SYSTEMATIC REVIEW

Dental stem cells in tooth repair: A systematic review [version 1; peer review: 2 approved with reservations]

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Abstract

**Background:** Dental stem cells (DSCs) are self-renewable teeth cells, which help maintain or develop oral tissues. These cells can differentiate into odontoblasts, adipocytes, cementoblast-like cells, osteoblasts, or chondroblasts and form dentin/pulp. This systematic review aimed to summarize the current evidence regarding the role of these cells in dental pulp regeneration.

**Methods:** We searched the following databases: PubMed, Cochrane Library, MEDLINE, SCOPUS, ScienceDirect, and Web of Science using relevant keywords. Case reports and non-English studies were excluded. We included all studies using dental stem cells in tooth repair whether in vivo or in vitro studies.

**Results:** Dental pulp stem cell (DPSCs) is the most common type of cell. Most stem cells are incorporated and implanted into the root canals in different scaffold forms. Some experiments combine growth factors such as TDM, BMP, and G-CSF with stem cells to improve the results. The transplant of DPSCs and stem cells from apical papilla (SCAPs) was found to be associated with pulp-like recovery, efficient revascularization, enhanced chondrogenesis, and direct vascular supply of regenerated tissue.

**Conclusion:** The current evidence suggests that DPSCs, stem cells from human exfoliated deciduous teeth, and SCAPs are capable of providing sufficient pulp regeneration and vascularization. For the development of the dental repair field, it is important to screen for more effective stem cells, dentine releasing therapies, good biomimicry scaffolds, and good histological markers.

**Keywords**
tooth repair, dental stem cell, pulp regeneration, SCAPs, SHEDs

Open Peer Review

Invited Reviewers

<table>
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<tr>
<th>Invited Reviewers</th>
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<tr>
<td>1. Sesha Hanson-Drury, D.D.S, University of Washington, Seattle, USA</td>
</tr>
<tr>
<td>2. Hannele Ruohola-Baker, University of Washington, Seattle, USA</td>
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<td>2. Weidong Tian, Sichuan University, Chengdu, China</td>
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</table>

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Introduction
Regenerative dentistry is designed to recover dental anatomy and function. Regenerative endodontics procedures (REPs) of a damaged tooth are a series of biological processes aimed to restore the dental pulp’s physiological functions, cure periapical lesions, and substitute pulp-dentin complex cells and dentin\(^2\). Three components are involved in these techniques: stem cells, growth and bio-materials, which are often known as scaffolds or templates\(^3\).

The dental pulp consists of nerves, blood vessels and connective tissue to maintain teeth’s integrity. The nerves of the pulp can mediate pain, blood flow control, recruit immunocompetent cells, and act as a mesenchymal stem cell (MSCs) niche\(^4\). Loss of tooth pulp stops the development of permanent root teeth that can weaken the periodontal connection and lead to teeth loss. Recent animal studies indicate that vascular dental pulp can be regenerated by cell-based therapy\(^5\).

Dental stem cells (DSCs) are self-renewable teeth cells, which help maintain or develop oral tissues\(^6\). In the literature, there are various types of dental adult stem cells, such as dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells or stem cells of the developing root apical papilla (SCAPs), dental follicle stem cells (DFSCs), and dental MSCs (DMSCs)\(^7\). Such cells can differentiate into dentine/pulp, odontoblast, adipocyte, cementoblast-like, osteoblast, and chondroblast cells. DSC regenerative potential is explained through both natural and experimental conditions\(^8\)\(^9\). Differentiated dentinoblasts, also called secondary odontoblasts, produce new dentine in response to dental cell injury. This regenerative process is called reparative tertiary dentinogenesis\(^10\). This process of dentinogenesis was suggested to be used in the recruitment of endogenous DSCs. Most recent animal studies have investigated the role of DSCs in the regeneration of dental pulp tissues.

Bakhtiar et al.\(^11\) conducted a systematic review on 47 studies that investigate the role of stem cell therapy in regeneration of dentine-pulp complex; the current systematic review aimed to update this previous systematic review, presenting 57 articles, and summarizes the current evidence regarding the efficacy of dental stem cells in dental pulp regeneration in animal models.

Methods
We report this manuscript following the preferred reporting items systematic reviews and meta-analysis (PRISMA statement) guideline\(^12\). All methods used in this review were conducted in strict accordance with the Cochrane Handbook for Systematic Reviews of Interventions\(^13\).

Literature search strategy
We searched the following databases from January 2000 to June 2019: PubMed, Cochrane Library, MEDLINE, SCOPUS, ScienceDirect, and Web of Science using the following keywords (((Dental pulp stem cells OR DPSCs OR stem cells OR human exfoliated deciduous teeth OR SHEDs OR Periodontal ligament stem cells OR developing root apical papilla OR SCAPs OR dental follicle stem cells OR DFSCs OR dental mesenchymal stem cells OR DMSCs) AND (pulp OR pulpal tissue OR pulp treatment OR pulpal therapy) AND (endodontic treatment OR deciduous teeth OR permanent teeth OR primary teeth OR dentition))) to identify the relevant studies.

Study selection process and eligibility criteria
Two authors screened the titles and abstracts of retrieved literature records. For titles and abstracts that deemed relevant to the research question, the full-text articles of these records were obtained and screened for eligibility according to the following criteria:

We included studies that meet the following PICOS criteria:

1) Population: Both in vitro and in vivo studies that investigate the endodontic regeneration following treatment with dental pulp stem cells.
2) Intervention/Comparator: studies that use all of the following types of stem cell in the regeneration of dental pulp tissue: DPSCs, SHEDs, SCAPs or DMSCs.
3) Outcomes: pulpal regeneration or repair.
4) Study design: All in vivo, in vitro, animal, or human studies.

We excluded all of the following studies: 1) Case reports and case series; and 2) non-English studies. In the case of multiple reports for the same study population, we analyzed data from the most updated dataset. Any discrepancies were resolved by discussion and consensus between reviewers.

Data extraction
Data extraction was performed manually and data were entered into a structured Microsoft Excel sheet (For Windows, Professional Plus version 2016). We extracted data of the following domains: 1) Characteristics of study design; 2) Baseline criteria of the included population; and 3) Study outcomes. There was not sufficient data for meta-analysis.

Results
Search strategy results
The electronic search retrieved 4433 unique articles. After removing duplications, 2780 articles were enrolled in the title/abstract screening. This led to the retrieval and screening of 327 full-text articles for eligibility. Studies that were not eligible with our criteria were excluded. In total 57 articles were included in the qualitative synthesis. A flow diagram of the selection process is shown in Figure 1. A summary of characteristics, models, and populations of the included studies and their key outcomes are shown in Table 1. Variation of the extracted data is reported in Table 2.

Types of stem cells
In this systematic review, we reviewed multiple types of stem cells, such as dental follicle stem cell (DFSC), bone marrow mesenchyme stem cell (BMSC), periodontal ligament stem cell (PDLSC), dental pulp extracellular matrix (DPEM), adipose-derived stem cell (ADSC), DPSC, SCAP, and SHED. The majority
of the studies (n=31; 46%) used DPSCs for regeneration of dentine-pulp complexes. Two out of eight studies, in which stem cells were transplanted into the renal capsule, used DPSCs. Moreover, DPSCs were used by 19 out of 40 studies on subcutaneous transplantation, three out of four studies on intra-canal transplantation, and two studies about allogeneic direct implantation into socket.

SCAP was reported in eight studies, six studies with subcutaneous implantation, and two studies on renal capsule transplantation. There are no experiments utilizing transplanted SCAP into root canal. DFSC is reported in six studies, five subcutaneous implantation studies, and one into-socket transplantation. Across four experiments that were all on subcutaneous transplants used SHEDs. Four experiments used BMSCs; two were transplanted to the renal capsule, one to the subcutaneous, and the other to the root canal. Three trials tried to regenerate periodontal ligament (PDL) tissue using PDLSCs; two subcutaneously transplanted and one transplanted into a socket for extraction.

Dental pulp stem cells

All included experiments utilizing DPSCs were isolated from human healthy pulp tissues, usually orthodontics, to be used in an animal model. Stem cells from exposed pulp have also been reported to be more likely to differentiate into osteoblastic cells than dentinogenic ones. In this review, 20 articles used DPSCs in mice models, three in rat models, four in dogs, and three in pigs. It was observed that DPSC transplantation was associated with regeneration of pulp-like tissue, successful revascularization, enhanced chondrogenesis, and tissue regeneration with direct vascular supply. However, two studies reported that DPSCs formed an inflamed pulp-like tissue.

Stem cells from apical papilla

SCAPs were commonly isolated from immature third molars. Wang et al. reported that SCAPs have greater generation of mineralized tissue than those with DPSCs and higher differentiation of osteo/odontoblast in the supplemental medium kpho4. Furthermore, SCAPs have been reported to have re-vascularizing properties, heterotopic dental pulp/dentin complex formation, faster proliferation and mineralization, and more efficient migration and telomerase than DPSCs.

Periodontal ligament stem cells

PDLSCs demonstrated a significant role in maintenance of MSC characteristics after implantation. In addition, the dentin tissue structure produced by dental follicle cell (DFC) was more complete. In the included experiments, Gao et al. used PDL for regeneration of a fresh bio-root. They developed an effective bio-root of PDL tissue, using a mixture of DPSC-hydroxyapatite wrapped in a layer of PDLSCs. These freshly produced miniature pig roots, both in mineral components and biomechanical characteristics, had comparable characteristics to natural teeth, but only 20% of the samples attained success, whilst titanium implants were 100% effective.

Bone marrow derived mesenchymal stem cells

Murakami et al. showed that BMSCs generated a potential pulp, but with less volume. Zhang et al. proposed applying endogenous BM-MSC to a subcutaneous root canal tooth to a regenerative tissue after the systemic homing in the root canal, driven by the use of the stromal cell-derived factor-1 (SDF-1).
## Table 1. Summary of included studies. This table was adapted from a previous systematic review with written permission from the authors and under a Creative Commons Attribution 4.0 International License.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Cell type</th>
<th>Dose &amp; dosage</th>
<th>Animal</th>
<th>Route of administration</th>
<th>Co-administrative factors</th>
<th>TERM approach</th>
<th>Main results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>DPSC</td>
<td>Not declared</td>
<td>NA</td>
<td>Allogenic transplantation into renal capsule</td>
<td>NA</td>
<td>PLA/HA/TCP &amp; CDHA</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Mice</td>
<td>DPSC</td>
<td>5 x 10⁶ cells/ml</td>
<td>NA</td>
<td>Xenograft subcutaneous tooth slice</td>
<td>NA</td>
<td>HA/TCP Cell sheet/POW</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Mice</td>
<td>Mice</td>
<td>250,000/190 μL</td>
<td>NA</td>
<td>Pre ameloblast CM</td>
<td>NA</td>
<td>HA/TCP Cell sheet/POW</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Mice</td>
<td>Mice</td>
<td>2 x 10⁶ cells/ml</td>
<td>NA</td>
<td>BMP-7</td>
<td>NA</td>
<td>Chitosan/collagen</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Mice</td>
<td>Mice</td>
<td>5 x 10⁶ cells/ml</td>
<td>NA</td>
<td>BMP-7</td>
<td>Fibrin gel CBB</td>
<td>HATCP Cell sheet/POW</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Mice</td>
<td>Mice</td>
<td>1 x 10⁶ cells/ml</td>
<td>NA</td>
<td>BMP-7</td>
<td>HA/TCP Cell sheet/POW</td>
<td>HA/TCP Ceramic powder</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Pig</td>
<td>Pig</td>
<td>3 x 10⁶ cells/ml</td>
<td>NA</td>
<td>BMP-7</td>
<td>NF-PPLA</td>
<td>HA/TCP Powder</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Pig</td>
<td>Pig</td>
<td>2 x 10⁶ Cells</td>
<td>NA</td>
<td>BMP-7</td>
<td>NF-PPLA</td>
<td>PLGA + TCP</td>
<td>Differentiation of human DPSCs</td>
<td>Wang et al. (2013)</td>
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</tbody>
</table>

### Notes:
- **TIME approach**: 2 weeks
- **Route of administration**: 6–10 weeks
- **Main results**: Differentiation of human DPSCs
- **Reference**: This table was adapted from a previous systematic review with written permission from the authors and under a Creative Commons Attribution 4.0 International License.
<table>
<thead>
<tr>
<th>Cell type</th>
<th>Animal model</th>
<th>Dose &amp; dosage</th>
<th>Route of administration</th>
<th>Co-administrative factors</th>
<th>TERM approach</th>
<th>Time point</th>
<th>Main results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPSC</td>
<td>Rabbit</td>
<td>$5 \times 10^6$ cells/ml</td>
<td>Autologous transplantation into the extracted socket</td>
<td>Collagen gel</td>
<td>Collagen gel</td>
<td>12 weeks</td>
<td>Similar tooth structure by different stem cells close to a normal living bone</td>
<td>Hung et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>$8 \times 10^5$ cells/ml</td>
<td>Xenograft intracanal Transplantation</td>
<td>PLLA Nanofibrous spongy microsphere</td>
<td>PLLA Nanofibrous spongy microsphere</td>
<td>4 weeks</td>
<td>Enhanced vasculization</td>
<td>Kung et al. (2016)</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>$1 \times 10^6$ cells/ml</td>
<td>Autologous intercanal Transplantation</td>
<td>BMP-2</td>
<td>BMP-2</td>
<td>12-26 weeks</td>
<td>Normal pulp-like tissue and apical secondary dentin formation</td>
<td>Nakatsuka and Iohara (2014)</td>
</tr>
<tr>
<td></td>
<td>Beagles</td>
<td>$2.0 \times 10^7$ cells</td>
<td>Autologous transplantation into the pulp canal</td>
<td>Collagen gel</td>
<td>Collagen gel</td>
<td>24 Week</td>
<td>Generation of pulp-like tissues</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>$5 \times 10^5$ cells</td>
<td>Subcutaneous implantation in mouse of treated human cells</td>
<td>PuraMatrix/MTA</td>
<td>PuraMatrix/MTA</td>
<td>4 weeks</td>
<td>Enhancement of vascularized pulp tissue &amp; vessels</td>
<td>Dissnayaka et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>$8 \times 10^5$ cells</td>
<td>Autologous transplantation into the pulp canal</td>
<td>Collagen/PGA</td>
<td>Collagen/PGA</td>
<td>4-8 weeks</td>
<td>Enhance chondrogenesis and partially inhibit ossification in engineered cartilage</td>
<td>Dai et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>$1 \times 10^6$ cells</td>
<td>Subcutaneous implantation in mouse of human cells</td>
<td>TCP</td>
<td>TCP</td>
<td>8 weeks</td>
<td>Enhance chondrogenesis and partially inhibit ossification in engineered cartilage</td>
<td>Zhou et al. (2015)</td>
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<td>Mice</td>
<td>$1 \times 10^6$ cells</td>
<td>Subcutaneous implantation in mouse of human cells</td>
<td>GCSF</td>
<td>GCSF</td>
<td>4 weeks</td>
<td>Enhance chondrogenesis and partially inhibit ossification in engineered cartilage</td>
<td>Schmaltz et al. (2016)</td>
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<tr>
<td></td>
<td>Dog</td>
<td>$1 \times 10^5$ cells</td>
<td>Autologous intercanal Transplantation</td>
<td>ND/NA</td>
<td>ND/NA</td>
<td>4 weeks</td>
<td>Angiogenesis, neurogenesis and pulp regeneration induction</td>
<td>Ishizuka et al. (2015)</td>
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<td></td>
<td>Rat</td>
<td>$NA$</td>
<td>Femur and autologous implantation of rat tooth chambers</td>
<td>Collagen/TE</td>
<td>Collagen/TE</td>
<td>4 weeks</td>
<td>Successful revascularization and tissue regeneration with direct vascular supply</td>
<td>Srisuwan et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>$1 \times 10^5$ cells</td>
<td>Subcutaneous implantation in mouse of human cells</td>
<td>TDM</td>
<td>TDM</td>
<td>4 weeks</td>
<td>Enhance chondrogenesis and partially inhibit ossification in engineered cartilage</td>
<td>Murakami et al. (2013)</td>
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<td></td>
<td>Rat</td>
<td>$NA$</td>
<td>Femur and autologous implantation of rat tooth chambers</td>
<td>FGF2/VEGF/POGF</td>
<td>FGF2/VEGF/POGF</td>
<td>4 weeks</td>
<td>Successful revascularization and tissue regeneration with direct vascular supply</td>
<td>Srisuwan et al. (2013)</td>
</tr>
<tr>
<td>Animal model</td>
<td>Cell type</td>
<td>Dose &amp; dosage</td>
<td>Route of administration</td>
<td>Co-administrative factors</td>
<td>Term approach</td>
<td>Main results</td>
<td>Reference</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Rat</td>
<td>SCAP</td>
<td>$1 \times 10^6$ cells</td>
<td>Xenograft transplantation into renal capsule</td>
<td>SCAP pellets / root segment</td>
<td>SCAP pellets / root segment</td>
<td>MTA regulates dentinogenesis of SCAPs</td>
<td>Yan et al. (2014)</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$3 \times 10^6$ cells</td>
<td>Xenograft Subcutaneous injection</td>
<td>BMP-2, rhPAlg</td>
<td>SCAP pellets / root segment</td>
<td>More mineralized tissue generation &amp; higher osteoblast differentiation in supplemented K2PO4 medium</td>
<td>Wang et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$1 \times 10^6$ cells</td>
<td>Xenograft Subcutaneous Transplantation</td>
<td>NA</td>
<td>HA/TCP ceramic powder, fibrin gel</td>
<td>Blood vessel formation &amp; negligible inflammation</td>
<td>Yang et al. (2017)</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$2 \times 10^5$ cells</td>
<td>Xenograft Subcutaneous Transplantation</td>
<td>VEGF</td>
<td>HA/TCP ceramic powder, fibrin gel</td>
<td>Negligible inflammation</td>
<td>Wang et al. (2016)</td>
<td></td>
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<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$1 \times 10^7$ cells</td>
<td>Xenograft Subcutaneous Transplantation</td>
<td>Poly(dex)</td>
<td>HA/TCP ceramic powder, fibrin gel</td>
<td>Dentin formation odontoblast presses inserted to dental tubules</td>
<td>Jin and Choung (2016)</td>
<td></td>
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<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$2 \times 10^4$ cells</td>
<td>Subcutaneous transplantation</td>
<td>NMPC</td>
<td>HA/TCP ceramic powder, fibrin gel</td>
<td>Mineralized tissue with embedded cells resembling odontoblasts</td>
<td>Wang et al. (2016)</td>
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<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$5 \times 10^6$ cells</td>
<td>Xenograft subcutaneous Transplantation</td>
<td>NA</td>
<td>HA/TCP</td>
<td>MTA regulates dentinogenesis of SCAPs</td>
<td>Yan et al. (2014)</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$2 \times 10^5$ cells</td>
<td>Xenograft subcutaneous Transplantation</td>
<td>WNT3A/BMP9</td>
<td>HA/TCP, BMP-2</td>
<td>Function of vascularized pulpal-like tissue</td>
<td>Hilkens et al. (2017)</td>
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<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$1 \times 10^7$ cells</td>
<td>Xenograft subcutaneous Transplantation</td>
<td>BMP-2, rhPAlg</td>
<td>HA/TCP, BMP-2</td>
<td>Mineralization &amp; DPC generation equally in SHED Fresh and SHED Cryo</td>
<td>Ma et al. (2012)</td>
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<tr>
<td>Mice</td>
<td>SCAP</td>
<td>$3 \times 10^6$ cells</td>
<td>Xenograft subcutaneous Transplantation</td>
<td>BMP-2, rhPAlg</td>
<td>HA/TCP</td>
<td>MTA regulates dentinogenesis of SCAPs</td>
<td>Zhang et al. (2015)</td>
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<tr>
<td>Mice</td>
<td>SHED</td>
<td>$4 \times 10^6$ cells</td>
<td>Xenograft subcutaneous Transplantation</td>
<td>NA</td>
<td>PLGA</td>
<td>Mineralization &amp; DPC generation equally in SHED Fresh and SHED Cryo</td>
<td>Wang et al. (2016)</td>
<td></td>
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<tr>
<td>Mice</td>
<td>SHED</td>
<td>$2 \times 10^6$ cells</td>
<td>Xenograft subcutaneous Transplantation</td>
<td>BMP-2, rhPAlg</td>
<td>HA/TCP</td>
<td>Mineralization &amp; DPC generation equally in SHED Fresh and SHED Cryo</td>
<td>Wang et al. (2016)</td>
<td></td>
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<tr>
<td>Mice</td>
<td>SHED</td>
<td>$5 \times 10^6$ cells</td>
<td>Subcutaneous implantation in mouse of treated human tooth slice</td>
<td>BMP-2, rhPAlg</td>
<td>HA/TCP</td>
<td>Mineralization &amp; DPC generation equally in SHED Fresh and SHED Cryo</td>
<td>Wang et al. (2016)</td>
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<tr>
<td>Mice</td>
<td>SHED</td>
<td>$5 \times 10^6$ cells</td>
<td>Subcutaneous implantation in mouse of treated human tooth slice</td>
<td>BMP-2, rhPAlg</td>
<td>HA/TCP</td>
<td>Mineralization &amp; DPC generation equally in SHED Fresh and SHED Cryo</td>
<td>Wang et al. (2016)</td>
<td></td>
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</tbody>
</table>

**Notes:**
- SCAP: Stem Cell Adipocyte Precursor
- SHED: Skeletal Multipotential Enamel-Derived Cell
<table>
<thead>
<tr>
<th>Animal model</th>
<th>Route of administration</th>
<th>Co-administrative factors</th>
<th>TERM approach</th>
<th>Main results</th>
</tr>
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<tr>
<td>BMSC</td>
<td>Allograft transplantation into renal capsules</td>
<td>NA</td>
<td>Lyophilized hydrogel</td>
<td>Local mineralization production of dentin-like tissue</td>
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<td>DFSC</td>
<td>Xenograft subcutaneous transplantation</td>
<td>Collagen</td>
<td>TDM</td>
<td>Polarized cells penetrating into dentin wall</td>
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<td>PDLSC</td>
<td>Xenograft subcutaneous transplantation</td>
<td>APES/TDM/DPEM</td>
<td>TDM</td>
<td>Potential pulp regeneration in MADC &amp; MEMDC but in less volume</td>
</tr>
<tr>
<td>ADSC</td>
<td>Xenograft subcutaneous transplantation</td>
<td>Human TDM and CDM</td>
<td>TDM</td>
<td>Complete mineralization of dentin-like tissue</td>
</tr>
<tr>
<td>MADC &amp; MEMDC</td>
<td>Xenograft subcutaneous transplantation</td>
<td>DPEM</td>
<td>TDM</td>
<td>Potential pulp regeneration in MADC &amp; MEMDC but in less volume</td>
</tr>
<tr>
<td>Rat</td>
<td>5 × 10^5 cells</td>
<td>10^4 cells/ml</td>
<td>BMP-2</td>
<td>Generation of uniform pulp-like tissue, predentin matrix formation</td>
</tr>
<tr>
<td>DFSC</td>
<td>Allogeneic transplantation into the renal capsule</td>
<td>AGS</td>
<td>TDM</td>
<td>More mechanical properties dentinogenic protein release by CDM</td>
</tr>
<tr>
<td>RPSC</td>
<td>Allogeneic transplantation into the renal capsules</td>
<td>NA</td>
<td>TDM</td>
<td>Typical dentinogenesis by iRPSC, bone-like tissues by mRPSC</td>
</tr>
<tr>
<td>DMSC</td>
<td>Allogeneic transplantation into the renal capsules</td>
<td>Collagen gel</td>
<td>TDM</td>
<td>Generation of bio-root with normal pulp and dentin-like matrix and natural biomechanical structure in low rate.</td>
</tr>
<tr>
<td>Human</td>
<td>Autologous transplantation into the extracted socket</td>
<td>Vitamin C</td>
<td>TDM</td>
<td>Generation of bio-root with normal pulp and dentin-like matrix and natural biomechanical structure in low rate.</td>
</tr>
<tr>
<td>MADC &amp; MEMDC</td>
<td>Xenograft subcutaneous transplantation</td>
<td>BMP-2</td>
<td>TDM</td>
<td>Typical dentinogenesis by iRPSC, bone-like tissues by mRPSC</td>
</tr>
</tbody>
</table>

**Cell type**
- BMSC: Bone marrow stromal cells
- DFSC: Dental fibroblasts
- PDLSC: Periodontal ligament stem cells
- ADSC: Adipose-derived stem cells
- MADC & MEMDC: Mandibular and maxillary dentin precursor cells
- RPSC: Rat pulp stem cells
- DMSC: Dental mesenchymal stem cells

**Route of administration**
- Allograft transplantation into renal capsules
- Xenograft subcutaneous transplantation
- Direct implantation into socket

**Co-administrative factors**
- Collagen
- Lyophilized hydrogel
- BMP-2

**TERM approach**
- APES/TDM/DPEM
- TDM
- NA

**Main results**
- Local mineralization production of dentin-like tissue
- Polarized cells penetrating into dentin wall
- Potential pulp regeneration in MADC & MEMDC but in less volume
- More mechanical properties dentinogenic protein release by CDM
- Typical dentinogenesis by iRPSC, bone-like tissues by mRPSC
- Generation of bio-root with normal pulp and dentin-like matrix and natural biomechanical structure in low rate.
<table>
<thead>
<tr>
<th>Cell type</th>
<th>Animal model</th>
<th>Dose &amp; dosage</th>
<th>Route of administration</th>
<th>Co-administrative factors</th>
<th>TERM approach</th>
<th>Time point</th>
<th>Main results</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Mesenchymal  | Mice         | $2 \times 10^5$ cells | Rat to mice Transplantation interoanal capsule | hBMP4
hBMP7         | PLGA          | 8 weeks    | Enamel and dentin-like tissues generation in two integrated layers with amelogenin expression and ameloblastin | Jiang et al. (2014) |
| UCMSC        | Mice         | $5 \times 10^4$ cells/well | Xenograft subcutaneous Transplantation | hTDM | TDM | 8 Weeks | Formation of layers of cells and calcifications | Chen et al. (2015) |
| Human DP Progenitors | Mice   | $10^6$ cells/50 μl | Xenograft subcutaneous transplantation Stem cell factor (SCF) | Collagen sponge | 4 weeks | Induction of cell homing, angiogenesis, and tissue remodeling | Pan et al. (2013) |
| Dermal multipotent cells | Mice | $2.0 \times 10^6$ cells | Xenograft subcutaneous transplantation Embryonic and neonatal TGC-CM | Fibrin gel | 4 weeks | Bone-like structure formation from embryonic TGC-CM | Huo et al. (2010) |
| DBCs         | Porcine      | $1 \times 10^7$ cells | Autotransplantation into swine’s original alveolar socket | GCHT Gelatin-chondroitin-hyaluronan-tricopolymer | 36 weeks | Successful rate of tooth regeneration from DBCs/GCHT scaffolds was about 33.3% | Kuo et al. (2007) |
| NA           | Beagles      | NA            | Cell homing | SDF-1α Silk/Fibrin | 12 weeks | Pulp tissue generation and mineralization along dentinal wall | Yang et al. (2015) |

BC, blood clot; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; BMSC, bone marrow mesenchymal stem cell; CC, costal chondrocyte; CDHA, calcium carbonate hydroxyapatite; CNCC, cranial neural crest cell; CXCL14, chemokine (CXC motif) ligand-14; DFSC, dental follicle stem cell; DPSC, dental pulp stem cell; FGF, fibroblast growth factor; GCSF, granulocyte colony-stimulating factor; GF, growth factors; HA, hydroxyapatite; IPS, induced pluripotent stem cell; MCP1, monocyte chemoattractant protein-1; MSC, mesenchymal stem cell; MTA, mineral trioxide aggregate; NA, not exogenously added; ND, not determined; NGF, nerve growth factor; PCL, polycaprolactone; PGA, polyglycolic acid; PGDF, platelet-derived growth factor; PLGA, poly(lactide-co-glycolide acid; PLLA, poly-l-lactic acid; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; REP, regenerative endodontic procedure; SCAP, stem cell of the apical papilla; SHED, stem cell from human exfoliated deciduous teeth; TCP, tricalcium phosphate; TDM, treated dentin matrix; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; DBCs, dental bud cells
Table 2. Variations of extracted data from reviewed articles.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of studies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td></td>
</tr>
<tr>
<td>DPSCs</td>
<td>31 (46)</td>
</tr>
<tr>
<td>SCAP</td>
<td>8 (12)</td>
</tr>
<tr>
<td>DFSC</td>
<td>6 (9)</td>
</tr>
<tr>
<td>BMSC</td>
<td>4 (6)</td>
</tr>
<tr>
<td>SHED</td>
<td>4 (6)</td>
</tr>
<tr>
<td>PDLSC</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>ADSC</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (10.4)</td>
</tr>
<tr>
<td>Scaffold</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>15 (22.3)</td>
</tr>
<tr>
<td>TDM</td>
<td>9 (13.4)</td>
</tr>
<tr>
<td>HA/TCP</td>
<td>10 (15)</td>
</tr>
<tr>
<td>PLLA</td>
<td>6 (9)</td>
</tr>
<tr>
<td>PLGA</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Atelocollagen</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Fibrin gel</td>
<td>8 (12)</td>
</tr>
<tr>
<td>CBB</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Silk fibroin</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Growth factors</td>
<td></td>
</tr>
<tr>
<td>TDM</td>
<td>9 (13.4)</td>
</tr>
<tr>
<td>BMP</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>G-CSF</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>SDF-1</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>VEGF</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>b-FGF</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Other</td>
<td>35 (52.2)</td>
</tr>
<tr>
<td>Transplantation site</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>40 (59.7)</td>
</tr>
<tr>
<td>Inter canal</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Renal capsule</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Into socket</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Animals</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>44 (65.6)</td>
</tr>
<tr>
<td>Rat</td>
<td>7 (10.4)</td>
</tr>
<tr>
<td>Pig</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Dog</td>
<td>7 (10.4)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Beagles</td>
<td>2 (2.9)</td>
</tr>
</tbody>
</table>

*The number of included studies is 57 studies; however, the number of trials is 67 trials. Therefore, each study may include one or more types of cells.

BC, blood clot; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; BMSC, bone marrow mesenchymal stem cell; CC, costal chondrocyte; CDHA, calcium carbonate hydroxapatite; CNCC, cranial neural crest cell; CXCL14, chemokine (CXC motif) ligand-14; DFSC, dental follicle stem cell; DPSC, dental pulp stem cell; FGF, fibroblast growth factor; GCFS; granulocyte colony-stimulating factor; GF, growth factors; HA, hydroxapatite; IPS, induced pluripotent stem cell; MCP1, monocyte chemotactic protein-1; MSC, mesenchymal stem cell; MTA, mineral trioxide aggregate; NA, not exogenously added; ND, not determined; NGF, nerve growth factor; PCL, polycaprolactone; PGA, polyglycolic acid; PGDF, platelet-derived growth factor; PLGA, poly(lactic-co-glycolic acid); PLLA, poly-L-lactic acid; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; REP, regenerative endodontic procedure; SCAP, stem cell of the apical papilla; SHED, stem cell from human exfoliated deciduous teeth; TCP, tricalcium phosphate; TDM, treated dentin matrix; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

The use of dentine-matrix-scaffolding cells was associated to the differentiation of the stem cells in a dentinal tubule in polarized odontoblast-like cells.

Stem cells from human exfoliated deciduous teeth

SHEDs, which rare derived from extracted deciduous teeth, were used in mice models in four studies. It was observed that the capability of mineralization of SHEDs was higher than DPSCs. Casagrande et al. reported that SHEDs express markers of odontoblastic differentiation (DSPP, DMP-1, MEPE).

Discussion

This is the largest and most updated systematic review aiming to investigate the role of DSCs in tooth repair. We found that multiple DSCs have a potent role in tissue regeneration and vascularization of dental pulp-like tissues. Most of the included research assessed the 4–8 week dentine-pulp regeneration following transplant. These studies used the ectopic models of dentine pulp-complex. A few studies used long-term evaluation of up to 36 weeks after surgery. However, there are some studies that had multiple time points for evaluation.

Like other tissues, three primary components are required to regenerate a necrotic pulp: 1) vital root canal cells, which can distinguish into normal pulp cells, 2) morphogenic and growth factors to activate and encourage cell distinction, and 3) a matrix that ensures an environment that maintains their vitality and growth and supports cells in a mechanical way.

Growth factors, drugs, bioactive products, glycosaminoglycans and other small molecules and motifs of peptide are considered promotive healing factors that can be used for stem cells and matrix to improve the effectiveness of stem cell therapy for dentine-pulp regeneration and biodegradation. Growth factors have a short half-life; therefore, degradable materials are required to control their release.

Recent studies for dentine pulp regeneration have been done in various types of stem cells from various sources in body. DPSCs are the preferred cells in the majority of these studies and have demonstrated their capacity to regenerate the complex dentine. Although the great tendency for dentine-pulp complex regeneration, SCAP and SHED were rarely administered. DPSCs and SHEDs were evaluated with adequate or partly successful histological outcomes in various REP research. DPSC, collagen or poly(lactic/glycolic acid and scarce factors with or without growth factors are optimized when compared to REPs with growth factors but without amplified stem cells. Several dentin therapies demonstrate further excellent outcomes, which should be followed by platelet-rich plasma/platelet-rich fibrin (PRP/PRF) or collagen gels in REPs and improved biomimicry to maintain various levels of the variables that release factors to activate and encourage cell distinction, and 3) a matrix that ensures an environment that maintains their vitality and growth and supports cells in a mechanical way.

Recent studies for dentine pulp regeneration have been done in various types of stem cells from various sources in body. DPSCs are the preferred cells in the majority of these studies and have demonstrated their capacity to regenerate the complex dentine.

Page 10 of 17
Besides dental sources, non-dental cells, such as the MSCs derived from bone marrow and adipose stem cells, are able to regenerate the pulp tissue.

Generally, our study showed that adult stem cells appear to be able to regenerate dentine-pulp complexes; therefore, the criteria of selection should be considered the most cost-effective and cheapest, particularly when the main obstacle is the expense. Moreover, our findings demonstrated that the human body is a wealthy source of stem cells; therefore, the third molars or any orthodontic tooth originated from a human body are excellent sources of stem cells. As regards cells circulating, these cells migrate to sites and engage in a recovery process in the presence of chemotactic gradients, as their capacity for root canal migration was shown.

This study showed two limitations; 1) we could not conduct a meta-analysis due to insufficient data; and 2) we failed to find a suitable tool to assess the quality of included studies and risk of bias.

In conclusion, the current evidence suggests that the DPSCs, SHEDs, and SCAPs are capable of providing a sufficient pulp regeneration and vascularization. Nevertheless, the efficacy of stem cell transplantation in therapy locations and their cost may be obstacles to their clinical use. Scaffolds and biomaterials provide a useful strategy for stronger incorporation of stem cells and development factors together with monitored regeneration rates. Hence, we suggest future studies to concentrate on offering definite guidance on appropriate and preferable biomaterial characteristics for use in regenerative endodontics.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Reporting guidelines


Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

References


24. Hung CN, Mar K, Chang HC, et al.: A comparison between adipose tissue and...
Published Abstract | Publisher Full Text | Free Full Text

Published Abstract | Publisher Full Text | Free Full Text

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Version 1

Reviewer Report 23 June 2020

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Weidong Tian
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In this review, the authors summarized recent studies of dental pulp regeneration using stem cells. In general, it is a timely and detailed review of stem cells used in dental regeneration. But this manuscript needs to be carefully modified.

Comments:
1. In the abstract and Table 2, the authors described “TDM (treated dentin matrix)” as a growth factor. In fact, TDM is a matrix containing growth factors. “Growth factors derived from TDM” is more suitable.

2. The language of this manuscript should be checked and polished by a native English speaker.

3. According to the methods, the authors searched the databases from January 2000 to June 2019: Since numbers of studies of dental pulp regeneration have published after June 2019, it is recommended for the authors to update their data to June 2020.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Partly

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
Yes
Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Dental regeneration

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 21 January 2020
https://doi.org/10.5256/f1000research.23175.r56928

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Institute for Stem Cell and Regenerative Medicine, School of Medicine, University of Washington, Seattle, WA, 98195, USA

The manuscript “Dental stem cells in tooth repair: A systematic review” is a review by Tadros et al. on a very timely topic, tooth regeneration. However, this paper does not read like a review but rather as a catalogue of papers. This review requires conceptual work towards synthesizing the great findings in the field. For example, many notable, recent papers are excluded from this review. In addition, the following comments need to be addressed.

- Provide citations for every statement (e.g. “Loss of tooth pulp stops the development of permanent root teeth that can weaken the periodontal connection and lead to teeth loss” Page 3, Paragraph 2.)

- Spelling and grammar review. Multiple incidences of unclear sentences (e.g. “Although the great tendency for dentine-pulp complex regeneration, SCAP and SHED were rarely administered.” Page 10, Paragraph 5) or incorrect word used (“SHEDs, which rare derived from extracted deciduous teeth” Page 10, Paragraph 1).

- Develop appropriate tool to assess the quality of included studies and risk of bias such as that described in Cochrane Handbook for Systematic Reviews of Interventions.
  - How titles and abstracts were “deemed relevant” to the research question.
  - Why were studies excluded/removed?
  - What databases/sources were used?
  - What search terms were used?
  - Studies included:
    - Reviews are not listed though there are reviews included.
    - Humans are not listed in populations studied though human studies are cited.
(e.g. “All included experiments utilizing DPSCs were isolated from human healthy pulp tissues…” Page 4, Paragraph 3).

- Why there was insufficient data for meta-analysis.
- Meaning of “usually orthodontics” Page 4, Paragraph 3. Does this mean teeth were extracted due to orthodontic treatment plans?
- “Fresh bio-root” and “success” when discussing PDLSCs and “potential pulp” on Page 4, Paragraph 4 and 5.
- TERM approach.
- “Scarce factors” and “excellent outcomes” discussed on Page 10, Paragraph 5.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
No

Are sufficient details of the methods and analysis provided to allow replication by others?
No

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** stem cells and regeneration

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.
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