INTERNATIONAL
ENDODONTIC JOURNAL

doi:10.1111/iej.12868

REVIEW

# Dental pulp regeneration *via* cell homing

**S. Eramo[1], A. Natali[1], R. Pinna[2] & E. Milia[2]**

[1]Department of Surgery and Biomedical Sciences, University of Perugia, Perugia; and [2]Department of Biomedical Sciences, University of Sassari, Sassari, Italy

## Abstract

**Eramo S, Natali A, Pinna R, Milia E.** Dental pulp regeneration *via* cell homing. *International Endodontic Journal*, **51**, 405–419, 2018.

The typical treatment for irreversibly inflamed/necrotic pulp tissue is root canal treatment. As an alternative approach, regenerative endodontics aims to regenerate dental pulp-like tissues using two possible strategies: cell transplantation and cell homing. The former requires exogenously transplanted stem cells, complex procedures and high costs; the latter employs the host's endogenous cells to achieve tissue repair/regeneration, which is more clinically translatable. This systematic review examines cell homing for dental pulp regeneration, selecting articles on *in vitro* experiments, *in vivo* ectopic transplantation models and *in situ* pulp revascularization. MEDLINE/PubMed and Scopus databases were electronically searched for articles without limits in publication date. Two reviewers independently screened and included papers according to the predefined selection criteria. The electronic searches identified 46 studies. After title, abstract and full-text examination, 10 articles met the inclusion criteria. *In vitro* data highlighted that multiple cytokines have the capacity to induce migration, proliferation and differentiation of dental pulp stem/progenitor cells. The majority of the *in vivo* studies obtained regenerated connective pulp-like tissues with neovascularization. In some cases, the samples showed new innervation and new dentine deposition. The *in situ* pulp revascularization regenerated intracanal pulp-like tissues with neovascularization, innervation and dentine formation. Cell homing strategies for pulp regeneration need further understanding and improvement if they are to become a reliable and effective approach in endodontics. Nevertheless, cell homing currently represents the most clinically viable pathway for dental pulp regeneration.

**Keywords:** cell homing, endodontics, pulp regeneration, scaffold, signalling molecules, tissue engineering.

*Received 19 May 2017; accepted 14 October 2017*

## Introduction

Dental pulp trauma or infections, frequently manifested as pulpitis, are commonly treated by removing the pulp and replacing it with inorganic materials (gutta-percha and sealer cement) *via* root canal treatment (RCT). Pulp extirpation may make endodontically treated teeth brittle, susceptible to postoperative fractures and reinfections due to coronal leakage or periapical microleakage (Ingle & Bakland 2002, Dammaschke *et al.* 2003). Moreover, they lose pulpal sensation to hot/cold stimulation and the ability to detect secondary infections (Ingle & Bakland 2002, Dammaschke *et al.* 2003, Caplan *et al.* 2005).

As an alternative approach, regenerative endodontics aims to replace inflamed/necrotic pulp tissue with regenerated pulp-like tissue. Two strategies can be applied towards dental pulp regeneration: cell transplantation and cell homing. The former approach is cell-based, which means transplanting exogenous stem cells loaded onto scaffolds incorporated with signalling molecules into the root canal system of the

Correspondence: Stefano Eramo, Department of Surgery and Biomedical Sciences, University of Perugia, Piazza Lucio Severi 1 - 06132, Perugia, Italy (Tel.: 39-3381704211; e-mail: stefano.eramo@unipg.it).

host to allow regeneration. The transplanted cells are collected from the host (autologous) or from other individuals (allogenic) and may be either processed (separation from tissues) or grown in cultures to increase their numbers. In this case, stem cells are the key for tissue regeneration. About 10 years after the discovery of dental pulp stem cells (Gronthos *et al.* 2000), pulp/dentine regeneration was accomplished using exogenously transplanted dental stem cells in small and large animals (Huang *et al.* 2010, Iohara *et al.* 2011). However, cell-based therapy faces many hurdles in clinical translation because complex procedures need to be followed, such as tooth extraction, pulp extirpation, *in vitro* cell culture, selection of stem/progenitor cell populations, *ex vivo* cell expansion, storage and shipping. Also, there are other concerns including potential contamination and development of tumorigenesis during *ex vivo* cell manipulation (Yildirim *et al.* 2011, Kim *et al.* 2012). Therefore, cell transplantation for pulp regeneration therapy is likely to suffer from a lack of clinical viability, difficulty with regulatory approval and the high costs, besides the risks of immune rejection, pathogen transmission and tumorigenesis during engraftment. Despite its scientific validity, dental pulp regeneration using cell transplantation is unlikely to be economically viable or competitive with current RCT or dental implants.

From a therapeutic viewpoint, cell homing may circumvent many of the challenges associated with cell transplantation. In tissue regeneration, cell homing is defined as active recruitment of endogenous cells, including stem/progenitor cells, into an anatomic compartment (Laird *et al.* 2008, Mao *et al.* 2010). The concept of cell homing is to achieve tissue repair/regeneration through chemotaxis of host endogenous cells to injured tissue *via* biological signalling molecules. Compared with stem cell transplantation, cell homing strategies might be easier to perform clinically, because there is no need to isolate and manipulate stem cells *in vitro* (Kim *et al.* 2013, Huang & Garcia-Godoy 2014, Xiao & Nasu 2014).

In this cell-free approach, bioactive scaffolds impregnated with growth factors are injected into pulpless root canals to induce the migration, proliferation and differentiation of endogenous stem/progenitor cells residing around the root apex. The possible cell sources include dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAP) and bone marrow stem cells (BMSCs). Then, two preconditions are necessary to achieve pulp regeneration: effective root canal disinfection and appropriate size of the apical foramen (especially in mature teeth with closed apices), which should be as small as possible, without affecting cell migration, neovascularization and reinnervation (Laureys *et al.* 2013).

Cytokines are critical signalling molecules participating in pulp regeneration, because they mobilize endogenous cells and regulate the proliferation and differentiation of stem/progenitor cells (Moretti *et al.* 2015, Wang *et al.* 2017). To exploit the innate healing potential of endogenous cells, several types of signalling molecules have been tested *in vitro* and added to the scaffolds in animal models *in vivo*: amongst them, stromal cell-derived factor (SDF-1$\alpha$), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF). The growth factors employed should have three functions: (i) promote angiogenesis in the root canal; (ii) enhance migration of endogenous stem cells and (iii) induce mineralization (Kao *et al.* 2009, Chieruzzi *et al.* 2016). Therefore, a suitable combination of scaffold and growth factors should be selected, and the scaffold should be easily manipulated in clinical practice. The use of biological signalling molecules for cell homing makes pulp regeneration more clinically translatable, because the delivery of growth factors is not nearly as complex and costly as cell transplantation. Essentially, the cell homing approach circumvents some of the safety, manufacturing and technical issues associated with cell transplantation (Kim *et al.* 2010, Suzuki *et al.* 2011).

Pulp revascularization of immature permanent teeth could be considered a type of cell homing strategy for pulp regeneration. It is a two-step regeneration-based protocol for immature teeth with necrotic pulps, proposed in clinical practice over the past decade (Thibodeau & Trope 2007, Wigler *et al.* 2013). In this approach, the root canal system is first disinfected with a combination of antibiotics or calcium hydroxide; then, it is filled with a blood clot from bleeding provoked from periapical tissues (European Society of Endodontology 2016, Galler 2016). The blood clot acts as a scaffold, and the growth factors inside it could recruit stem cells, most likely from the periapical papilla (Lovelace *et al.* 2011). Several clinical case reports described good outcomes with pulp revascularization of immature teeth, such as radiographic signs of tooth maturation and symptom reduction.

Nevertheless, histological studies have shown that most of the tissues formed were not pulp but including cementum, periodontal and bone-like tissues (Becerra *et al.* 2014). Therefore, further studies are needed to promote the formation of pulp-like tissues and to apply this therapeutic approach to mature teeth.

The regenerated pulp-like tissues should be connective tissues that (i) deposit new dentine with a regulated rate, a process named dentinogenesis, (ii) show similar cell density and architecture to the natural pulp and (iii) have vascularization and (iv) innervation (Fawzy El-Sayed *et al.* 2015). Remarkable advances have been made in the understanding of dental pulp biology at the cellular and molecular levels and towards pulp/dentine regeneration (Mao & Prockop 2012), but the existing *in vivo* animal studies have not yet found a valid pathway applicable to humans. The major hurdle that cell homing faces for pulp regeneration *in vivo* is represented by the shortage of endogenous cells in defect sites (Kim *et al.* 2012, 2013). Periapical cell numbers are reduced in teeth with necrotic pulps and periapical lesions. SCAP possess a key benefit concerning their apical location that supports tissue survival during pulp necrosis (Huang *et al.* 2008). Nevertheless, no therapy promoting regeneration of the pulp/dentine complex in cases of pulp necrosis currently exists (Bottino *et al.* 2017).

The aim of this systematic review was to select and analyse the studies published on dental pulp regeneration through cell homing strategies. A distinction was made between *in vitro* experiments with stem cells and signalling molecules, *in vivo* ectopic transplantation animal models and *in situ* pulp revascularization, to provide a global overview of current knowledge on cell homing in regenerative endodontics.

## Review

### Materials and methods

*Search strategy*

This systematic review was performed according to the PRISMA Statement (Moher *et al.* 2009). A first systematic literature search for articles related to pulp regeneration *via* cell homing in endodontics, without limits in publication date, was conducted in the databases MEDLINE/PubMed and Scopus, using a combination of the following terms: [Pulp regeneration] AND [Cell homing]. The search fields were 'All fields' in MEDLINE/PubMed, whilst 'Title, Abstract and Keywords' in Scopus. The search results were imported into a computerized database Review Manager 5.2. The search results from each of the electronic databases of MEDLINE/PubMed and Scopus were combined, and duplicated publications were eliminated.

*Criteria for selecting studies*

After completing the search, articles for review were selected based on:

- Original data protocols
- Document type: articles
- Dental pulp regeneration
- Cell homing
- English language

*Exclusion criteria*

The reasons for exclusion were defined as follows:

- Studies without original and/or actual data
- Studies with data from previous publications
- Cell transplantation
- Reviews
- Opinion papers, editorials and book chapters

In this way, removing irrelevant citations according to the selected criteria, a preliminary set of potentially relevant publications was created.

*Screening and selection*

Using a screening guide based on eligibility criteria, two reviewers (AN and SE) screened independently the registered titles and abstracts, authors and references in two separate files (one for included abstracts and the other one for excluded abstracts). The full text of all potentially eligible studies in at least one screening was retrieved. Reviewers then evaluated the full text for inclusion using a screening guide, and a second reviewer (AN) screened all the findings. When disagreement occurred, a third reviewer (RP) was consulted.

*Data extraction*

An *ad hoc* data extraction form was designed to record data from the selected studies. For the *in vitro* experiments articles, the recorded fields were: Authors and Year, Employed cells/tissues, Source and Methods, Results (Table 1). For the *in vivo* ectopic transplantation models papers, the registered fields were: Authors and Year, Specimens, Test subjects, Site and Trial time, Scaffold and Growth factors, Results (Table 2). For the *in situ* pulp revascularization papers, the

**Table 1** Systematic review of *in vitro* experiments papers

| Authors and year | Employed cells/tissues | Source and methods | Results | Qualities and relevance of studies included score |
|---|---|---|---|---|
| Suzuki *et al.* (2011) | DPSCs | Human third molars<br>3D cell migration assay and RT-PCR analysis | SDF1 or bFGF recruited significantly more cells than the control group<br>BMP7 had little effect on cell recruitment | ++ |
| Pan *et al.* (2013) | Mandibles DP cells | Mice<br>Human teeth<br>Immunohistochemical and RT-PCR analyses<br>Immunofluorescence staining<br>Culture with osteogenic induction medium<br>MTT assay<br>Culture in a trans-well system<br>Protein extraction and Western blot analysis | High levels of both SCF and its receptor c-Kit were found<br>DP cells possess dentinogenic/osteogenic potential<br>SCF stimulated proliferation, migration and cytoskeletal reorganization<br>SCF modulated DP cell functions | +++ |
| Takeuchi *et al.* (2015) | MDPSCs PdLF, BM, HUVEC and TGW | Human third molars<br>Cell banks<br>Real-time horizontal chemotaxis assay<br>Spectrophotometry<br>Flow cytometry<br>Angiogenic differentiation<br>Neurogenic differentiation<br>Odontogenic differentiation | bFGF and G-CSF did not significantly differ in inducing migration, proliferation, anti-apoptotic effect, angiogenic and neurogenic differentiation<br>G-CSF promoted mineralization, whilst bFGF completely suppressed it | +++ |
| Yang *et al.* (2015) | DPSCs | Human third molars<br>Immunofluorescence staining<br>Flow cytometry<br>Colony-forming assay<br>Odontogenic/osteogenic induction<br>Adipogenic induction<br>CCK-8 assay<br>Cell migration assay:<br>1. *In vitro* wound healing assay<br>2. Transmigration assay<br>Immunofluorescence staining<br>Transmission electron microscopy<br>Protein extraction and Western blot analysis | SDF-1α had no effect on DPSC viability<br>It significantly promoted DPSC migration<br>It optimized focal adhesion formation and stress fibre assembly | +++ |
| Liu *et al.* (2015) | SCAP and DPSCs | Human impacted immature third molars<br>Immunofluorescence staining<br>Immunocytofluorescence<br>RT-PCR analysis<br>Flow cytometry<br>CCK-8 assay<br>Trans-well migration assay<br>3D cell migration assay | CXCR4 was expressed in both SCAP and DPSC cultures<br>SDF-1α had no significant effect on CXCR4 expression and SCAP proliferation, but it significantly promoted SCAP migration | +++ |
| Li & Wang (2016) | BMSCs[2] | SD rats<br>CCK-8 assay<br>Trans-well migration assay<br>RT-PCR analysis | PDGF-BB, NGF and BDNF promoted BMSC[2] proliferation<br>PDGF-BB significantly promoted BMSC[2] migration | +++ |

**Table 1** Continued

| Authors and year | Employed cells/tissues | Source and methods | Results | Qualities and relevance of studies included score |
|---|---|---|---|---|
| Ruangsawasdi *et al.* (2016) | hMSCs | Cell bank<br>μ-slide chemotaxis 3D assays<br>WST-1 assay<br>Odonto/osteogenic differentiation<br>ELISA | SCF increased hMSC directional migration, proliferation and odonto/osteogenic differentiation | +++ |

+++, high level; ++, medium level; +, low level.

evaluated fields were: Authors and Year, Test subjects, Teeth, Scaffold, Trial time, Methods, Results (Table 3).

*Quality assessment*

All studies meeting the inclusion criteria then underwent quality assessment. Two examiners (AN and SE) read the papers independently. The qualities and relevance of each study were graded as follows: high (+++), medium (++) or low (+) using a study-quality checklist. External validity, internal validity and study precision were analysed to obtain an overall assessment of quality. The assessment was used as a basis for the discussion between the two examiners to grade the studies. In the case of disagreement, all authors discussed the paper until a consensus was reached.

Table 4 gives the abbreviations used throughout the paper.

## Results

The electronic searches identified 46 studies. Figure 1 summarizes the selection procedure of the papers. Subsequently, during the review of titles, abstracts and full texts, 36 studies were excluded. The final analysis included 10 articles that conformed to the criteria for the present review.

*In vitro experiments*

A total of seven studies reported *in vitro* experiments for cell homing strategy in dental pulp regeneration.

To study the chemotactic effect of different cytokines on human dental stem/progenitor cells, Suzuki *et al.* (2011) established a 3D *in vitro* cell migration assay and quantitatively studied the recruitment of DPSCs in response to SDF-1, bFGF and BMP7. SDF-1 or bFGF recruited significantly more cells into 3D collagen gel scaffolds than the control group without cytokine delivery in 7 days, as demonstrated by the increased DNA content inside the gel. Instead, BMP7 had little effect on cell recruitment, failing to show an advantage over the control group, but it was highly effective in promoting mineralization of cultured DPSCs. BMP7 might have initiated cell differentiation at the expense of cell migration. Moreover, cell membrane receptors for SDF-1, bFGF and BMP7 were upregulated in treated DPSCs. As a result, dental pulp stem/progenitor cells may be selectively recruited by chemotactic cytokines and act as endogenous cell sources for pulp regeneration and mineralization.

Also, Yang *et al.* (2015) investigated the effects of SDF-1α on DPSC migration, together with the role of autophagy involved in this process. *In vitro* addition of SDF-1α had no effect on DPSC viability, but significantly promoted their migration in a concentration-dependent manner and optimized focal adhesion formation and stress fibre assembly, which have a critical function in cell migration. Immunostaining analysis and TEM observation strongly suggested the activation of autophagy in SDF-1α-treated DPSCs. Furthermore, DPSC migration was abolished in the presence of autophagy inhibitors and promoted in the presence of autophagy activator. Therefore, autophagy was induced and positively associated with DPSC migration induced by SDF-1α. SDF-1α has an essential function in the mobilization and recruitment of stem/progenitor cells.

The chemotactic effect of the cytokine SDF-1α was further studied on SCAP by Liu *et al.* (2015) using *in vitro* transmigration models. SDF-1α is widely expressed and mediates cell migration through its binding with CXC chemokine receptor 4 (CXCR4). CXCR4 expression was detected in both the paravascular region of the apical papilla and the pulp tissue, as well as in both SCAP and DPSCs in cultures. Most cultured SCAP harboured intracellular CXCR4,

**Table 2** Systematic review of *in vivo* ectopic transplantation models papers

| Authors and year | Specimens, test subjects, site and trial time | Scaffold and growth factors | Results | Qualities and relevance of studies included score |
|---|---|---|---|---|
| Kim *et al.* (2010) | Human mature incisors and canines; mice dorsum; 3 weeks | 2 mg $mL^{-1}$ neutralized collagen gel; 10 ng $mL^{-1}$ VEGF-2 and/or 100 ng $mL^{-1}$ bFGF, 10 ng $mL^{-1}$ PDGF, 50 ng $mL^{-1}$ NGF and 100 ng $mL^{-1}$ BMP7 | bFGF and/or VEGF-2 yielded recellularized and revascularized connective tissue<br>bFGF or VEGF-2 or PDGF with NGF and BMP7 generated cellularized and vascularized pulp-like tissue | +++ |
| Suzuki *et al.* (2011) | Human teeth; rats dorsum; 3 weeks | Collagen gel; bFGF | bFGF induced recellularization and revascularization of pulp-like tissue | + |
| Pan *et al.* (2013) | Mice dorsum; 4 weeks<br>Mice dorsum; 1 week | Collagen sponge; 50 ng $mL^{-1}$ SCF<br>Collagen sponge; 50 ng $mL^{-1}$ SCF | SCF had a highly significant effect on cell number and angiogenesis<br>SCF induced sponge remodelling and collagen fibre neogenesis | ++ |
| Takeuchi *et al.* (2015) | Porcine second incisors; mice; 3 weeks | Collagen; 15 μg $mL^{-1}$ bFGF or 15 μg $mL^{-1}$ G-CSF | No significant difference between bFGF and G-CSF in pulp-like tissue regeneration and angiogenesis | +++ |
| Yang *et al.* (2015) | Human teeth root fragments; nude mice dorsum; 8 weeks | Porous 3D silk fibroin (1.5–2 $mm^3$); none or 100 ng SDF-1α | In the group with scaffold alone no tissue was formed. In the SDF-1α group vascularized connective tissues with collagen deposition were found | ++ |
| Zhang *et al.* (2015) | Human mandibular first premolars; mice dorsum; 3 weeks | 2 mg $mL^{-1}$ neutralized collagen gel; none or 100 ng $mL^{-1}$ SDF-1 | Dental pulp-like tissue and new vessels were regenerated in both groups | +++ |
| Li & Wang (2016) | Human incisors and premolars; SD rats dorsum; 2 to 4 months | 1.8 mg $mL^{-1}$ neutralized collagen I gel; PDGF-BB (50 ng $mL^{-1}$), NGF (50 or 100 ng $mL^{-1}$) and BDNF (50 or 100 ng $mL^{-1}$) | Well-vascularized pulp-like tissue was regenerated in both the H and the L group. Newly formed nerve fibres only in the H group | +++ |
| Ruangsawasdi *et al.* (2016) | Root sections of human immature third molars; SD rats calvaria and dorsum; 6 weeks | 0.3% fibrin gel; none | Specimens placed at the calvaria showed pulp-like tissue and odontoblast-like cells | +++ |
| Ruangsawasdi *et al.* (2017) | Human bilateral immature premolars; SD rats calvaria; 6 or 12 weeks | 0.4% fibrin gel; none or 15 μg $mL^{-1}$ SCF | SCF increased the tissue ingrowth at 6 weeks but not at 12 weeks. However, at 12 weeks the tissue was more mature | +++ |

+++, high level; ++, medium level; +, low level.

**Table 3** Systematic review of *in situ* pulp revascularization papers

| Authors and year | Test subjects | Teeth | Scaffold | Trial time | Methods | Results | Qualities and relevance of studies included score |
|---|---|---|---|---|---|---|---|
| Yang *et al.* (2015) | Beagle dogs | Mature second and third lower premolars | Blood clot (control group) or SDF-1α-loaded silk fibroin | 3 months | Induction of apical periodontitis<br>Instrumentation and irrigation of the canals with 2.5% NaOCl and 17% EDTA<br>Intracanal dressing of triple antibiotic paste consisting of ciprofloxacin, metronidazole and minocycline (20 mg $mL^{-1}$ each), then teeth sealing for 4 weeks<br>After irrigation, bleeding induction into the canals by gentle over-instrumentation | In both groups, regenerated intracanal tissues (rCT and rMT) were evident, with little nerve bundles<br>SDF-1α silk fibroin scaffolds improved the *de novo* ingrowth of pulp-like tissues | +++ |

+++, high level; ++, medium level; +, low level.

whereas only a low percentage of them expressed it on the cell surface. Although SDF-1α had no significant effect on CXCR4 expression or SCAP proliferation, it significantly promoted SCAP migration in a dose-dependent manner, reaching the peak at 100 ng $mL^{-1}$ SDF-1α. This effect was abolished by anti-CXCR4 antibodies. Notably, SCAP began expressing surface CXCR4 after transmigration. The 3D migration assay revealed that SDF-1α significantly enhanced SCAP migration into the 3D collagen gel. These findings suggest that the SDF-1α gradient may induce translocation of cytoplasmic CXCR4 to the membrane, thus increasing the population of SCAP expressing CXCR4. SCAP can be chemoattracted to migrate *via* the SDF-1α/CXCR4 axis. Therefore, SDF-1α may be clinically used to induce CXCR4-expressing SCAP residing in the apical papilla of an immature permanent tooth to migrate into the root canal space as an endogenous cell source for pulp regeneration *via* the SDF-1α/CXCR4 axis.

Another signalling molecule extensively investigated in the cell homing approach has been SCF, a powerful chemokine capable of recruiting progenitor cells. Pan *et al.* (2013) examined whether SCF and its receptor c-Kit are expressed in teeth and whether they may be useful as homing agents for the recruitment of dental pulp progenitors. Immunohistochemical analysis reported high levels of both SCF and c-Kit in differentiating dental pulp (DP) cells and in the subodontoblastic layer of Höhl. SCF expression pattern in odontoblasts and DP cells indicates that SCF may play a role in dentinogenesis. *In vitro* studies using human DP progenitors showed they possess dentinogenic/osteogenic potential, because they can be induced towards an odontoblast/osteoblast lineage using osteogenic medium. More interestingly, SCF stimulated proliferation in a dose-dependent manner, promoted migration and enhanced cytoskeletal reorganization of DP progenitors, modulating their functions through MEK/ERK and PI3K/Akt pathways. These results suggest that SCF acts as a potent and fast acting chemotactic agent related to DP cells.

Similarly, Ruangsawasdi *et al.* (2017) wanted to test the effect of SCF on human mesenchymal stem cells (hMSCs). They set up *in vitro* experiments in which hMSCs were exposed to SCF at various concentrations for assessing cell directional migration, proliferation and differentiation towards odonto/osteoblasts by 3D-chemotaxis slides, WST-1 assay and alkaline phosphatase activity, respectively. The presence of SCF increased hMSC directional migration, with a

**Table 4** Abbreviations

| | |
|---|---|
| ALP | Alkaline phosphatase |
| Atg5 | Autophagy-related protein 5 |
| BDNF | Brain-derived neurotrophic factor |
| bFGF | Basic fibroblast growth factor |
| BM | Human bone marrow-derived mesenchymal stem cells |
| BMP2 | Bone morphogenetic protein 2 |
| BMP7 | Bone morphogenetic protein 7 |
| BMSCs[1] | Bone marrow stromal cells |
| BMSCs[2] | Bone mesenchymal stem cells |
| BSP | Bone sialoprotein |
| CCK-8 | Cell counting kit-8 |
| CD-34 | Cluster of differentiation 34 |
| CM | Conditioned medium |
| Col1/COL1 | Collagen type I |
| CXCR4 | CXC chemokine receptor 4 |
| DMP1 | Dentine matrix protein 1 |
| DP | Dental pulp |
| DPSCs | Dental pulp stem cells |
| DSP | Dentine sialoprotein |
| DSPP | Dentine sialophosphoprotein |
| EDTA | Ethylene-diaminetetraacetic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| G-CSF | Granulocyte colony-stimulating factor |
| GDNF | Glial cell-derived neurotrophic factor |
| GFP | Green fluorescent protein |
| H | High concentration |
| hMSCs | Human mesenchymal stem cells |
| HUVEC | Human umbilical vein endothelial cells |
| L | Low concentration |
| LC3 | Microtubule-associated protein 1A/1B-light chain 3 |
| MAP2 | Microtubule-associated protein 2 |
| MDPSCs | Mobilized dental pulp stem cells |
| MEK/ERK | Mitogen-activated protein kinase/extracellular-signal regulated kinase |
| MMP2 | Matrix metalloproteinase 2 |
| MTT | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| NaOCl | Sodium hypochlorite |
| NGF | Nerve growth factor |
| OPN | Osteopontin |
| PDGF(-BB) | Platelet-derived growth factor (BB) |
| PdLF | Human periodontal ligament fibroblasts |
| PI3K/Akt | Phosphatidylinositol-3-kinase/protein kinase B |
| rCT | Regenerated connective tissue |
| rMT | Regenerated mineralized tissue |
| RT-PCR | Real-time polymerase chain reaction |
| Runx2 | Runt-related transcription factor 2 |
| S-100 | 100% soluble protein |
| SCAP | Stem cells from the apical papilla |
| SCF | Stem cell factor |
| SCID | Severe combined immune deficient |
| SD | Sprague-Dawley |
| SDF1(α) | Stromal cell-derived factor 1(α) |
| TEM | Transmission electron microscope |
| TGW | Human neuroblastoma cell line |
| VEGF(-2) | Vascular endothelial growth factor (2) |
| vWF | Von Willebrand factor |
| WST-1 | Water-soluble tetrazolium-1 |

significant effect when 2.5 and 12.5 μg $mL^{-1}$ SCF were loaded in one of the reservoirs. SCF at a concentration of 100 ng $mL^{-1}$ showed a significant increase in hMSC proliferation, whilst differentiation was significantly affected only at very high concentrations of 1 μg $mL^{-1}$ SCF. *In vitro* results also showed that SCF can be freed from the current fibrin gel formulation used in pulp regeneration strategies: in particular, its release kinetics was low during the first 3 days and completed after 7 days. As a whole, SCF influenced stem cell migration, proliferation and differentiation; thus, it could facilitate chemotaxis-induced cell homing in dental pulp regeneration.

Besides those listed, some other signalling molecules are indicated to act as homing factors for pulp regeneration. Takeuchi *et al.* (2015) compared the *in vitro* effects of bFGF with those of G-CSF to assess the potential utility of bFGF as an alternative to G-CSF for pulp regeneration. In this study, five different types of cells were examined, amongst which PdLF, BM, HUVEC and TGW were used as representative of endogenous cells surrounding the tooth, whilst MDPSCs, a subset of DPSCs known to have high angiogenic and regenerative potential, were used as DPSCs. *In vitro* experiments outlined that bFGF and G-CSF did not significantly differ in inducing migration, proliferation, anti-apoptotic effects, vascular endothelial differentiation and neurite outgrowth. Instead, regarding odontogenic induction, G-CSF promoted mineralization in DPSC subsets, whilst bFGF completely suppressed it. Nevertheless, bFGF may be a potent homing/migration factor in pulp regeneration therapy similar to the influence of G-CSF.

Li & Wang (2016) investigated the chemotactic effects of PDGF-BB and the cytobiological effects of NGF and BDNF on the culture of bone mesenchymal stem cells (BMSCs[2]) from rats *in vitro*. The proliferation, migration and differentiation of rat BMSCs[2] treated with different dosages of PDGF-BB, NGF and BDNF were evaluated using CCK-8, trans-well and RT-PCR assays. The results showed that such cytokines markedly promoted BMSC[2] proliferation in time- and dose-dependent manners; the optimum concentration of PDGF-BB for enhancing cell proliferation was 50 ng $mL^{-1}$, whilst it was 100 ng $mL^{-1}$ with NGF and BDNF. Moreover, PDGF-BB significantly promoted BMSC[2] migration in a dose-dependent manner, whereas no significant difference was found between the number of migrated cells and the growth factors concentration in the NGF- and BDNF-treated groups. PDGF-BB, NGF and BDNF significantly enhanced the

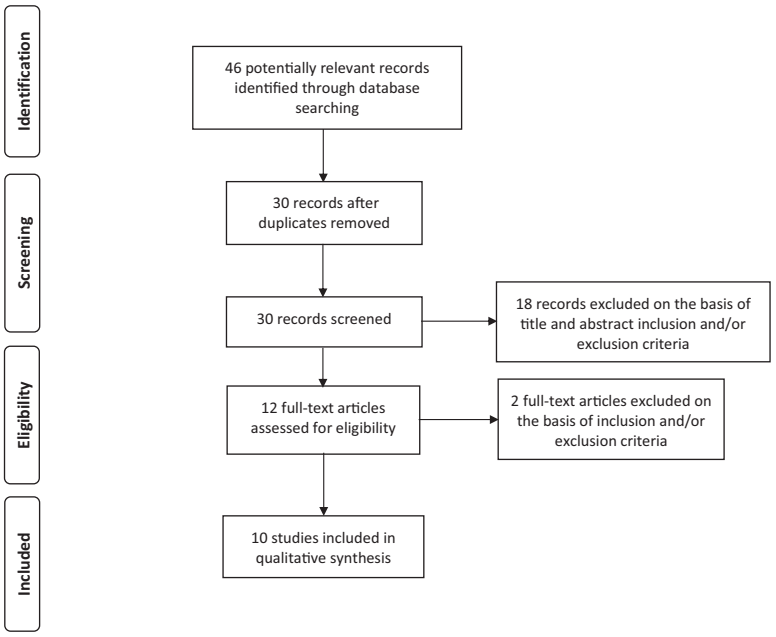


**Figure 1** Search flowchart according to the PRISMA Statement.

expressions of OPN and Runx2, which are specific genes of osteogenic induction, and also those of MAP-2 and β-III-tubulin, markers of neurogenic induction, in a dose-dependent manner in treated BMSCs[2]. These findings suggest that PDGF-BB, NGF and BDNF have the potential to mobilize endogenous cells and regulate the proliferation and differentiation of mesenchymal stem/precursor cells useful for pulp regeneration *via* cell homing.

*In vivo ectopic transplantation models*

A total of nine selected papers evaluated ectopic transplantation animal models to study pulp regeneration through cell homing *in vivo*.

Kim *et al.* (2010) induced dental pulp-like tissue regeneration *via* a cell homing technique using human teeth transplanted in a mouse model. Human-extracted canines and incisors were endodontically treated without root filling materials. The emptied root canals were filled with collagen scaffolds impregnated with combinations of different molecules: bFGF, VEGF or PDGF with a basal set of NGF and BMP7. Then, the teeth were transplanted subcutaneously into mice dorsum for 3 weeks. Upon retrieval of the specimens, delivery of bFGF and/or VEGF yielded newly formed recellularized and revascularized connective tissue with abundant cells that integrated to the native dentinal walls in the root canals. Furthermore, combined delivery of bFGF or VEGF or PDGF with basal NGF and BMP7 generated cellularized and vascularized tissues positive of VEGF antibody staining, with new dentine formation over the surface of native dentinal walls in some, but not all, specimens. Newly regenerated dental pulp tissues were dense with disconnected cells surrounded by extracellular matrix. Multiple blood vessels with endothelial-like cell lining were present. Microscopic images showed that the entire root canal, from root apex to pulp chamber, was filled with vascular pulp-like tissues with innervation and odontoblast layers integrated to dentinal wall. In quantitative ELISA, co-delivery of bFGF or VEGF or PDGF with basal NGF and BMP7 yielded von Willebrand factor, dentine sialoprotein and endogenous NGF, thus suggesting that ectopically homed cells elaborate angiogenesis, mineralization and neurogenesis in regenerated dental pulp-like tissue. The study of Kim *et al.* (2010) was the first to use an ectopic model to demonstrate the regeneration of pulp-like tissue through the migration, proliferation and differentiation of host endogenous cells in endodontically treated root canals of real size, native human teeth. This regenerative process is thought to be guided by growth factors that filled the emptied root canals: bFGF and PDGF acted as biological signals for chemotaxis, PDGF and VEGF for vasculogenesis/angiogenesis, NGF for neural growth and

BMP7 for odontoblast differentiation and mineralization. These multiple cytokines, alone or in combination, induced cell homing, angiogenesis and mineralized tissue formation. The present study represents a founding model for recellularization and revascularization in endodontically treated root canals *in vivo*, although in an ectopic model, and a starting point towards a clinically translatable cell homing approach for dental pulp regeneration in humans.

The same group has also achieved pulp regeneration and angiogenesis in an ectopic tooth transplantation model with injection of bFGF alone in the empty root canal (Suzuki *et al.* 2011). Clinically extracted human teeth were endodontically treated as in patients, but without gutta-percha root filling. Then, collagen gel was injected into the root canals with or without delivery of bFGF, followed by *in vivo* teeth implantation into the dorsum of rats. After 3 weeks, bFGF induced recellularization and revascularization of the root canals, which were filled with connective tissue with abundant cells that integrated with the dentinal wall. Delivery of bFGF resulted in cellular migration and pulp-like tissue formation in the ectopic tooth transplantation model, thus suggesting a significant role of bFGF in the migration of endogenous progenitor cells for pulp regeneration. The present findings confirm that dental pulp regeneration is likely to occur by the recruitment and *in situ* differentiation of host stem/progenitor cells homed by chemotactic cytokines.

The *in vivo* regenerative potential of bFGF was compared with that of G-CSF in an ectopic tooth transplantation model by Takeuchi *et al.* (2015). They employed extracted porcine teeth, whose roots were cut out and injected with collagen and bFGF or G-CSF. Then, each root was transplanted subcutaneously in SCID mice. After 3 weeks, both growth factors determined the regeneration of pulp-like tissues with dentine formation along the dentinal wall. No significant differences were observed between bFGF and G-CSF in the total volume, in the cell density and in the capillary density of regenerated pulp tissues. As regards odontoblastic differentiation, the number of enamelysin-positive cells and DSPP-positive cells was similar in bFGF transplants to that in G-CSF transplants. On the whole, this study demonstrated the potential utility of bFGF in pulp and dentine regeneration *in vivo* through cell homing in comparison with G-CSF, even though further studies are needed in an orthotopic transplantation model to confirm that bFGF can be the alternative to G-CSF.

Pan *et al.* (2013) investigated the homing capacities of the cytokine SCF performing *in vivo* subcutaneous implantation experiments. SCF was dropped on collagen sponges which were then implanted into the dorsum of nude mice for 1 or 4 weeks. After 4 weeks, SCF effect on implant cell number and angiogenesis was highly significant: a sevenfold increase in the cell number and a more than ninefold increase in capillaries. Instead, its effect on collagen synthesis and scaffold remodelling was assessed after 1 week of implantation: there was a remarkable degradation of the scaffold thick collagen fibres that were replaced with thin collagen fibres forming smaller subunits. This finding of collagen sponge remodelling and collagen fibre neogenesis was supported by a significant increase in MMP2, collagen I and III gene expression. The regenerated pulp/dentine complex also displayed a new layer of predentine along demineralized dentine surfaces and oval-shaped round cells parallel to the dentine surface. Therefore, when subcutaneously implanted with collagen sponges, SCF enhanced cell homing, angiogenesis and tissue remodelling. The study showed that SCF use in dental pulp regeneration not only induced migration, proliferation and chemotaxis, but also promoted other benefits related to tissue engineering: neovascularization, degradation of exogenous collagen scaffolds and new collagen matrix synthesis. These benefits indicate SCF suitability as a potent aid in dental pulp regeneration.

Yang *et al.* (2015) set up a similar *in vivo* ectopic transplantation model to study the involvement of autophagy in SDF-1α-mediated dental pulp regeneration. They employed extracted human teeth roots, which were sectioned into 3-mm segments, irrigated and filled with silk fibroin scaffolds loaded with or without SDF-1α. The constructs were then subcutaneously implanted into the dorsum of immunocompromised nude mice for 8 weeks. Upon retrieval, no tissue was formed in the SDF-1α-free group, as only small clusters of cells were observed at the end of the scaffold. On the contrary, tooth fragments implanted with SDF-1α-loaded scaffolds had vascularized connective tissues with collagen matrix deposition in the canals, but no new dentine deposition. Moreover, immunostaining revealed the localization of autophagy-related proteins Atg5 and LC3 in the regenerated tissue.

The possible role of systemic bone marrow stromal cells (BMSCs[1]) in dental pulp regeneration and the enhancing effect of SDF-1 on cell recruitment and angiogenesis were evaluated *in vivo* by Zhang *et al.*

(2015). Extracted human premolars were sectioned along the crown-root junction, endodontically treated and disinfected. The roots were loaded with neutralized collagen gel with or without SDF-1 and implanted into subcutaneous pockets in the dorsum of mice. Before implant, BMSCs[1] labelled with green fluorescent protein (GFP) were transplanted into each mouse *via* the tail vein. Three weeks later, specimens were harvested for histological and immunohistochemical analyses. Ectopic dental pulp-like tissue and new blood vessels were regenerated in both the SDF-1 and SDF-1-free groups. In the SDF-1-free group, root canals filled with collagen scaffold alone revealed a small amount of cells and blood vessels and the residual collagen scaffold exhibited a grid-like morphology. In contrast, in the SDF-1 group abundant cells and a large number of blood vessels were found and the grid-like morphology of the scaffold disappeared. The difference in the newly formed blood vessels area between the SDF-1 group (6.13%) and the SDF-1-free group (1.52%) was statistically significant. GFP-positive BMSCs[1] could be observed in both groups, but their number in the SDF-1 group was significantly higher than in the SDF-1-free group. SDF-1 delivery homed significantly more GFP+ cells than without SDF-1 delivery, suggesting that SDF-1 induces a strong chemotaxis on BMSCs[1] towards the root canal. ALP expression also was found in both groups; however, it was stronger in the SDF-1 group than in the SDF-1-free group. This was the first study to provide direct evidence that systemic BMSCs[1] can home to the root canal *via* the bloodstream and participate in pulp-like tissue regeneration in ectopic endodontically treated human teeth. Furthermore, intracanal application of SDF-1 may improve BMSC[1] homing efficiency and angiogenesis.

Also, Ruangsawasdi *et al.* (2017) investigated whether SCF could induce cell homing in a pulpless immature root canal and promote regeneration of a functional pulp. Human-extracted immature premolars were accessed and irrigated, then filled with fibrin hydrogels with or without SCF and implanted on top of rat calvariae for 6 or 12 weeks. To assess the effect of SCF on tissue ingrowth, histologic analyses were performed: at 6 weeks, SCF increased the tissue ingrowth extent (21% ± 5% of the pulp space) compared to fibrin gel alone (6% ± 2%). Therefore, tissue ingrowth was accelerated by SCF application, and the morphology of ingrown tissue was also affected: it reached up to the middle third of the root canal, it had begun to form vessel structures, and the newly formed mineral matrix reached along the entire dentinal wall. The root apex was covered by connective tissue together with abundant blood vessels. In contrast, at 12 weeks, the addition of SCF did not result in a significant improvement of tissue ingrowth (47% ± 15% with SCF compared to 38% ± 13% without SCF), but the formed tissue in SCF-treated specimens was more mature. The ingrown tissue occupied over two-thirds of the root canal; odontoblast-like cells were lined along the dentinal wall and extended their processes further down the dentinal tubules. Moreover, revascularization appeared to be improved and a highly dense connective tissue resembling mineralized tissue had developed at the apical opening. In terms of gene transcription, RT-PCR analysis showed that the levels of DMP1, Col1 and VEGF were significantly upregulated, whilst those of DSPP and NGF were not affected by SCF. Overall, SCF increased tissue ingrowth at 6 weeks and formed a better developed pulp-like tissue at 12 weeks, thus showing the ability to recruit cells into immature human teeth. These results suggest that SCF can accelerate cell homing and pulp/dentine maturation in human immature teeth, thereby promising to improve current cell homing strategies in endodontic tissue engineering.

Focusing on other cytokines, Li & Wang (2016) combined PDGF-BB, NGF and BDNF in an *in vivo* approach for regenerating ectopic dental pulp-like tissue *via* cell homing. Extracted human incisors and premolars were disinfected and endodontically treated (just cleaned and shaped root canals). The cytokines were added at low concentration (L group) or high concentration (H group) to neutralized collagen scaffolds, which were injected into the pulp chambers and root canals. Next, the teeth were implanted subcutaneously into rat dorsa for 2 to 4 months. Upon retrieval, a histologic analysis was employed to identify the regenerated tissue: well-vascularized pulp-like tissue was present in both the groups, with more small vessels in the H group. At 4 months after transplantation, vascularization in the H group was similar in density and orientation to that of the normal pulp. CD-34 immunohistochemical analysis confirmed the presence of newly formed vessels in both the groups. However, S-100 immunohistochemical staining detected positive signals only in the H group, which indicated newly formed nerve fibres. Without using exogenous cells, the researchers successfully achieved the revascularization and reinnervation of regenerated pulp-like tissue by the combined use of PDGF-BB, NGF

and BDNF *via* cell homing. This study represents the first attempt to combine PDGF-BB and NGFs (NGF and BDNF) to obtain pulp-like tissue regeneration through a cell homing strategy. As a result, the study demonstrated that the combined delivery of PDGF-BB and NGFs is a promising *in vivo* approach for enhancing dental pulp-like tissue regeneration from endogenous stem/progenitor cells.

Unlike previous studies, Ruangsawasdi *et al.* (2016) investigated the effects of the implantation site in an animal model for pulp regeneration. Pulp-like tissue formation in teeth placed at the calvaria of rats was compared to that obtained at the dorsal area. Human immature third molars were standardized to a length of $3 \pm 1$ mm, root canals were cleaned with NaOCl and EDTA, and a fibrin gel scaffold was prepared without growth factors and injected into the canals. Four specimens with similar size of the pulp space were implanted per rat: two of them were placed on top of calvarial bone, whilst the others were implanted at the dorsum (control group). Six weeks after, the samples were examined for histological composition, immunoreactivity to dentine sialoprotein (DSP) and bone sialoprotein (BSP), percentage of tissue ingrowth and gene expression (DSPP, COL1, NGF and VEGF). Histologic analysis revealed that all specimens contained a newly formed tissue. In teeth placed at the calvaria, more than half of root canal was occupied by a highly vascularized and innervated connective soft tissue showing the typical four cell layers of dental pulp. Odontoblast-like cells were found in intimate contact with regenerated dentine at the dentinal wall, where DSP and BSP immunoreactivities were intense. In contrast, tooth specimens placed at the dorsum showed various types of ingrown tissue, such as adipose tissue with vacuoles and dense collagen tissue, and remnants of the fibrin gel. Tissue ingrowth analysis revealed that the area of ingrown tissue was significantly larger in the samples from calvarias (median of 90% related to the total pulp area) compared to those from dorsa (median of 57%). By gene expression analysis, DSPP levels were significantly higher in teeth from the calvaria compared to counterparts from the dorsum, especially in cells from the dentinal wall. Instead, COL1, NGF and VEGF expressions did not differ between the two implantation sites. These findings suggest that the calvarial site is superior to the dorsum to study *in vivo* pulp regeneration of human teeth ectopically transplanted in the rat. The calvarial space may provide a microenvironment similar to the tooth socket with various potential cell sources, including bone and periosteum. Therefore, it might be a better site for implanting teeth than the dorsum in cell homing studies.

*In situ pulp revascularization*

Only one paper dealt with *in situ* dental pulp revascularization in animals.

Yang *et al.* (2015) investigated the possible use of SDF-1α-loaded silk fibroin scaffolds without cell transplantation for *in situ* pulp revascularization accompanied by autophagy of pulpectomized mature teeth in dog. Mature premolars with complete apical closure of two beagle dogs were selected, and periapical lesions were induced. After root canal instrumentation and disinfection, either SDF-1α-loaded scaffolds or nothing (control group) were inserted into the canals following the induction of blood clot by gentle over-instrumentation. Three months after surgery, histological analysis of the specimens was performed. In both the control group and the SDF-1α-loaded scaffold group, regenerated intracanal tissues were evident, characterized as regenerated connective tissue (rCT) and regenerated mineralized tissue (rMT), whilst little nerve bundles were observed. In the control group, tissues were mainly rMTs deposited along the dentinal wall and in the middle of the canal, and rCTs with few blood vessels. Instead, in the SDF-1α group, there was mainly rCT in the middle of the canal with a thin layer of rMT deposited along the dentinal wall. Furthermore, a fibrous matrix similar to normal canine pulp was observed. As a whole, the SDF-1α-loaded scaffold group formed pulp-like tissues with neovascularization and dentine formation along the native dentinal wall and, compared with the control group, no mineralization was observed in the centre of the tissues. The formation of pulp-like tissues might be attributed to SDF-1α role in neovascularization and mineralization, as well as to its chemotactic function; nevertheless, innervation of the new tissue should be further clarified. The presence of autophagy in the regenerated tissues was examined by immunostaining: Atg5 and LC3 (autophagy-related proteins) were positively located in both the groups, but the number of positive cells was much higher in the SDF-1α group than in the control group. Therefore, SDF-1α triggered autophagy activation. The study demonstrated that SDF-1α-loaded silk fibroin scaffolds could effectively promote *in situ* pulp revascularization accompanied by autophagy of

pulpectomized mature dog teeth, thus establishing a model for possible clinical application of SDF-1α in regenerative endodontics.

## Conclusion

The *in vitro* experiments included in this review showed that multiple cytokines, alone or in combination, have the potential to induce many biological effects on human DPSCs and SCAP, amongst which migration, proliferation and differentiation are essential to achieve dental pulp regeneration. Not all described cytokines are needed in a single formulation; therefore, further studies should determine whether a specific subset, or even a single cytokine, can effectively recruit stem/progenitor cells from the host and promote their differentiation into multiple cell lineages to regenerate dental pulp. In this way, regenerative endodontics by cell homing could be clinically easier to perform and also economically convenient.

The *in vivo* experiments also employed different cell homing molecules, in both mature and immature teeth, from humans or animals. Almost all the studies managed to obtain regenerated connective pulp-like tissues in the emptied root canals and pulp chambers without transplanted cells, exploiting only host endogenous cells. Remarkably, all the samples had new vascularization, thus supporting the concept that angiogenesis is a priority in dental pulp regeneration. Moreover, in some cases, newly formed nerve fibres and new dentine deposition were observed, meaning that pulp regeneration was completed by neural and odontoblastic tissues.

Finally, the *in situ* pulp revascularization experiment achieved the regeneration of intracanal pulp-like tissues with neovascularization, innervation and dentine formation in an animal model. This represents a landmark for future studies aiming to apply regenerative endodontics to mature human teeth as well.

Despite the developments of the last few years, dental pulp regeneration *via* cell homing needs to be further understood and improved. Cell homing strategies need to be tested in orthotopic models, first animal and then human, and prospective clinical trials are necessary to identify their indications and contraindications. Several aspects need to be clarified to make it achievable and predictable in dental practice. The success of pulp regeneration relies on the clinical and biological conditions of the teeth; therefore, cell homing strategies will not be consistently successful in every condition, such as pulp necrosis. Root canal treatment currently remains the standard of care for mature teeth with necrotic pulps and closed apices (Huang 2011).

Despite such issues, cell homing currently represents the most clinically viable pathway for dental pulp regeneration (He *et al.* 2017), because it recruits the patient's own stem/progenitor cells through biological cues to restore the lost pulp tissues.

On the whole, the comprehensive knowledge on cell homing for dental pulp regeneration, which results from the present literature review, deals with:

- the validity of the scaffolds employed in the analysed studies; moreover, further improvements are in progress, such as nanofibrous technology and antibiotic addition (Bottino *et al.* 2017);
- the efficacy of growth factors, especially when dentine-derived, as reported in the latest update (Schmalz *et al.* 2017);
- the feasibility and safety of cell homing strategies in mature teeth with vital pulps from animals and subsequently from humans, although better characterization and standardization of the procedures are required (Galler & Widbiller 2017).

## Conflict of interest

The authors have stated explicitly that there are no conflict of interests in connection with this article.

## References

Becerra P, Ricucci D, Loghin S, Gibbs JL, Lin LM (2014) Histologic study of a human immature permanent premolar with chronic apical abscess after revascularization/revitalization. *Journal of Endodontics* **40**, 133–9.

Bottino MC, Pankajakshan D, Nör JE (2017) Advanced scaffolds for dental pulp and periodontal regeneration. *Dental Clinics of North America* **61**, 689–711.

Caplan DJ, Cai J, Yin G, White BA (2005) Root canal filled versus non-root canal filled teeth: a retrospective comparison of survival times. *Journal of Public Health Dentistry* **65**, 90–6.

Chieruzzi M, Pagano S, Moretti S *et al.* (2016) Nanomaterials for tissue engineering in dentistry. *Nanomaterials* **6**, 134–54.

Dammaschke T, Steven D, Kaup M, Ott KH (2003) Long-term survival of root-canal-treated teeth: a retrospective study over 10 years. *Journal of Endodontics* **29**, 638–43.

European Society of Endodontology (2016) European Society of Endodontology position statement: revitalization procedures. *International Endodontic Journal* **49**, 717–23.

Fawzy El-Sayed KM, Jakusz K, Jochens A, Dörfer C, Schwendicke F (2015) Stem Cell transplantation for pulpal

regeneration: a systematic review. *Tissue Engineering Part B: Reviews* **21**, 451–60.

Galler KM (2016) Clinical procedures for revitalization: current knowledge and considerations. *International Endodontic Journal* **49**, 926–36.

Galler KM, Widbiller M (2017) Perspectives for cell-homing approaches to engineer dental pulp. *Journal of Endodontics* **43**, S40–5.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 13625–30.

He L, Zhong J, Gong Q *et al.* (2017) Regenerative endodontics by cell homing. *Dental Clinics of North America* **61**, 143–59.

Huang GT (2011) Dental pulp and dentin tissue engineering and regeneration – advancement and challenge. *Frontiers in Bioscience (Elite Edition)* **3**, 788–800.

Huang GT, Garcia-Godoy F (2014) Missing concepts in *de novo* pulp regeneration. *Journal of Dental Research* **93**, 717–24.

Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S (2008) The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and BioRoot engineering. *Journal of Endodontics* **34**, 645–51.

Huang GT, Yamaza T, Shea LD *et al.* (2010) Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an *in vivo* model. *Tissue Engineering Part A* **16**, 605–15.

Ingle JI, Bakland LK (2002) Structure and function of the dentin-pulp complex. In: Ingle JI, Bakland LK, eds. *Endodontics*, 5th edn. Hamilton-London: BC Decker Inc, pp 121–43.

Iohara K, Imabayashi K, Ishizaka R *et al.* (2011) Complete pulp regeneration after pulpectomy by transplantation of CD105 + stem cells with stromal cell-derived factor-1. *Tissue Engineering Part A* **17**, 1911–20.

Kao RT, Murakami S, Beirne OR (2009) The use of biologic mediators and tissue engineering in dentistry. *Periodontology* **50**, 127–53.

Kim JY, Xin X, Moioli EK *et al.* (2010) Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. *Tissue Engineering Part A* **16**, 3023–31.

Kim SG, Zhou J, Ye L *et al.* (2012) Regenerative endodontics: barriers and strategies for clinical translation. *Dental Clinics of North America* **56**, 639–49.

Kim SG, Zheng Y, Zhou J *et al.* (2013) Dentin and dental pulp regeneration by the patient's endogenous cells. *Endodontic Topics* **28**, 106–17.

Laird DJ, von Andrian UH, Wagers AJ (2008) Stem cell trafficking in tissue development, growth, and disease. *Cell* **132**, 612–30.

Laureys WG, Cuvelier CA, Dermaut LR, De Pauw GA (2013) The critical apical diameter to obtain regeneration of the pulp tissue after tooth transplantation, replantation, or regenerative endodontic treatment. *Journal of Endodontics* **39**, 759–63.

Li L, Wang Z (2016) PDGF-BB, NGF and BDNF enhance pulp-like tissue regeneration *via* cell homing. *RSC Advances* **6**, 109519–27.

Liu JY, Chen X, Yue L, Huang GT, Zou XY (2015) CXC chemokine receptor 4 is expressed paravascularly in apical papilla and coordinates with stromal cell-derived factor-1α during transmigration of stem cells from apical papilla. *Journal of Endodontics* **41**, 1430–6.

Lovelace TW, Henry MA, Hargreaves KM, Diogenes A (2011) Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. *Journal of Endodontics* **37**, 133–8.

Mao JJ, Prockop DJ (2012) Stem cells in the face: tooth regeneration and beyond. *Cell Stem Cell* **11**, 291–301.

Mao JJ, Stosich MS, Moioli EK *et al.* (2010) Facial reconstruction by biosurgery: cell transplantation versus cell homing. *Tissue Engineering Part B: Reviews* **16**, 257–62.

Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Journal of Clinical Epidemiology* **62**, 1006–12.

Moretti S, Bartolommei L, Galosi C *et al.* (2015) Fine-tuning of Th17 Cytokines in Periodontal Disease by IL-10. *Journal of Dental Research* **94**, 1267–75.

Pan S, Dangaria S, Gopinathan G *et al.* (2013) SCF promotes dental pulp progenitor migration, neovascularization, and collagen remodeling - potential applications as a homing factor in dental pulp regeneration. *Stem Cell Reviews and Reports* **9**, 655–67.

Ruangsawasdi N, Zehnder M, Patcas R, Ghayor C, Weber FE (2016) Regenerative dentistry: animal model for regenerative endodontology. *Transfusion Medicine and Hemotherapy* **43**, 359–64.

Ruangsawasdi N, Zehnder M, Patcas R *et al.* (2017) Effects of stem cell factor on cell homing during functional pulp regeneration in human immature teeth. *Tissue Engineering Part A* **23**, 115–23.

Schmalz G, Widbiller M, Galler KM (2017) Signaling molecules and pulp regeneration. *Journal of Endodontics* **43**, S7–11.

Suzuki T, Lee CH, Chen M *et al.* (2011) Induced migration of dental pulp stem cells for *in vivo* pulp regeneration. *Journal of Dental Research* **90**, 1013–8.

Takeuchi N, Hayashi Y, Murakami M *et al.* (2015) Similar *in vitro* effects and pulp regeneration in ectopic tooth transplantation by basic fibroblast growth factor and granulocyte-colony stimulating factor. *Oral Diseases* **21**, 113–22.

Thibodeau B, Trope M (2007) Pulp revascularization of a necrotic infected immature permanent tooth: case report and review of the literature. *Pediatric Dentistry* **29**, 47–50.

Wang F, Jiang Y, Huang X *et al.* (2017) Pro-inflammatory cytokine TNF-α attenuates BMP9-induced osteo/

 

odontoblastic differentiation of the stem cells of dental apical papilla (SCAPs). *Cellular Physiology and Biochemistry* **41**, 1725–35.

Wigler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD (2013) Revascularization: a treatment for permanent teeth with necrotic pulp and incomplete root development. *Journal of Endodontics* **39**, 319–26.

Xiao L, Nasu M (2014) From regenerative dentistry to regenerative medicine: progress, challenges, and potential applications of oral stem cells. *Stem Cells and Cloning: Advances and Applications* **7**, 89–99.

Yang JW, Zhang YF, Wan CY *et al.* (2015) Autophagy in SDF-1α-mediated DPSC migration and pulp regeneration. *Biomaterials* **44**, 11–23.

Yildirim S, Fu SY, Kim K *et al.* (2011) Tooth regeneration: a revolution in stomatology and evolution in regenerative medicine. *International Journal of Oral Science* **3**, 107–16.

Zhang LX, Shen LL, Ge SH *et al.* (2015) Systemic BMSC homing in the regeneration of pulp-like tissue and the enhancing effect of stromal cell-derived factor-1 on BMSC homing. *International Journal of Clinical and Experimental Pathology* **8**, 10261–71.