which soluble dentin molecules significantly enhanced odontogenic
Dental stem cells in three-dimensional regeneration

DFPCS

The dental follicle is the connective tissue surrounding the enamel organ and dental papilla that forms a vascular fibrous sac. In 2005, Morsczeck et al. isolated DFPCs from the dental follicle of human third molar teeth, which were found to express the stem cell markers Notch and Nestin. Their potential for osteogenic, adipogenic, chondrogenic, and neural differentiation was further confirmed. Subsequently, DFPCs were applied for tissue regeneration, such as the regeneration of the salivary glands, dental roots, and bone tissue.

Application in 3D tissue regeneration

Among the applications of dental stem cells in tissue regeneration, only a few studies have introduced DFPCs to 3D tissue regeneration. DFPCs cultured in a 3D rotatory culture system displayed many follicle markers, such as CD44, CD90, CD146, CD31, CD34, and CD45Ag. Furthermore, their differentiation potential was increased when DFPCs were cultured in a 3D dynamic culture system. For the generation of 3D tissue constructs, DFPCs were seeded in 3D porous scaffolds of collagen-nanohydroxyapatite/phosphoserine (collagen-nano-HA/OPS) biocomposite cryogels and implanted into the subcutaneous layer of nu mice. These 3D DFPC-loaded collagen-nano-HA/OPS constructs exhibited greater osteogenic differentiation with higher levels of osteopontin secretion.

Clinical applications of dental stem cells

The use of dental stem cells for autologous or allogeneic transplantation has been introduced into clinical practice. The biological safety of dental stem cells requires strict regulation. Standard examinations for viruses, pathogenic microorganisms, or any sources with animal origins are necessary. Due to the immune response, a same-species origin of the stem cell culture system is recommended for cell therapy. According to the Clinical Gov website, there are fewer than 10 cases of the use of dental stem cells in clinical applications, implying a gap in the application of dental stem cells between basic research and clinical practice. There is a scarcity of data for the use of decellularized biological membranes for preparing 3D dental regenerative constructs, which is a crucial approach for regenerative dentistry. Indeed, dental stem cells are not the most suitable stem cell choice for tissue regeneration due to harvest contamination, small cell amounts available per patient and invasive harvesting approaches. However, the regenerative potential of dental stem cells is still supported by several clinical results. A clinical study reported that most clinical trials based on the use of DPSCs cells were performed for bone regeneration, periodontitis, and dental pulp regeneration, whereas trials involving the use of periodontal PDLSCs were conducted to study periodontal disease treatment. No clinical trials that used DFPCs were found. Overall, dental stem cells are not commonly used to treat human diseases. Identical to the original issues hindering stem cell therapy, ethical concerns and cell sources are the main obstacles. Moreover, the survival of grafted dental stem cells exhibited different results after long-term follow-up observations. Autologous PDLSCs were detected after 8 wk in an ovine periodontal defect model, whereas donor PDLSCs implanted into recipient mice were untraceable two weeks after implantation. Whether autologous or allogeneic stem cell sources affect the survival rate of transplanted cells remains to be further investigated.

Conclusion

Dental-derived stem cells with mesenchymal stem cell properties are promising cell sources for tissue regeneration. Comparisons among these five types of dental-derived stem cells showed that DPSCs, SHEDs, and PDLSCs present a higher growth potential than BMSCs. Moreover, SCAP and DPSCs showed weaker adipogenic differentiation than BMSCs. Regardless of whether the different types of dental stem cells have osteogenic or odontogenic potential, each cell type presents unique differentiation potentials in the corresponding tissue type. Although dental stem cells present
demonstrated potential for adipogenesis, chondrogenesis, and neurogenesis, most of their clinical utility lies in the field of regenerative dentistry. With the trend of 3D tissue engineering, the application of dental stem cells to 3D tissue reconstruction has been emphasized. In this review, many basic research and preclinical studies were presented to support the idea that dental stem cells can be applied in a feasible approach to translational medicine and are available resources for 3D tissue regeneration.

Acknowledgements

The authors thank Miss Kuo I and the Center for Big Data Analytics and Statistics at Chang Gung Memorial Hospital for creating the illustrations used herein.

References

1. Bojic S, Volarevic V, Ljubic B, Stojkovic M. Dental stem cells–characteristics and potential. Histol Histopathol 2014; 29: 699-706 [PMID: 24446280 DOI: 10.14670/HH-29.699]

2. Ercal F, Pekozuer GG, Kose GT. Dental Stem Cells in Bone Tissue Engineering: Current Overview and Challenges. Adv Exp Med Biol 2018; 1107: 113-127 [PMID: 29498025 DOI: 10.1007/5994_2018_171]

3. Yao L, Flynn N. Dental pulp stem cell-derived chondrogenic cells demonstrate differential cell motility in type I and type II collagen hydrogels. Spine J 2018; 18: 1070-1080 [PMID: 29452287 DOI: 10.1016/j.spinee.2018.02.007]

4. Knight E, Przyborski S. Advances in 3D cell culture technologies enabling tissue-like structures to be created in vitro. J Anat 2015; 227: 746-756 [PMID: 25411113 DOI: 10.1111/joa.12257]

5. Fantini D. Easypubmed: Search and retrieve scientific publication records from pubmed. R package version 2.13. 2019. [cited 3 April 2021]. In: CRAN.R-project [Internet]. Available from: https://CRAN.R-project.org/package=easyPubMed

6. Rani J, Shah AB, Ramachandran S. pubmedminer: an R package with text-mining algorithms to analyse PubMed abstracts. J Biocomput 2015; 40: 671-682 [PMID: 26564970 DOI: 10.1007/s12038-015-9552-2]

7. Team RC. R: A language and environment for statistical computing. R foundation for statistical computing, vienna, austria. 2020. [cited 3 April 2021]. In: CRAN.R-project [Internet]. Available from: https://www.R-project.org/

8. Gronthos S, Mankani M, Brahim 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]

9. Mortada I, Mortada R. Dental pulp stem cells and osteogenesis: an update. Cytotechnology 2018; 70: 1479-1486 [PMID: 29938380 DOI: 10.1007/s10616-018-0225-5]

10. Shi S, Robey PG, Gronthos S. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone 2001; 29: 532-539 [PMID: 11728923 DOI: 10.1016/s8756-3282(01)00612-3]

11. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, Jin Y. Odontogenic capability: bone marrow stromal stem cells vs dental pulp stem cells. Biol Cell 2007; 99: 465-474 [PMID: 17371295 DOI: 10.1042/BC20070013]

12. Kumar A, Kumar V, Rattan V, Jha V, Bhattacharyya S. Secretome Cues Modulate the Neurogenic Potential of Bone Marrow and Dental Stem Cells. Mol Neurobiol 2017; 54: 4672-4682 [PMID: 27422132 DOI: 10.1007/s12035-016-0011-3]

13. Mohanram Y, Zhang J, Tsiridis E, Yang XB. Comparing bone tissue engineering efficacy of HDPSCs, HBMSCs on 3D biomimetic ABM-15 scaffolds in vitro and in vivo. Cytotechnology 2020; 72: 715-730 [PMID: 32820463 DOI: 10.1007/s10616-020-00414-7]

14. Kim SH, Kim YS, Lee SY, Kim KH, Lee YM, Kim WK, Lee YK. Gene expression profile in mesenchymal stem cells derived from dental tissues and bone marrow. J Periodontal Implant Sci 2011; 41: 192-200 [PMID: 21934428 DOI: 10.5051/jpis.2011.41.4.192]

15. Hoo J, Kim DS, Jang H, Kim HR, Kang HW. Bioprinting of three-dimensional-dentin-pulp complex with local differentiation of human dental pulp stem cells. J Tissue Eng 2019; 10: 2041731419845849 [PMID: 31205671 DOI: 10.1177/2041731419845849]

16. Kuang R, Zhang Z, Jin X, Hu J, Shi S, Ni L, Ma PX. Nanofibrous spongy microspheres for the delivery of hypoxia-primed human dental pulp stem cells to regenerate vascularized dental pulp. Acta Biomater 2016; 33: 225-234 [PMID: 26826529 DOI: 10.1016/j.actbio.2016.01.032]

17. Ishoh Y, Sasaki JI, Hashimoto M, Katata C, Hayashi M, Imazato S. Pulp Regeneration by 3-dimensional Dental Pulp Stem Cell Constructs. J Dent Res 2018; 97: 1137-1143 [PMID: 29702010 DOI: 10.1177/0022034518772260]

18. Soares DG, Zhang Z, Mohamed F, Eyster TW, de Souza Costa CA, Ma PX. Simvastatin and nanofibrous poly(l-lactic acid) scaffolds to promote the odontogenic potential of dental pulp cells in
Hsiao HY et al. Dental stem cells in three-dimensional regeneration

an inflammatory environment. Acta Biomater 2018; 68: 190-203 [PMID: 29294374 DOI: 10.1016/j.actbio.2017.12.037]

Cooke ME, Ramirez-Garcia Lula JR, Rangel-Berridi K, Park H, Nazhat SN, Weber MH, Henderson JE, Rosenzweig DH. 3D Printed Polyurethane Scaffolds for the Repair of Bone Defects. Front Bioeng Biotechnol 2020; 8: 557215 [PMID: 33195122 DOI: 10.3389/fbioe.2020.557215]

Rubert Pérez CM, Stephanopoulos N, Sur S, Lee SS, Newcomb C, Stupp SI. The powerful functions of peptide-based biomatrix tiles for regenerative medicine. Ann Biomed Eng 2015; 43: 501-514 [PMID: 25366903 DOI: 10.1016/j.sij.2014.04.1166-5]

Akkouch A, Zhang Z, Rouabhia M. Engineering bone tissue stimulated using human dental pulp stem cells and an osteogenic collagen-hydroxyapatite-poly (lactide-co-e-caprolactone) scaffold. J Biomater Appl 2014; 28: 922-936 [PMID: 23640860 DOI: 10.1177/0885328213486705]

Xia K, Chen Z, Chen J, Xu H, Xu Y, Yang T, Zhang Q, RGD- and VEGF-Mimetic Peptide Epitope-Functionalized Self-Assembling Peptide Hydrogels Promote Dentin-Pulp Complex Regeneration. Int J Nanomedicine 2020; 15: 6631-6647 [PMID: 32982223 DOI: 10.2147/IJIN.S253576]

Suzuki T, Lee CH, Chen M, Zhao W, Fu SY, Qi JJ, Chotkowski G, Eisig SB, Wong A, Mao JJ. Induced migration of dental pulp stem cells for in vivo pulp regeneration. J Dent Res 2011; 90: 1013-1018 [PMID: 21586666 DOI: 10.1016/j.sij.2014.05.014]

Nam S, Won JE, Kim CH, Kim HW. Odontogenic differentiation of human dental pulp stem cells stimulated by the calcium phosphate porous granules. J Tissue Eng 2011; 2011: 812547 [PMID: 21772958 DOI: 10.4061/2011/812547]

Galler KM, Hartgerink JD, Cavender AC, Schmalz G, D'Souza RN. A customized self-assembling peptide hydrogel for dental pulp tissue engineering. Tissue Eng Part A 2012; 18: 176-184 [PMID: 21827280 DOI: 10.1089/ten.TEA.2011.0222]

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: 10.1089/biom.2013.0991]

Li J, Chen M, Wei X, Hao Y, Wang J. Evaluation of 3D-Printed Polycaprolactone Scaffolds Coated with Freeze-Dried Platelet-Rich Plasma for Bone Regeneration. Materials (Basel) 2017; 10 [PMID: 28773189 DOI: 10.3390/ma10070831]

Liu YJ, Su WT, Chen PH. Magnesium and zinc borate enhance osteoblastic differentiation of stem cells from human exfoliated deciduous teeth in vitro. J Biomater Appl 2018; 32: 765-774 [PMID: 29119879 DOI: 10.1177/0885328217740730]

Wiesmann HP, Tkotz T, Joos U, Zierold K, Stratmann U, Szuwart T, Plate U, Höhling HJ. Magnesium in newly formed dentin mineral of rat incisor. J Bone Miner Res 1997; 12: 380-383 [PMID: 9076580 DOI: 10.1359/jbmr.1997.12.3.380]

Qu T, Jing J, Jiang Y, Taylor RJ, Feng QJ, Geiger B, Liu X. Magnesium-containing nanostructured hybrid scaffolds for enhanced dental tissue regeneration. Tissue Eng Part A 2014; 20: 2422-2433 [PMID: 24593189 DOI: 10.1089/ten.TEA.2013.0741]

Tognarini I, Soraice S, Zonefrati R, Galli G, Gozzini A, Carbonell Sala S, Thyron GD, Carossino AM, Tanini A, Mavilia C, Azzari C, Saba F, Facchinetti A, Capanna R, Brandi ML. In vitro differentiation of human mesenchymal stem cells on Ti6Al4V surfaces. Biomaterials 2008; 29: 809-824 [PMID: 18022639 DOI: 10.1016/j.biomaterials.2007.04.043]

Galli D, Benedetti L, Bongio M, Mialiardi V, Silvani G, Ceccarelli G, Ronzoni F, Conte S, Benazzo F, Graziano A, Papaccio G, Sampaolo M, De Angelis MG. In vitro osteoblastic differentiation of human mesenchymal stem cells and human dental pulp stem cells on poly-L-lysine-treated titanium-6-aluminium-4-vanadium. J Biomed Mater Res A 2011; 97: 118-126 [PMID: 21370441 DOI: 10.1002/jbmr.32996]

Mittelbrunn M, Sánchez-Madrid F. Intercellular communication: diverse structures for exchange of genetic information. Nat Rev Mol Cell Biol 2012; 13: 328-335 [PMID: 22510790 DOI: 10.1038/nrm3335]

Mantz BN, Groves JT. Spatial organization and signal transduction at intercellular junctions. Nat Rev Mol Cell Biol 2010; 11: 342-352 [PMID: 20354536 DOI: 10.1038/nrm2883]

Xiao L, Tsutsui T. Three-dimensional epithelial and mesenchymal cell co-cultures form early tooth epithelium invagination-like structures: expression patterns of relevant molecules. J Cell Biochem 2012; 113: 1875-1885 [PMID: 22234822 DOI: 10.1002/jcb.24056]

Jin GZ, Kim HW. Co-culture of Human Dental Pulp Stem Cells and Endothelial Cells Using Porous Biopolymer Microcarriers: A Feasibility Study for Bone Tissue Engineering. Tissue Eng Regen Med 2017; 14: 393-401 [PMID: 30603495 DOI: 10.1089/ten.sr.2017.017.0061-2]

Mironov V, Boland T, Trusk T, Forcaga G, Markwald RR. Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol 2003; 21: 157-161 [PMID: 12679063 DOI: 10.1016/S0167-7799(03)0033-7]

Yu H, Zhang X, Song W, Pan T, Wang H, Ning T, Wei Q, Xu HHK, Wu B, Ma D. Effects of 3-dimensional Bioprinting Alginate/Gelatin Hydrogel Scaffold Extract on Proliferation and Differentiation of Human Dental Pulp Stem Cells. J Endod 2019; 45: 706-715 [PMID: 31056297 DOI: 10.1016/j.joen.2019.03.004]

Park JY, Shim JH, Choi SA, Jang J, Kim M, Lee SH, Cho DW. 3D printing technology to control BMP-2 and VEGF delivery spatially and temporally to promote large-volume bone regeneration. J Mater Chem B 2015; 3: 5415-5425 [PMID: 23226513 DOI: 10.1039/c5tb006377]

Hilkens P, Bronckaers A, Ratajczak J, Gervois P, Wolfs E, Lambrecht I. The Angiogenic Potential
of DPSCs and SCAPs in an In Vivo Model of Dental Pulp Regeneration. Stem Cells Int 2017; 2017: 2582080 [PMID: 29018483 DOI: 10.1155/2017/2582080]

41 Dubey N, Ferreira JA, Malda J, Bhaduri SB, Bottino MC. Extracellular Matrix/Ammorphous Magnesium Phosphate Bionik for 3D Bioprinting of CranioMaxillofacial Bone Tissue. ACS Appl Mater Interfaces 2020; 12: 23752-23763 [PMID: 32325748 DOI: 10.1021/acsami.0c03511]

42 Hsiao D, Hsu SH, Chen RS, Chen MH. Characterization of designed directional polyacrylic acid 3D scaffolds for neural differentiation of human dental pulp stem cells. J Formos Med Assoc 2020; 119: 268-275 [PMID: 31155229 DOI: 10.1016/j.jfma.2019.05.011]

43 Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Eng 2006; 12: 2813-2823 [PMID: 17518650 DOI: 10.1089/ten.2006.12.2813]

44 Longoni A, Utomo L, van Hooijdonk IE, Bittermann GK, Vetter VC, Kruit Spanjer EC, Ross J, Rosenberg AJ, Gawlitta D. The chondrogenic differentiation potential of dental pulp stem cells. Eur Cell Mater 2020; 39: 121-135 [PMID: 32083715 DOI: 10.22203/ecm.039a08]

45 Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells 2008; 26: 1787-1795 [PMID: 18499892 DOI: 10.1634/stemcells.2007-0079]

46 Askari N, Yaghooobi MM, Shamsara M, Esmaili-Mahani S. Human Dental Pulp Stem Cells Differentiated into Oligodendrocyte Progenitors Using the Expression of Olig2 Transcription Factor. Cells Tissues Organs 2014; 200: 93-103 [PMID: 25966902 DOI: 10.1159/000381668]

47 Ghasemi Hamidabadi B, Rezvani Z, Nazm Bojnordi M, Shirinzadeh H, Seifalian AM, Joghataei MT, Razaghpour M, Aibakhashi A, Yazdanpanah A, Salimi M, Mozafari M, Urbanaska AM, Reis RL, Kanduc D, Gholipourmalekabadi M. Chitosan-Intercalated Montmorillonite/Poly(vinyl alcohol) Nanofibers as a Platform to Guide Neuronal Differentiation of Dental Pulp Stem Cells. ACS Appl Mater Interfaces 2017; 9: 11392-11404 [PMID: 28117963 DOI: 10.1021/acsami.6b14253]

48 Luo L, Alhashari AA, Wang X, Jin L, Zhang Y, Zheng L, Xia J, Xu H, Zhao Y, Xiao J, He Y, Ye Q. Effects of Transplanted Heparin-Poloxamer Hydrogel Combining Dental Pulp Stem Cells and bFGF on Spinal Cord Injury Repair. Stem Cells Int 2018; 2018: 239852 [PMID: 29765407 DOI: 10.1155/2018/239852]

49 Zheng K, Feng G, Zhang J, Xing J, Huo D, Lian M, Zhang W, Wu W, Hu Y, Lu X, Feng X. Basal fibroblast growth factor promotes human dental pulp stem cells cultured in 3D porous chitosan scaffolds for neural differentiation. Int J Neurosci 2021; 131: 625-633 [PMID: 32186218 DOI: 10.1080/00207454.2020.1744592]

50 Guo S, Redenski I, Landau S, Szklanny A, Merdler U, Levenberg S. Prevascularized Scaffolds Bearing Human Dental Pulp Stem Cells for Treating Complete Spinal Cord Injury. Adv Healthc Mater 2020; 9: e2000974 [PMID: 32902147 DOI: 10.1002/adhm.202000974]

51 Luzuriaga J, Izurzun J, Irautzorria I, Unda F, Ibarretxe G, Pineda JR. Vasculogenesis from Human Dental Pulp Stem Cells Grown in Matrigel with Fully Defined Serum-Free Culture Media. Biomedicines 2020; 8 [PMID: 33182239 DOI: 10.3390/biomedicines8110188]

52 Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Sakamoto K, Tauchi R, Wako K, Nigama S, Hibi H, Kodamatsu K, Ishiguro N, Ueda M. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest 2012; 122: 80-90 [PMID: 22133879 DOI: 10.1172/JCI59251]

53 Miura M, Grontos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED cells: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003; 100: 5807-5812 [PMID: 12716973 DOI: 10.1073/pnas.0309736100]

54 Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, Caplan AI, Cerruti HF. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 2006; 184: 105-116 [PMID: 17409736 DOI: 10.1159/000099617]

55 Koyama N, Okubo Y, Nakao K, Bessho K. Evaluation of pluripotency in human dental pulp cells. J Oral Maxillofac Surg 2009; 67: 501-506 [PMID: 19231772 DOI: 10.1016/j.joms.2008.09.011]

56 Laino G, Graziano A, d'Aquino R, Pirozzi G, Lanza V, Valiante S, De Rosa A, Naro F, Vivarelli E, Antonov EN, Popova AV, Konstantinova EV, Karalkin PA, Kholodenko IV. Differentiate into Oligodendrocyte Progenitors Using the Expression of Olig2 Transcription Factor. Cells Tissues Organs 2013; 212: 1023-1031 [PMID: 20240019 DOI: 10.1159/000342126]

57 Vakhrušev IV, Antonen EO, Popova AV, Konstantinova EV, Karalkin PA, Khloodenko IV, Lupatov AY, Popov VK, Bagnatashvili VN, Yarygin KN. Design of tissue engineering implants for bone tissue regeneration of the basis of new generation poly lactoglycolide scaffolds and multipotent mesenchymal stem cells from human exfoliated deciduous teeth (SHED cells). Bull Exp Biol Med 2012; 153: 143-147 [PMID: 22808514 DOI: 10.1007/s10517-012-1663-2]

58 Feng X, Xiong J, Feng G, Sang A, Shen B, Xu Y, Jiang J, Liu S, Tan W, Gu Z, Li L. Age-dependent impaired neurogenic differentiation capacity of dental stem cell is associated with Wnt/β-catenin signaling. Cell Mol Neurobiol 2013; 33: 1023-1031 [PMID: 24043508 DOI: 10.1007/s10517-013-9965-0]

59 Sugimura-Wakayama Y, Katagiri W, Osugi M, Kawai T, Ogata K, Sakaguchi K, Hibi H. Peripheral Nerve Regeneration by Secretomes of Stem Cells from Human Exfoliated Deciduous Teeth. Stem Cells Dev 2015; 24: 2687-2699 [PMID: 26154068 DOI: 10.1089/scd.2015.0104]
Hsiao HY et al. Dental stem cells in three-dimensional regeneration

60 Yamagata M, Yamamoto A, Kako E, Kaneko N, Matsubara K, Sakai K, Sawamoto K, Ueda M. Human dental pulp-derived stem cells protect against hypoxic-ischemic brain injury in neonatal mice. Stroke 2013; 44: 551-554 [PMID: 23238858 DOI: 10.1161/STROKEAHA.112.676759]

61 Zhu W, Liang M. Periodontal ligament stem cells: current status, concerns, and future prospects. Stem Cells Int 2015; 2015: 972313 [PMID: 25861283 DOI: 10.1155/2015/972313]

62 Iwasaki K, Komaki M, Yokoyama N, Tanaka Y, Taki A, Kimura Y, Takeda M, Oda S, Izumi Y, Morita I. Periodontal ligament stem cells possess the characteristics of pericytes. J Periodontol 2013; 84: 1425-1433 [PMID: 23240762 DOI: 10.1002/j.2012.1205417]

63 See BM, Miura M, Grontos S, Bartold PM, Batouli S, Brahim 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)16227-0]

64 Liu W, Konermann A, Guo T, Jäger A, Zhang L, Jin Y. Canonical Wnt signaling differently regulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions. Biochim Biophys Acta 2014; 1840: 1125-1134 [PMID: 24231680 DOI: 10.1016/j.bbagen.2013.11.003]

65 Deng C, Sun Y, Liu H, Wang W, Wang J, Zhang F. Selective adipogenic differentiation of human periodontal ligament stem cells stimulated with high doses of glucose. PLoS One 2018; 13: e0199603 [PMID: 29979705 DOI: 10.1371/journal.pone.0199603]

66 Kim YT, Park JC, Choi SH, Cho KS, Im GI, Kim BS, Kim CS. The dynamic healing profile of human periodontal ligament stem cells: histological and immunohistochemical analysis using an ectopic transplantation model. J Periodontal Res 2012; 47: 514-524 [PMID: 22308970 DOI: 10.1111/j.1600-0765.2011.01463.x]

67 Horiiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF, Kudo A. Identification and characterization of a novel protein, peristin, with restricted expression to perioseum and periodontal ligament and increased expression by transforming growth factor beta. J Bone Miner Res 1999; 14: 1239-1249 [PMID: 10404027 DOI: 10.1359/jbmr.1999.14.7.1239]

68 Ansari S, Diniz IM, Chen C, Sarrión P, Tamayol A, Wu BM, Mosheravinia A. Human Periodontal Ligament- and Gingiva-derived Mesenchymal Stem Cells Promote Nerve Regeneration When Encapsulated in Alginate/Hyaluronic Acid 3D Scaffold. Adv Healthc Mater 2017; 6 [PMID: 29076281 DOI: 10.1002/adhm.201700670]

69 Duan X, Ji M, Deng F, Sun Z, Lin L. Effects of connective tissue growth factor on human periodontal ligament fibroblasts. Arch Oral Biol 2017; 84: 37-44 [PMID: 28941713 DOI: 10.1016/j.archoralbio.2017.09.010]

70 Thomadakis G, Ramoshebi LN, Crooks J, Rueger DC, Ripamonti U. Immunolocalization of Bone Morphogenetic Protein-2 and -3 and Osteogenic Protein-1 during murine tooth root morphogenesis and in other craniofacial structures. Connect Tissue Res 2002; 43: 257-264 [PMID: 12489168 DOI: 10.1080/03008207.2002.9900127]

71 Cho H, Tarafder S, Fogge M, Kao K, Lee CH. Periodontal ligament stem/progenitor cells with protein-releasing scaffolds for cementum formation and integration on dentin surface. Cell Tissue Res 2016; 57: 488-495 [PMID: 27215800 DOI: 10.1007/s00468-016-19147-8]

72 Liu Y, Yang R, Shi S. Systemic infusion of mesenchymal stem cells improves cell-based bone regeneration via upregulation of regulatory T cells. Tissue Eng Part A 2015; 21: 498-509 [PMID: 25159486 DOI: 10.1089/ten.TEA.2013.0673]

73 Cao Y, Xiong J, Mei S, Wang F, Zhao Z, Wang S, Liu Y. Aspirin promotes bone marrow mesenchymal stem cell-based calvarial bone regeneration in mini swine. Stem Cell Res Ther 2015; 6: 210 [PMID: 26519141 DOI: 10.1186/s13287-015-0200-4]

74 Du J, Mei S, Guo L, Su Y, Wang H, Liu Y, Zhao Z, Wang S. Platelet-rich fibrin/aspirin complex promotes alveolar bone regeneration in periodontal defect in rats. J Periodontal Res 2018; 53: 47-56 [PMID: 28862325 DOI: 10.1111/jre.12485]

75 Kramer JL. Decreasing the organ donor shortage. Am J Forensic Med Pathol 1995; 16: 257; author reply 259-257; author reply 260 [PMID: 7495271 DOI: 10.1111/j.1222-4956.1995.tb00574.x]

76 Pan J, Deng J, Luo Y, Yu L, Zhang W, Tan X, You Z, Liu Y. Thermosensitive Hydrogel Delivery of Human Periodontal Stem Cells Oversexpressing Platelet-Derived Growth Factor-BB Enhances Alveolar Bone Defect Repair. Stem Cells Dev 2019; 28: 1620-1631 [PMID: 31663419 DOI: 10.1089/scd.2019.0184]

77 Ravindran S, Huang CC, George A. Extracellular matrix of dental pulp stem cells: applications in pulp tissue engineering using somatic MSCs. Front Physiol 2014; 4: 395 [PMID: 24432005 DOI: 10.3389/fphys.2013.00395]

78 Ma Y, Ji Y, Huang G, Ling K, Zhang X, Xu F. Bioprinting 3D cell-laden hydrogel microarray for screening human periodontal ligament stem cell response to extracellular matrix. Biofabrication
Advanced 3D Models Cultured to Investigate Mesenchymal Stromal Cells of the Human Dental Pulp of porcine dental follicle progenitor cells.

Tsuchiya S
[PMID: 21227403] DOI: 10.1016/j.archoralbio.2012.10.008

Liu C, Xiong H, Chen K, Huang Y, Yin X. Long-term exposure to pro-inflammatory cytokines inhibits the osteogenic/dentinogenic differentiation of stem cells from the apical papilla. Int Endod J 2016; 49: 950-959 [PMID: 26383494 DOI: 10.1111/iej.12551]

Nada OA, El Backly RM. Stem Cells From the Apical Papilla (SCAP) as a Tool for Endogenous Tissue Regeneration. Front Bioeng Biotechnol 2018; 6: 103 [PMID: 30087893 DOI: 10.3389/fbioe.2018.00103]

Kitamura C, Nishihara T, Terashita M, Tabata Y, Washio A. Local regeneration of dentin-pulp complex using controlled release of fgf-2 and naturally derived sponge-like scaffolds. Int J Dent 2012; 10: 190561 [PMID: 22174717 DOI: 10.1155/2012/190561]

Wang W, Dang M, Zhang Z, Hu J, Eyster TW, Ni L, Ma PX. Dentin regeneration by stem cells of apical papilla on injectable nanofibrous microspheres and stimulated by controlled BMP-2 release. Acta Biomater 2016; 36: 63-72 [PMID: 26971664 DOI: 10.1016/j.actbio.2016.03.015]

Xiao M, Qiu J, Kuang R, Zhang B, Wang W, Yu Q. Synergetic effects of stromal cell-derived factor-1α and bone morphogenetic protein-2 treatment on odontogenic differentiation of human stem cells from apical papilla cultured in the VitroGel 3D system. Cell Tissue Res 2019; 378: 207-220 [PMID: 31152245 DOI: 10.1007/s00441-019-03045-3]

Battagyi EJ, Rupp J, Iuelas-Arispe L, Sage EH, Pech M. PDGF-BB modulates endothelial proliferation and angiogenesis in vitro via PDGF beta-receptors. J Cell Biol 1994; 125: 917-928 [PMID: 7514607 DOI: 10.1083/jcb.125.4.917]

Lange S, Heger J, Euler G, Wartenberg M, Piper HM, Sauer H. Platelet-derived growth factor BB stimulates vasculoogenesis of embryonic stem cell-derived endothelial cells by calcium-mediated generation of reactive oxygen species. Cardiovasc Res 2009; 81: 159-168 [PMID: 18806276 DOI: 10.1093/ctvr/cv258]

Deng J, Pan J, Han X, Yu L, Chen J, Zhang W, Zhu L, Huang W, Liu S, You Z, Liu Y. PDGFBB-modified stem cells from apical papilla and thermosensitive hydrogel scaffolds induced bone regeneration. Chem Biol Interact 2020; 316: 108931 [PMID: 31874163 DOI: 10.1016/j.chembiol.2019.108931]

Penn JW, Grobbelaar AO, Rolfe KJ. The role of the TGF-β family in wound healing, burns and scarring: a review. Int J Burns Trauma 2012; 2: 18-28 [PMID: 22928164]

Somoza RA, Acevedo CA, Albornoz F, Luz-Crawford P, Carrión F, Young ME, Weinstein-Oppenheimer C. TGFβ3 secretion by three-dimensional cultures of human dental pulp apical papilla mesenchymal stem cells. J Tissue Eng Regen Med 2017; 11: 1045-1056 [PMID: 25690385 DOI: 10.1002/term.2906]

Na S, Zhang H, Huang F, Wang W, Ding Y, Li D, Jin Y. Regeneration of dental pulp/dentine complex with a three-dimensional and scaffold-free stem-cell sheet-derived pellet. J Tissue Eng Regen Med 2016; 10: 261-270 [PMID: 23365018 DOI: 10.1002/term.1686]

Salehi S, Cooper P, Smith A, Ferracane J. Dentin matrix components extracted with phosphoric acid enhance cell proliferation and mineralization. Dent Mater 2016; 32: 334-342 [PMID: 26777093 DOI: 10.1016/j.dental.2015.11.004]

Athirasala A, Tahayeri A, Thrivikraman G, França CM, Monteiro N, Tran V, Ferracane J, Bertassoni L.E. A dentin-derived hydrogel bioink for 3D bioprinting of cell laden scaffolds for regenerative dentistry. Biofabrication 2018; 10: 024101 [PMID: 29320722 DOI: 10.1088/1758-5080/aa9b4e]

Morszeck 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: 155-163 [PMID: 15900265 DOI: 10.1016/j.mabios.2004.12.004]

Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Xu GT, Liang A, Liu S. Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells 2015; 33: 627-638 [PMID: 25447379 DOI: 10.1002/stem.1909]

Xu QL, Furushashi A, Zhang QZ, Jiang CM, Chang TH, Le AD. Induction of Salivary Gland-Like Cells from Dental Follicle Epithelial Cells. J Dent Res 2017; 96: 1035-1043 [PMID: 28541773 DOI: 10.1177/0022034517711146]

Chen G, Chen J, Yang B, Li L, Luo X, Zhang X, Feng L, Jiang Z, Yu M, Guo W, Tian W. Combination of aligned PLGA/Gelatin electrospun sheets, native dental pulp extracellular matrix and treated dentin matrix as substrates for tooth root regeneration. Biomaterials 2015; 52: 56-70 [PMID: 25818413 DOI: 10.1016/j.biomaterials.2015.02.011]

Tsuchiya S, Ohshima S, Yamakoshi Y, Simmer JP, Honda MJ. Osteogenic differentiation capacity of porcine dental follicle progenitor cells. Connect Tissue Res 2010; 51: 197-207 [PMID: 20053131 DOI: 10.3109/0308020903267542]

Steinberg N, Angiero F, Farronato D, Benzeri A, Cossefu G, Ottonello A, Kaigler D, Mazzoleni G. Advanced 3D Models Cultured to Investigate Mesenchymal Stromal Cells of the Human Dental Pulp
Follicle. *Tissue Eng Part C Methods* 2018; 24: 187-196 [PMID: 29327669 DOI: 10.1089/ten.TEC.2017.0428]

102 Salgado CL, Barrias CC, Monteiro FJM. Clarifying the Tooth-Derived Stem Cells Behavior in a 3D Biomimetic Scaffold for Bone Tissue Engineering Applications. *Front Bioeng Biotechnol* 2020; 8: 724 [PMID: 32671055 DOI: 10.3389/fbioe.2020.00724]

103 Healy L, Hunt C, Young L, Stacey G. The UK Stem Cell Bank: its role as a public research resource centre providing access to well-characterised seed stocks of human stem cell lines. *Adv Drug Deliv Rev* 2005; 57: 1981-1988 [PMID: 16290151 DOI: 10.1016/j.addr.2005.07.019]

104 Washio K, Iwata T, Mizutani M, Ando T, Yamato M, Okano T, Ishikawa I. Assessment of cell sheets derived from human periodontal ligament cells: a pre-clinical study. *Cell Tissue Res* 2010; 341: 397-404 [PMID: 20632035 DOI: 10.1007/s00441-010-1009-1]

105 Gombold J, Karakasidis S, Niksa P, Podczasy J, Neumann K, Richardson J, Sane N, Johnson-Leva R, Randolph V, Sadoff J, Minor P, Schmidt A, Duncan P, Sheets RL. Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products. *Vaccine* 2014; 32: 2916-2926 [PMID: 24681273 DOI: 10.1016/j.vaccine.2014.02.021]

106 Osathanon T. Transplantation of cryopreserved teeth: a systematic review. *Int J Oral Sci* 2010; 2: 59-65 [PMID: 20737931 DOI: 10.4248/IJOS10021]

107 Yamada Y, Nakamura-Yamada S, Konoki R, Baba S. Promising advances in clinical trials of dental tissue-derived cell-based regenerative medicine. *Stem Cell Res Ther* 2020; 11: 175 [PMID: 32398041 DOI: 10.1186/s13287-020-01683-x]

108 Menicanin D, Mrozik KM, Wada N, Marino V, Shi S, Bartold PM, Gronthos S. Periodontal-ligament-derived stem cells exhibit the capacity for long-term survival, self-renewal, and regeneration of multiple tissue types in vivo. *Stem Cells Dev* 2014; 23: 1001-1011 [PMID: 24351050 DOI: 10.1089/scd.2013.0490]

109 Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. *J Tissue Eng Regen Med* 2015; 9: 1205-1216 [PMID: 24850632 DOI: 10.1002/term.1899]
