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REVIEW ARTICLE

Advances in Research on Stem Cell-Based Pulp Regeneration

Hua-Nien Lee[1] · Cheng Liang[1] · Li Liao[1] · Wei-Dong Tian[1]

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Abstract Although root canal therapy is the most common and widely used treatment at clinical presentation, there are still some postoperative complications. As cell biology and tissue engineering techniques advance rapidly, the use of biological therapy to regenerate dental pulp has become a new trend; Relevant literatures in recent five years were searched using key words such as "root canal therapy", "Dental pulp stem cells", "Dental pulp regeneration", and "Cell homing" in PubMed, Web of Science, etc; Dental pulp stem cells (DPSCs) have multi-differentiation potential, self-renewal capability, and high proliferative ability. Stem cell-based dental pulp regeneration has emerged as a new research hot spot in clinical therapy. Recently, dental pulp-like structures have been generated by the transplantation of exogenous DPSCs or the induction of homing of endogenous DPSCs. Studies on DPSCs are important and significant for dental pulp regeneration and dental restoration; In this review, the existing clinical treatment methods, dental pulp regeneration, and DPSC research status are revealed, and their application prospects are discussed. The stem cell-based pulp regeneration exerts promising potential in clinical therapy for pulp regeneration.

Keywords Root canal therapy · Dental pulp stem cells · Dental pulp regeneration · Dental restoration · Cell homing

1 Introduction

The dental pulp locates in the root canal and communicates with the periapical tissues through a narrow apical foramen, which hinders the self-repairing capability when the dental pulp suffers from inflammation and injury under the influence of bacterial infection or tooth fracture. Currently, root canal therapy (RCT) is the most frequently used clinical manipulation for periapical lesions and dental pulp disorders. In RCT, the dental pulp tissue is extracted utilizing mechanical instruments, and the root canal is firmly cemented using inorganic materials. According to data from the American Dental Association, approximately 22 million RCT cases arise every year throughout the USA, incurring annual expenses of up to 20 $\sim$ 34 billion dollars. Although the success rate of treatment is 78–98% [1, 2], many obstacles remain in RCT.

Traditional RCT cannot promote the regeneration of pulp tissue and the re-development of the young permanent tooth root, making it easy to cause a tooth fracture. Besides, the development of the apical foramen of some young permanent teeth is hindered after treatment. The traditional apical induction method can only promote the formation of the apical mineralized tissue, and cannot produce the normal apical structure containing the pulp tissue, which leads to unsatisfactory long-term effects.

Hua-Nien Lee and Cheng Liang contribute to this article equally.

✉ Li Liao
lliao@scu.edu.cn

✉ Wei-Dong Tian
drtwd@sina.com

[1] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases and Engineering Research Center of Oral Translational Medicine, Ministry of Education and National Engineering Laboratory for Oral Regenerative Medicine, West China Hospital of Stomatology, Sichuan University, Chengdu 610044, China

After treatment of the affected teeth, the traditional filling material often discolors the crown, thus affecting its appearance [3]. A more ideal treatment to regenerate dental pulp is necessary for dental pulp disease.

The purpose of pulp regeneration is to form a functional dental pulp complex through the regeneration of dentin cells, reconstruction of the pulp blood flow, and innervation. The goal of pulp regeneration in young permanent teeth is to eliminate clinical symptoms, heal periapical lesions, regain pulp vitality, and strengthen the root canal wall.

In recent years, stem cell-based pulp regeneration has been developed as a new technology to treat pulp disease and has the potential to overcome many challenging clinical issues. Two decades ago, Gronthos et al. found that pulp contains stem cells and proposed the concept of dental pulp stem cells (DPSCs). DPSCs show self-renewal, proliferation, and multi-potent differentiation *in vitro*, and produce a dentin pulp complex after *in vivo* transplantation [4]. Research has shown that DPSCs possess a latent capacity to differentiate multi-directionally: for instance, differentiation into adipocytes, chondrocytes, osteoblasts, odontoblasts, nerve cells, and endothelial cells [5]. The findings reveal the possibility of extended application of DPSCs in tissue regeneration and cell therapy. Currently, DPSCs are a research focus in terms of tissue regeneration and engineering [6].

2 Pulp regeneration based on stem cell and tissue engineering techniques

The specific process of pulp regeneration based on stem cells and tissue engineering technology is to implant exogenous stem cells into the host root canal system to achieve pulp regeneration. The stem cells derived from allogeneic cells or hosts are isolated and amplified for pulp regeneration [7]. In the process of pulp regeneration, the biological scaffold materials can effectively maintain the survival of stem cells and vascular nerve regeneration. The microenvironment of pulp regeneration is also important for treatment. Stem cells can respond to changes in the microenvironment, undergo differentiation and division, and then repair tissue damage. Therefore, this article is aimed at reviewing the advances in stem cell-based pulp regeneration, summarizing the critical regenerative elements of stem cells, biomaterials, and the regenerative microenvironment, and finally discussing the barriers and prospects of the therapeutic application of stem cell-based pulp regeneration.

2.1 Dental pulp stem cells

Stem cells are partially differentiated cells that possess latent capacities to differentiate multi-directionally and to self-renew. In 2000, Gronthos et al. demonstrated the self-renewal ability DPSCs, which could be induced to differentiate into dentin-like cells from the third molar pulp group for the first time. This confirmed the existence of a stem cell population in dental pulp tissue [4]. DPSCs are undifferentiated mesenchymal cells with the ability of cell cloning and proliferation in pulp tissue. DPSCs have multiple differentiation potential and can differentiate into neuroectoderm-cells, adipocytes, odontoblasts, osteoblasts, chondrocytes, myoblasts, and other mesoderm-derived cells [8, 9]. DPSCs have a similar immunophenotype to bone marrow mesenchymal stem cells (BMSCs). Considering their plasticity, DPSCs have been used as seed cells in tissue engineering and regeneration in recent years [10, 11].

2.1.1 Proliferation and self-renewal ability

DPSCs have high proliferative ability and can be isolated and amplified from pulp tissue fragments attached to plastic Petri dishes and then transferred to other Petri dishes without interrupting cell proliferation. Stem cell characteristics are maintained *in vitro* for more than 20 generations. After adding bromodeoxyuridine to pulp tissue cultured *in vitro*, Gronthos found that DPSCs proliferated with higher hyperplasia than BMSCs[4]. A clone formation assay confirmed that DPSCs possessed strong self-renewal ability.

Some studies have used the gold standard, continuous *in vivo* transplantation, to validate DPSCs [12]. Human DPSCs in hydroxyapatite-tricalcium phosphate (HA/TCP) ceramic particles were implanted subcutaneously into nude mice for 6 weeks and formed dentin pulp-like tissue expressing the dentin-specific protein, dentin sialophosphoprotein, and dentine sialophosphate protein (DSPP). These mesenchymal cells in the transplants were then harvested and amplified *in vitro* and transplanted into nude mice again. These cells also produced human Alu positive dentin cells and dentin pulp complexes, indicating the ability of DPSCs to renew themselves *in vivo*.

2.1.2 Multidirectional differentiation potential

DPSCs have multiple differentiation potential under different induction conditions. A study of osteogenic differentiation showed that the DPSCs could differentiate into osteoblasts *in vitro* and *in vivo* under specific mineralization conditions. After subcutaneous transplantation into immunocompromised mice for 4 weeks, DPSCs generated

well-developed plate bone with bone cells in the bone lacunae. After lipogenic induction for 5 weeks, DPSCs generated lipid droplets and expressed two specific transcription factors in adipocytes [13]. DPSCs have the potential to differentiate into nerve cells. Researchers determined that DPSCs express glial fibrillary acidic protein and nestin, which provide nutritional support for dopamine-powered neurons. Importantly, after *in vivo* transplantation, DPSCs could generate odontoblast-like cells and pulp-like tissue, suggesting their potency for dental pulp regeneration [14]. DPSCs derived from deciduous teeth also possess robust regeneration capacities. Several studies found that stem cells from underage individuals have a stronger regeneration ability than adult MSCs, suggesting an alternative seed cell source in dental pulp regeneration [15].

2.2 Selection of biomaterials for dental pulp regeneration

The selection of a suitable scaffold material is essential for the success of pulp regeneration. The scaffolds commonly used in dental pulp tissue engineering can be divided into two categories: Natural scaffolds and artificial polymers.

The natural scaffolds used for pulp regeneration mainly include recombinant collagen and silk fibroin. Prescott et al. inoculated DPSCs onto a dentin matrix protein 1 (DMP1)-collagen scaffold and placed it into a dental implant in mice [16]. The formation of pulp-like tissue was observed six weeks later. Recently, platelet-rich plasma (PRP), platelet-rich fibrin, and blood clots have been used as scaffolds for pulp regeneration [17–19].

Artificial polymer scaffolds for dental pulp regeneration are mainly polyglycolic acid (PGA), polylactic acid (PLA), and poly lactic-co-glycolic (PLAGA). The selection of artificial polymerized porous scaffolds for stem cell survival and vascular and nerve regeneration is crucial for pulp regeneration. To reconstruct the pulp tissue, the scaffold material needs to have excellent biodegradability, such that the newly formed tissue can replace the original degraded scaffold to form a regenerative structure. Scaffold materials should also have appropriate pore size to facilitate the entrance of cell nutrients and oxygen [20]. The scaffold can also carry growth factors that are beneficial for stem cell proliferation, differentiation, and adhesion [21].

2.3 The micro-environment of pulp regeneration

The survival, proliferation, and differentiation of DPSCs *in vivo* depends on the appropriate microenvironment. Establishing a microenvironment conducive to regenerative process is the key to pulp regeneration.

2.3.1 Cytokines and signaling molecules

Cytokines and signaling molecules in the stem cell niche are essential for the proliferation, differentiation, and migration of DPSCs [22]. Suitable signaling molecules play a critical role in the regeneration of dental pulp. Several cytokines and signaling molecules have been applied in dental-pulp regeneration. A pulp cavity implanted with growth factors or chemokines guides homing cells more easily and promotes the regeneration of pulp tissue [7]. Furthermore, cytokines that induce pulp angiogenesis are also helpful for the survival of stem cell transplants [23, 24]. Some common cytokines and signaling molecules are described below (Table 1).

2.3.2 Inflammation

Inflammation is considered an important factor to induce pulp regeneration [25]. During the recovery process, inflammation stimulates DPSCs hidden in the blood vessels to migrate to the injured location, proliferate, and eventually differentiate into endothelial cells, which can form neovascularization [26]. Inflammation also promotes the regeneration course of dental pulp vessels by inducing the expression of basic fibroblast growth factor, vascular endothelial growth factor (VEGF), and transforming growth factor β (TGF-β) in the damaged pulp cells and endothelial cells [27–29]. Pulp cells isolated from dental caries strongly expressed vascular factors and can be used as seed cells for pulp vascular regeneration, suggesting that pulp cells in the inflammatory environment can guide vascular regeneration [30]. These studies implied that inflammation facilitates stem cell migration and differentiation, and enhances the expression of angiogenic factors, which helps to regenerate blood vessels.

2.3.3 Oxygen supply

DPSCs showed faster proliferation, a higher vascular formation ability, and a stronger vascular factor secretion ability when cultured under hypoxic conditions. Cultivated DPSCs and periodontal ligament stem cells in a hypoxic environment possess robust proliferation and differentiation abilities [31]. Hypoxia-inducible factor 1 (HIF-1) is expressed rapidly in DPSCs cultured in a hypoxic environment and regulates the expression of various vascular factor-related genes, including placental growth factor (PIGF), angiogenin, platelet-derived growth factor (PDGF), and vascular endothelial growth factors (VEGF). Meanwhile, HIF-1 also regulates stromal cell-derived factors, sphingosine phosphate, and their receptors [32]. When new angiogenesis and oxygen levels reverse to customary, HIF-1 expression declines, resulting in decreased

Table 1 Signal molecules used in dental pulp regeneration

Signal molecules	Effects
Exocrine	miR- 15/16, miR- 17, miR- 31, miR- 221/222, miR- 320a, miR- 424, miR- 126,
Stem cell factor	Induction of cell homing
Vascular endothelial growth factor	Activation of tyrosine kinase
	Promotes endothelial cell proliferation, migration, and capillary maturation
Granulocyte colony-stimulating factor	Promotes the vascular potential of stem cells
Fibroblast growth factor	Promotes endothelial cell proliferation and migration
Hypoxia-inducible factor 1	Intervenes in the expression of platelet-derived growth fctors, vascular endothelial
Stromal cell-derived factor 1	Encourages the SDF- 1α/CXCR4 signaling axis which participates in cell homing
Angiogenic hormone	Activation and stabilization of angiogenesis through competitive interactions with Tie-2
Blood platelet-derived growth factor	Promotes the migrating and proliferation of perivascular support cells

expression of a series of vascular factors [24, 33]. The experiments above showed that DPSCs have an enhanced vascular formation ability in a hypoxic environment, demonstrating that a hypoxic environment might be one of the most crucial conditions for pulp vascular regeneration.

2.3.4 Dental pulp revascularization

Blood transport reconstruction and vascular regeneration are essential for pulp regeneration. Dental pulp revascularization refers to the reintroduction of blood vessels in the root canal system. Pulp blood circulation regeneration promotes the regeneration of active pulp-like tissue in the root canal and assists the root to develop. The core of pulp revascularization is to provide a suitable matrix (such as an autologous blood clot or platelet-rich plasma) and concentrated growth factors to facilitate vascular regeneration [34].

2.3.5 Nerve regeneration

Dental pulp nerve regeneration can make the teeth respond protectively when they are stimulated by mechanical, temperature, or chemical elements, and thus can maintain the long-term survival of teeth. DPSCs also possess the potential to express nestin, a neuroepithelial stem cell surface marker, and to differentiate neuronally, which allows the generation of glial cells and neurons. Woods et al. confirmed that DPSCs have a special effect on the treatment of adult brain injury [35]. Studies have shown that the utilization of certain neurotrophic drugs and electrical stimulation positively increase the ability of nerve regeneration. Thus, the application of nerve induction conditions in stem cell masses and local injection of nerve regeneration drugs might represent a novel research direction in pulp regeneration.

3 Basic research and the clinical application of dental pulp regeneration

Currently, mesenchymal stem cells that are known to have differentiation potential include DPSCs, dental papilla stem cells, dental sac stem cells, bone marrow stromal stem cells, exfoliated deciduous tooth pulp stem cells, and other dentin-like cells. Among these stem cells that can be exploited for the regeneration of dental pulp, dentin, and periodontal ligament, DPSCs are discussed most frequently in related research. As eminently self-renewable, multiphase differentiating, and proliferative cells, DPSCs occupy an essential position in the regenerating and healing process of the dental pulp. We discuss the application of DPSCs in dental pulp regeneration from two perspectives, cell homing and stem cell transplantation, in the following section.

3.1 Basic research on pulp regeneration

3.1.1 Stem cell transplantation

Research has shown that dental pulp or other stem cell transplantation can produce ectopic pulp-like tissue [36–40]. Moreover, the number of transplanted cells can be controlled and the cell subspecies with the best potential efficacy for pulp regeneration can be selected. DPSCs and stem cells from human exfoliated deciduous teeth (SHED) are the most widely used mesenchymal stem cell (MSC) seed cells. There are four main applications of exogenous MSC transplantation for pulp regeneration: (1) Simple MSC transplantation and organic or synthetic scaffolds; (2) MSC co-transplantation with microvascular endothelial cells to provide additional vascularization; (3) MSC pretreatment or transplantation in combination with growth

factors; and (4) Transplantation with self-organized MSC aggregates or granules [39, 41].

Preliminary studies by Kuo et al. and Iohara et al. showed that implantation of DPSCs with bone morphogenetic protein 2 (BMP2) into incisors of canine teeth achieved histologically effective pulp regeneration [40, 42]. A follow-up study conducted by the same team in 2009 tested pulp regeneration using CD31-, CD146- subtypes of SP cells after complete pulp cutting. The pulp regeneration potential of piglet autogenous DPSCs in the jaw micro-environment was tested in Kodonas et al. in 2012 [43], which confirmed that DPSCs can successfully regenerate pulp-like tissue with characteristics similar to normal pulp morphology. Iohara et al. showed that the cell layer had the typical columnar morphology of polarized dentin cells and extended to the dentine tubules to the cementum–enamel junction [44].

3.1.2 Cell homing

Cell homing represents an alternative option for dental pulp regeneration. Stem cell homing can supply host endogenous cells to the injured tissue [45]. Cell homing in pulp regeneration refers to the recruitment of host endogenous stem cells into tooth root canals by the induction of signal molecules to form pulp-tooth-like tissues. Cell homing avoids the cell processing steps needed for cell transplantation and makes full use of the patient's stem cells, which to some extent reduces the difficulty and risk of operation [46].

In recent studies, stem cell homing was induced through apical hemorrhage. For immature dead pulp teeth, inducing bleeding can cause a large number of undifferentiated mesenchymal stem cells to pour into the root canal [47]. The new tissue that grows into the root canal is periodontal ligament-like, odontoid, or bone-like, which may be related to stem cells derived from residual living pulp and the apical papilla [48].

Other cell-independent methods applied growth factors and cytokines to induce the migration of endogenous stem cells into the root canal. For example, granulocyte colony-stimulating factor (G-CSF) was used to stimulate the mobilization of DPSCs to promote DPSCs-based pulp regeneration [15], suggesting that autologous cell therapy is feasible. Although the therapeutic effect still needs to be confirmed in further studies, its feasibility was proven by the recent proof-of-concept research, indicating a broad prospect of clinical application [49].

3.2 Clinical application of pulp regeneration

Some progress has been made in animal experiments, after which several pioneer studies have been performed to explore the possibility of stem cell-based dental pulp regeneration in the clinic. In 2017, Nakashima et al. performed in situ transplantation of DPSCs in five patients with irreversible pulpitis and monitored them for up to 24 weeks [50]. Using cone-beam computed tomography and magnetic resonance imaging, experts confirmed that three of the five patients showed complete pulp regeneration and dentin formation. Besides, four of the five patients were positive for electric pulp testing (EPT) at 24 weeks after follow-up, indicating that the regenerated pulp restored nerve activity.

One randomized clinical trial using SHED aggregates for functional pulp regeneration in population cavities in 2018 provided stronger evidence (Fig. 1) [51]. In that study, children aged 7 to 12 years old with traumatic teeth received transplantation of the aggregates of autologous SHEDs. The trial included 36 patients with pulp necrosis, including 26 in the treatment group and 10 in the control group. All patients were followed up for 12 months. Xuan et al. found that SHED transplantation regenerated the entire pulp tissue with blood vessels (as demonstrated using laser Doppler flowmeter) and nerves (as shown by radiological and histological examination) [51]. A more important clinical finding was that the root length of immature permanent teeth and the width of the apical foramen decreased after SHED implantation, which indicated that the regenerated pulp has the normal function of maintaining the continuous development of the root. Studies by Nakashima and Xuan et al. reported that pulp stem cell application has no adverse effect or toxicity, which confirms their clinical safety [51, 52]. These results provide the first clinical evidence that pulp stem cell transplantation can regenerate complete pulp tissue with physiological and neurovascular functions.

4 Key steps of pulp stem cell transplantation

To achieve the ultimate goal of pulp regeneration, many possible factors should be taken into consideration besides the difficulty of reconstructing dental pulp. Considering the possible effects of mechanical instruments and chemical disinfection preparations on subsequent pulp regeneration, the preparation of the pulp cavity and procedure of stem cell transplantation are also important. The process of pulp stem cell transplantation includes opening pulp to expose the root canal and remove the infected pulp. Followed by selecting the reagent for washing and the sealing agent, washing and disinfecting the root canal, and seal to ensure that the pulp cavity cannot be infected. After preparation, the cultured DPSCs are implanted into the internal root canal cavity, and the crown is closed using materials selected to prevent the infection of the DPSCs. The

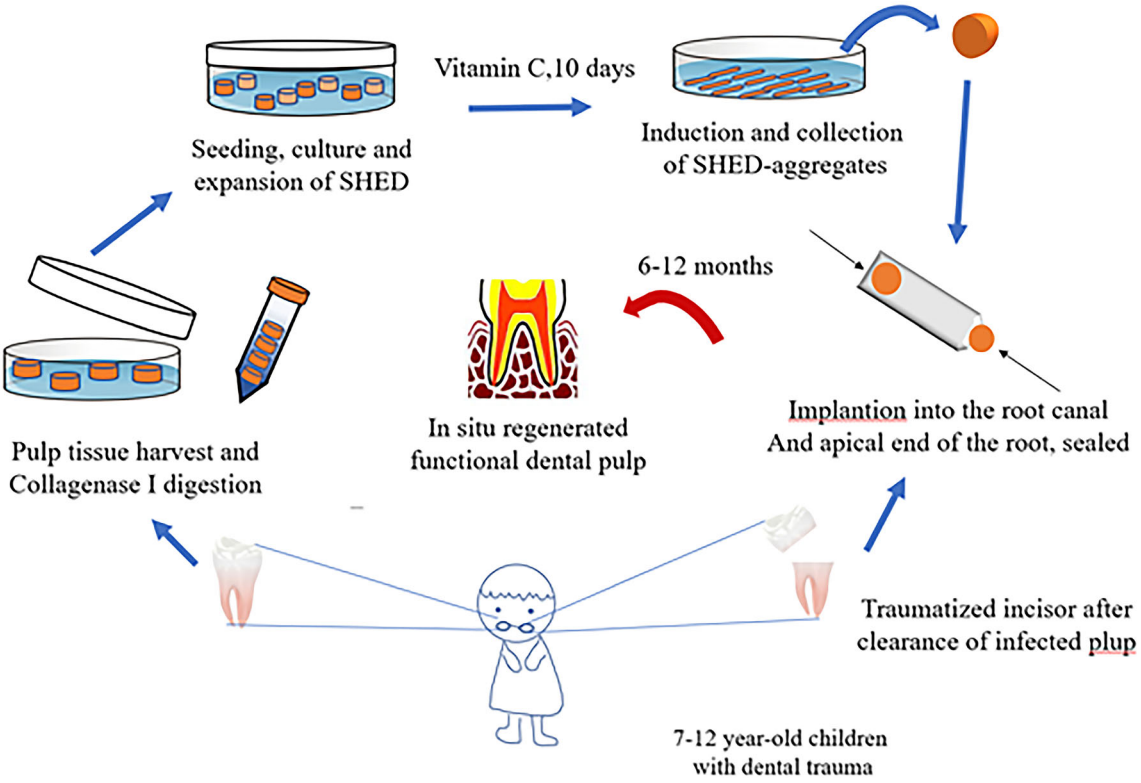

Fig. 1 The schematic diagram of SHED aggregates for functional pulp regeneration in a randomized clinical trial

following sections will focus on the treatment of the pulp cavity, root canal preparation, disinfection, flushing agent selection, selecting materials for crown sealing.

4.1 Treatment of the dental pulp cavity

The primary manipulation for successful pulp vascular regeneration is to thoroughly eliminate microbes and necrotic tissue from the pulp cavity. Under local anesthesia, dentists exploit a rubber barrier to isolate pulpitis teeth and then eradicate the saprophytic pulp, followed by careful meterage of the working length of the root canal. After repeatedly rinsing the pulp cavity, doctors stretch the sterile paper tip to dry the pulp cavity, add the root canal medicine, and then seal the tooth temporarily for 2–6 weeks. Full and thorough root canal disinfection, microenvironmental matrix promotion (inducing blood clot formation), and tight crown closure are essential elements for new tissues to generate in the root canal.

4.2 Root canal washing

Flushing plays an important role in primary root canal disinfection. Flushing agents should have the greatest bactericidal and bacteriostatic effect and have the least cytotoxicity to stem cells and fibroblasts to maintain their vitality, proliferation, and differentiation abilities. At present, there are several kinds of flushing agents in clinical application:(1) hydrogen peroxide: Its foaming effect can remove root canal exudation and necrotic tissue, and has a hemostatic function. (2) Sodium hypochlorite: Sodium hypochlorite is the most extensively applied root canal flushing AGENT, which can dissolve the organic material in the saprophytic pulp tissue and smear layer, combined with high-efficiency bacteriostasis and low cytotoxicity [53]. (3) Chlorhexidine has a broad-spectrum antibacterial effect, similar to sodium hypochlorite. In particular, it can inhibit gram-positive bacterial infection. (4) Ethylenediamine Tetraacetic Acid (EDTA) is a potent chelating agent and is regularly used in combination with other flushing agents, such as sodium hypochlorite solution, in clinical application. Chelation using metal ions is necessary for bacterial growth; therefore, it has certain anti-microbial properties [54–63]. (5) MTAD is a new type of root canal flushing solution, which comprises doxycycline, citric acid, and detergent (polysorbitol ester-80). MTAD can effectively remove the smear layer, does not destroy the physical properties of dentin, and has a strong anti-microbial effect.

However, several studies found that the flushing agents can affect the regeneration of DPSCs. Ring et al. found that sodium hypochlorite is not biocompatible and will kill

DPSCs, preventing their adhesion to the root canal surface [56]. Chen et al. supported the view that if sodium hypochlorite is used in dental pulp vascular regeneration, it should be mixed with normal saline to remove the residual toxicity that can weaken the regeneration reaction [58]. A recent study has showed that chlorhexidine irrigation has cytotoxic influences on human stem cells and interferes with the association of DPSCs to the pulp cavity wall. In addition to root canal flushing fluid, root canal irrigation also affects the success rate of pulp vascular regeneration [56]. Da et al. recommended using the Endovac system for sodium hypochlorite root tip negative pressure washing [59]. Nielsen et al. believed that negative pressure flushing of the Endovac system can break the gas plug in the apical region (the root canal is 3 mm from the root tip and a mass of air column produced by the hydrolysis of organic tissue by sodium hypochlorite solution), thus removing the debris [60].

4.3 Preparation of the root canal system

Many scholars agree that there is no need for mechanical preparation of the root canal system in the treatment of pulp revascularization, to maintain stem cell activity and avoid weakening the root wall [61–65]. Mechanical preparation might also destroy stem cells present in the apical region [66–68]. Chen et al. also supported the use of minimal root canal preparation in the root canal crown to facilitate the elimination of saprophytic pulp, thus ensuring no interference with pulp vascular regeneration [69]. However, it remains a challenge to transplant exogenous stem cells into the narrow and curved root canal. Novel equipment for stem cell transplantation is necessary to facilitate such surgery.

4.4 Disinfection of the root canal

Endodontic disinfection drugs affect the success of pulp vascular regeneration. Usually, Hoshino paste, consisting of tri-antibiotics minocycline, ciprofloxacin, and metronidazole, is recommended as a root canal disinfectant [70]. Comparative studies using these antibiotics, alone or in combination, found that for infected dentin and pulp periapical lesions, combined use can have a continuous bactericidal effect on all bacterial samples. Moreover, Sato et al. believed that removing the bacteria in the necrotic pulp of young permanent teeth created an environment conducive to the regeneration of blood vessels and cells [71]. However, the side effects, including discoloration of the crown, bacterial resistance, and allergic reactions, limited their application [72].

Calcium hydroxide has been used successfully used in general root canal disinfection. Compared with other antibiotic pastes, calcium hydroxide has antibacterial properties, does not discolor teeth, and promotes growth factors and biomolecular secretion from dentin. Several studies demonstrated that calcium hydroxide can promote root elongation, root canal wall thickening, and pulp vascular regeneration [72]. However, some studies suggested that the high PH value of calcium hydroxide could kill the stem cells in the remaining pulp or apical papilla, or change the nature of dentin [73, 74].

4.5 Root canal coronal sealing

To avoid the reinfection of the root canal caused by bacteria and to achieve pulp vascular regeneration, strict coronal sealing filling and sealing should be carried out [73]. According to the advice of most scholars, double-layer materials can be closed above blood clots, such as coronal filling using mineral trioxide aggregate (MTA) and resin bonding [62, 65, 69, 75–78]. Some scholars also recommend the sealing of three layers of materials, i.e., adding a layer of glass ions between the MTA and the composite resin as the second layer of closed materials, to seal the coronal more closely [79]. MTA is often in direct contact with the dental pulp tissue and blood clots within the root canal, reproduce organization in dental pulp vascular regeneration surgery, has beneficial biocompatibility, sealing stability, and strong bacteriostasis, as well as good hydrophilicity and unique hard-solid properties. However, the curing time of MTA is long, the cost is high, and the tooth tissue is easily discolored. Khetarpal et al. suggested the use of tricalcium silicate (Biodentine) instead of MTA placed above the blood clot [80]. Biodentine is a kind of dentin substitute with good biological activity and sealing characteristics. The time required to achieve hard fixation is shorter than that of MTA, the clinical operation process is simpler and the possibility of the color change of the coronal is avoided.

5 Summary

Using regenerative dental pulp therapy, it is possible to restore the intact structure of the affected teeth. The three-dimensional dental pulp-like structure formed by DPSCs could be transplanted *in vivo* and provides an induced micro-environment for blood flow reconstruction and nerve regeneration, thus ensuring the survival and function of the transplanted tissue. To achieve successful dental pulp regeneration, suitable seed cells, a scaffold, and a regenerative microenvironment are necessary to ensure the survival, migration, proliferation, and differentiation of stem cells. Moreover, the preparation method of a root canal, the selection of disinfection preparation, physical and chemical

washing methods, and the selection of washing preparation will lay a good foundation for the successful transplantation of stem cells in the clinic. The most challenging issue is how to optimize and integrate each composition process into a whole, and finally realize the regeneration of the pulp dentin complex. Thus, a multi-disciplinary and comprehensive research process is necessary for future dental pulp regeneration.

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Declarations

Conflict of interest The authors declaed that there is no conflicts of interest.

Ethical Statement There are no animal experiments carried out for this article.

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