Pomeranian J Life Sci 2021;67(2):10-17 doi: 10.21164/pomjlifesci.718

Tissue engineering application in regenerative endodontics

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ABSTRACT

Regenerative endodontics can be defined as a group of procedures that use tissue engineering, gene therapy, and stem cell-based approaches to regenerate tissue in tooth cavities. One of the main advantages of dental pulp revitalization compared to traditional endodontic treatments is that, by maintaining living tissue in the tooth cavity, teeth retain their defensive, nutritional, and sensory abilities which can provide protection against potential infection. This review discusses the application of stem cells in dental pulp regeneration, with an emphasis on tissue engineering strategies. In particular, tissue engineered scaffolds, which can provide a supportive structure for stem cell growth, will be examined. We discuss characteristics of an ideal scaffold, the use of growth factors which can have an important impact on cell proliferation and differentiation, and current therapies based on tissue engineering for dental pulp revitalization.

Keywords: growth factors; regenerative endodontics; scaffold; stem cells; tissue engineering.

INTRODUCTION

Regenerative endodontics can be defined as biologically-based procedures, designed to restore or replace damaged tissue structures with live viable tissues, ideally of the same origin, and allows the physiologic functions of the pulp-dentin complex [1, 2, 3]. The aim of regenerative endodontics is to preserve tooth function and viability by revitalizing pulp that has been damaged by disease, inflammation or trauma and to replace necrotic pulp tissue with new pulp-like tissue. Traditional endodontic therapy for irreversible pulpitis includes dental pulp extirpation under local anesthesia or in the case of pulp necrosis, an antiseptic root canal treatment. Regenerative endodontic approaches that involve cell homing and transplanting stem cells into the tooth cavity provide alternatives to conventional treatments [1, 4, 5, 6, 7].

Under normal physiological conditions, the tooth cavity is filled with pulp tissue. Dental pulp is an immature type of embryonic connective tissue that ends at the apex. The multifunctional tissue is responsible for regulating inflammation, as well as other defence reactions, and is a source of stem cells that repair and regenerate the endodontium [8, 9, 10, 11, 12, 13]. Pulp and periodontal tissue, referred to as mixed tissue or Black's tissue, lies between physiological and anatomical openings and has excellent regenerative properties [8]. Pulp is involved in the formation of dentin, provides nutrients, and contributes to the sensory and defensive processes of teeth. Keeping pulp alive and healthy provides the body with a defensive barrier and any disruption to this protective layer can lead to infection [8, 11, 12, 13]. Thus, maintaining pulp function can have a significant effect on the long-term preservation of teeth. Without viable pulp, the mechanical properties of teeth are reduced by up to 82% [8]. This is often the case after root canal treatment, particularly treatment which involves mesio-occlusion-distal preparations. As well as this, colonizing microorganisms in the apical delta or lateral canals cannot be completely removed after root canal procedures, often leading to reinfection. Pulp revitalization has advantages over traditional root canal treatments since revitalized pulp has defensive properties and triggers the immune system to combat microorganisms by recruiting natural killer cells, B and T lymphocytes, and antibodies [14, 15]. Moreover, exposure to X-rays (necessary control images) and complications related to filling the canal can be avoided, e.g., extrusion of material, underfilling of the canal, or thermal irritation of periapical tissues [1, 8, 16].

This paper presents the current state of knowledge on the use of tissue engineering in pulp regeneration procedures, paying particular attention to factors that determine the success of therapies including scaffolds, growth factors and stem cells. The PubMed database and collected literature were used as a source of peer-reviewed articles to present the topic of tissue engineering in regenerative endodontics.

TISSUE ENGINEERING

Tissue engineering is a branch of medicine that deals with the restoration, maintenance, or improvement of tissue and organ function [2, 3]. The term was 1st coined in the 1980s at a National

Science Foundation Conference in Washington and is defined as "the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function" [3]. In 1993, Lanza et al. defined tissue engineering as "an interdisciplinary field which applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve organ function" [2]. According to Lynch, 3 key elements, also known as the tissue engineering triad, are essential in the promotion of tissue regeneration: scaffolds (natural, synthetic), growth factors (signals, morphogens), and stem cells (Fig. 1) [3].

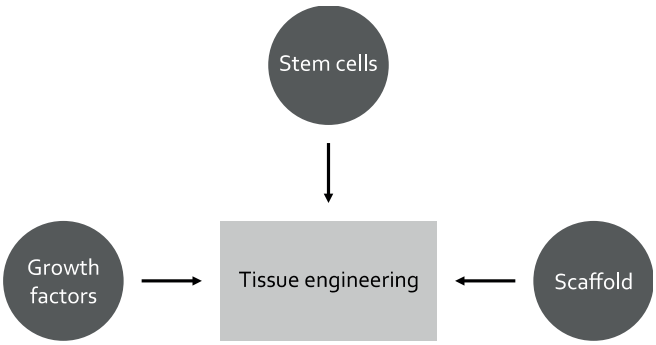

FIGURE 1. Elements of tissue engineering necessary for proper regenerative processes

SCAFFOLD

Scaffolds are 3D porous biomaterials providing the structural framework for cells to grow and produce de novo tissue. The scaffold itself should be biodegradable, without releasing toxic breakdown products, and the rate of degradation should be similar to the rate of new tissue formation [17, 18, 19, 20]. The ideal scaffold should allow effective transport of oxygen, nutrients and waste. The scaffold should preferably be a 3D replica of the extracellular matrix with a high porosity and an adequate pore size to facilitate cell seeding and diffusion throughout the whole structure and provide mechanical and biological support for stem cells inside the body. In addition, the scaffold should have adequate mechanical and physical strength and promote cell migration, proliferation, and differentiation. Scaffolds can be divided according to origin (biological or synthetic), form (porous sponges, solid blocks, sheets, hydrogels), presence of cells and degradability of matrices [18, 20]. Scaffolds used in pulp regeneration include induced blood clots, autologous platelet concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), synthetic polymers, bioactive ceramics and glass, and naturally derived biomaterials [21, 22].

Blood clots

A blood clot is considered a suitable scaffold for filling the intracanal space and provides a suitable framework for new tissue growth. The fibrin component traps cells necessary for tissue regeneration and allows fibroblasts and macrophages to migrate from the periapical region. Moreover, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are naturally present in blood clots [1, 20, 21, 22]. The limitations of using induced blood clots as scaffolds are mainly associated with their variability.

Thibodeau and Trope demonstrated the use of a blood clot scaffold for pulp revitalization in an immature permanent tooth with injury-induced necrosis [23]. A sterile instrument was used to induce bleeding inside the root canal and the resulting clot was protected with mineral trioxide aggregate (MTA). The blood clot provided the scaffold to support stem cells from the apical papilla (SCAP). The results of the procedure were positive: the patient did not report any pain, there was no reaction to percussion or palpation, and pockets of proper depth formed. In addition, root growth was observed in terms of length and thickness, and apical closure was confirmed.

The advantages of the intracanal blood clot technique are clinical simplicity, low cost and that this is an autologous scaffold which does not promote foreign body response. The main obstacles and challenges that complicate the use of this therapy are the limitations in inducing bleeding and hemostasis in some groups of patients [21, 22]. As well as this, in some cases, insufficient bleeding from the dental papilla results in the formation of an incomplete clot that cannot fulfil the scaffold function by itself [15, 24, 25]

Platelet-rich concentrates

Platelet-rich concentrates, such as PRP and PRF are autologous scaffolds from the patient's own blood that reduce the risk of cross-infection [24, 25]. The scaffolds are well suited to regenerative endodontics because they contain important growth factors including transforming growth factor beta (TGF-β), PDGF, VEGF, and insulin-like growth factor-1 (IGF-1), which stimulate migration of stem cells from the periapical region as well as the production of collagen [25, 26, 27, 28, 29, 30, 31]. Platelet-rich plasma represents an injectable scaffold that belongs to the 1st generation of platelet-rich concentrates and has a different fiber structure to PRF. To derive PRP, blood is collected from the patient and centrifuged with anticoagulant until the PRP fraction precipitates. Then, polymerization of the fibrin is artificially induced by adding bovine thrombin. To isolate PRF, the patient's blood is collected into sterile tubes just a few minutes before the procedure, without the addition of anticoagulant, and centrifuged at a low speed resulting in a 3D fibrin network with a high concentration of active agents [31, 32, 33].

Platelet-rich concentrates are being used more and more often in pulp regeneration procedures [26, 27, 31, 34, 35]. A systematic review of platelet-rich concentrates used in regenerative endodontics showed better results in pulp sensitivity tests when compared to traditional methods for tooth pulp revitalization [25].

Chen et al. studied the cytobiological effect of PRF on dental pulp stem cells (DPSCs) and subsequent production of

pulp-dentin complex. Fragments of freshly extracted human teeth (with the pulp removed) were subcutaneously implanted into 3 groups of mice: DPSCs/PRF (group I), DPSCs (group II), and PRF (group III). After 8 weeks, the fragments were removed and histologically analyzed. The analysis showed well-organized pulp-like tissue and a layer of mineralized dentin-like tissue in group I, whereas the pulp-like tissue was less well organized in group II with a thinner layer of dentin-like tissue. In group III, stem cells were observed but with no functional pulp-like tissue [34].

Jadhav et al. compared teeth treated with PRF or a blood clot as the scaffold and demonstrated differences in the healing of periapical tissues, apical closure, and thickening of root canal walls. Platelet-rich concentrate displayed a more favourable outcome [29]. Bezgin et al. demonstrated that more than 50% of the teeth from the PRP-based scaffold group reacted positively to pulp sensitivity tests compared to the control group in which the same reaction was only obtained for about 20% of teeth [27]. Pinto et al. placed PRF in the root canal of a tooth with bone loss due to inflammation of periapical tissues at the apex [35]. After 6 months, correct tooth responses to thermal and electrical stimuli were observed and blood flow was confirmed using a laser Doppler flowmetry.

The benefits of platelet-rich concentrate include increased rates of angiogenesis and revascularization, which are essential for a successful regenerative endodontic therapy, while the main limitations in the use of this scaffold is the blood collection procedure and the necessity of obtaining blood from paediatric patients.

Synthetic polymers

Synthetic polymers, such as polyglycolic acid (PGA), polylactic acid, and polylactide-co-glycolide), are characterized by good formability and predictable changes in porosity and degradation [36, 37]. Mooney et al. were the 1st to study the possible use of synthetic polymers in regenerative endodontics [38]. The authors cultured PGA with human fibroblasts *in vitro* and found that the scaffold increased blood vessel proliferation and fibroblast differentiation. However, subsequent studies showed other types of scaffolds, e.g., collagen sponge, are superior; therefore, synthetic polymers are currently not the preferred choice of scaffold in regenerative endodontics [39].

The advantages of synthetic polymers are non-toxicity, biodegradability and the variability in application where structural strength is fundamental. Unfortunately, synthetic polymers might be also responsible for chronic or acute inflammatory host response [40].

Bioactive ceramics

Bioactive ceramics, including hydroxyapatite, biphasic calcium phosphate, and beta-tricalcium phosphate, are biocompatible and create a positive environment for cell growth and proliferation owing to their inherent porosity [41, 42, 43, 44, 45]. In tissue regeneration strategies, bio-ceramic scaffolds look promising, however, thus far, only a few published studies have demonstrated positive effects on dental stem cells [46].

Bioactive glass

Bioactive glass is a synthesized biomaterial with an amorphous structure and high mechanical strength that has been shown to have a positive effect on angiogenesis. For tissue engineering, bioactive glasses based on borosilicate are recommended. During the degradation process, bioactive glasses release ions that induce angiogenesis and osteogenesis [47]. Studies on the use of bioactive glass in pulp regeneration procedures are still ongoing. Owing to the relatively small number of studies that have been published to date, bioactive glass scaffolds are not currently used in regenerative endodontics.

Natural materials

Natural scaffolds include collagen, hyaluronic acid (HYA), amniotic membrane, and polysaccharides.

Collagen sponge is often used as a scaffold in regenerative procedures and can create the right conditions for growth and proliferation of stem cells [48, 49].

Biocompatible and biodegradable HYA can be used as a 3D sponge and creates suitable conditions for both blood vessel proliferation and stem cell differentiation in pulp regeneration procedures. Moreover, when applied to uncovered pulp, HYA stimulates the production of reparative dentin [44, 48].

Amniotic membrane is characterized by good biocompatibility and low immunogenicity and contains many growth factors, making it an excellent scaffold for regeneration procedures. Amniotic membrane structure is similar to that of pulp and provides an appropriate environment for growth and the proliferation of stem cells [50].

Polysaccharides are easily accessible biocompatible compounds and, according to the latest research, promising scaffold materials for regenerative endodontic procedures [18, 51, 52]. In tissue engineering, polysaccharides such as agar, alginate, cellulose, and chitin are often used. Agar is mainly composed of agarose and offers a stable substrate for cell growth and differentiation [53]. Alginate is a natural scaffold with poor mechanical properties but can be used as an injectable hydrogel or porous skeleton presenting a natural structure that is rich in growth factors to enable cell proliferation [54, 55]. Cellulose is a linear polymer composed of glucose molecules that are synthesized by fungi and some bacteria. In tissue engineering, cellulose is used as a porous membrane to induce angiogenesis. Chitin is a compound built from N-acetylglucosamine monomers. Porous glycol chitin-based hydrogel scaffolds have been shown to promote stem cell differentiation, and are a promising material for regenerative processes [52, 56].

GROWTH FACTORS

Growth factors (signals, morphogens) are proteins that bind to certain cell receptors and play an important role in stem cell proliferation and differentiation. Most growth factors have a broad spectrum of influence on cells, however, some act more selectively [57]. Studies have shown that proteins in dentin induce tissue responses and play a key role in the repair

processes of the pulp-dentin complex. The release of growth factors from dentin is triggered by superficial demineralization. The most important growth factors released from dentin are presented in Table 1. The TGF-β family is an important group of growth factors associated with odontoblast differentiation and stimulation of dentin matrix secretion. Transforming growth factor beta is secreted by odontoblasts and stored in the dentin matrix in its active form through interaction with other matrix factors [58]. Bone morphogenetic proteins

TABLE 1. Growth factors in root dentin [11, 38, 42, 47, 57, 60]

Growth factors	Role in pulp regeneration
Bone morphogenetic protein 2, 4, 6 (BMP-2, BMP-4, BMP-6)	stimulates odontoblast differentiation, increases alkaline phosphatase activity, and induces DPSCs
Epidermal growth factor (EGF)	stimulates neurogenic differentiation of DPSCs and SCAPs
Fibroblast growth factor (FGF-2)	stimulates chemotaxis in stem cells and angiogenesis
Hepatocyte growth factor (HGF)	stimulates migration and proliferation of mesenchymal stem cells
Vascular endothelial growth factor (VEGF)	stimulates angiogenesis
Insulin-like growth factor 1, 2 (IGF-1, IGF-2)	stimulates proliferation and differentiation of DPSCs and SCAPs
Platelet-derived growth factor (PDGF)	stimulates angiogenesis, chemotactic migration of mesenchymal stem cells (MSCs), and odontoblast differentiation
Transforming growth factor beta 1, 2, 3 (TGF-β1, TGF-2, TGF-3)	stimulates odontoblast differentiation, differentiation of DPSCs, and tertiary dentin formation

DPSCs – dental pulp stem cells; SCAPs – stem cells from the apical papilla; MSCs – mesenchymal stem cells

(BMPs) are another important family of growth factors and are responsible for tooth development and regeneration [59]. Recombinant morphogenetic proteins 2, 3, and 7 (BMP-2, BMP-3, BMP-7) simulate reparative responses in dentin, while recombinant IGF-1 together with collagen promotes the production of dentin bridges.

Suzuki et al. analysed the migration and proliferation of DPSCs in response to a gel scaffold infused with stromal cell derived factor 1 (SDF-1), basic fibroblast growth factor (bFGF), and BMP-7. Compared to the control group, BMP-7 had little effect on stem cells, although it did significantly influence mineralization of DPSCs. On the other hand, SDF-1 and bFGF induced proliferation of a significantly higher number of stem cells [61]. The cytokine SDF-1 alpha has a positive influence on stem cell migration, which was demonstrated by Yang et al. [62] and Liu et al. [63].

Takeuchi et al. compared the effect of bFGF and granulocyte colony stimulating factor on pulp regeneration and showed that both factors can be used to successfully control the regeneration process [64]. Kim et al. studied the regeneration of pulp-like tissue by induced stem cells extracted from human teeth, endodontically prepared, and seeded onto collagen scaffolds along with bFGF, VEGF, PDGF, nerve growth factor, and BMP-7 [65]. The teeth were then subcutaneously implanted into the dorsal area. After 3 weeks, histological evaluation of the samples showed formation of a vascularized connective tissue that was rich in cells.

In 2017, Galler and Widbiller proposed a pulp regeneration procedure based on the principle of cell homing using endogenous growth factors compacted in root canal dentin. The procedure is initiated by chemo-mechanical root canal treatment and the conditioning of dentin in a 10–17% ethylenediaminetetraacetic acid (EDTA) solution. After removing the EDTA solution, the canal is flushed with saline and activated by ultrasound, which causes the release of dentine-derived growth factors. Since the number of growth factors increases with activation time, according to Galler and Widbiller, the ultrasound should last for at least 60 s. The saline solution now contains endogenous growth factors from the tooth cavity that can be mixed with a hydrogel scaffold (PRF) and injected into the damaged root canal. Before the scaffold is applied, the authors recommend bleeding the periapical region to ensure the biomaterial will be in contact with the host tissue. In addition, they suggest widening the apical foramen to 0.6 mm to facilitate revascularization for cases of permanent teeth that have reached full growth [66].

STEM CELLS

Stem cells are distinguished by their ability to self-renew through a virtually infinite number of cell divisions and to differentiate into specialized cells [21, 67, 68]. Adult stem cell populations can be collected from the patient and stored under appropriate conditions that allow them to maintain the ability to differentiate into other cell types [69]. In 2000, DPSCs were isolated for the first time [70, 71]. In 2007, scientists from the Tokyo University of Science used stem cells to generate new teeth in mice [72]. They extracted mesenchymal and epithelial cells from embryos and after 6 days in culture, the cells were implanted into adult mice in post-extraction sites. Fourty nine days post-transplant, the teeth were found to be anatomically and functionally complete and had integrated with surrounding tissue. However, only preclinical studies were performed.

The 1st bank of stem cells obtained from tooth pulp opened in 2004 at the National Hiroshima University of Japan. Extracted teeth are placed in a special kit and transported to the bank where the stem cells are isolated [73]. The cells are then frozen and stored so that they can be used in regenerative procedures later.

Types and occurrence of stem cells

Stem cells are grouped according to differentiation potential as totipotent, pluripotent, multipotent, and unipotent. Totipotent cells can form any of the cells and tissues of the body, as well as extraembryonic structures. Similarly, pluripotent cells have the ability to differentiate into all cells and tissues;

whereas multipotent cells can only differentiate into certain cell types with a common origin (this group includes most DPSCs). Lastly, unipotent cells are only capable of transforming into a single cell type [68, 74].

Based on their origin, stem cells can also be categorized as embryonic stem cells, fetal stem cells, and adult stem cells. Embryonic stem cells must be isolated from embryos that are between 1–5 days old and can differentiate into any cell type, which means theoretically, they can replace any damaged cells of the body and give rise to any type of new tissue [68]. Fetal cells have multipotent properties but can only form specific tissues and organs. Adult stem cells can have both multipotent or unipotent properties and can usually transform into other cell types of their tissue of origin. Importantly, the recipient and donor can be the same individual, thereby eliminating the risk of rejection. Adult stem cells, like SCAPs, do not require the destruction of an embryo and are therefore more frequently used in regenerative procedures [3, 68, 75].

TABLE 2. Potential sources of stem cells found in the dental system for pulp regeneration procedures [22, 60, 72, 73, 74, 77]

Cell type	Place of origin
Pulp stem cells (DPSCs)	dental pulp
Stem cells from the apical papilla (SCAPs)	apical region of immature tooth
Stem cells from human exfoliated deciduous teeth (SHED)	pulp of exfoliated deciduous tooth
Bone marrow stem cells (BMSCs)	bone marrow
Dental follicle stem cells (DFSCs)	periodontal ligaments
Inflamed periapical progenitor cells (iPAPCs)	periapical area of tooth apex
Dental follicle progenitor cells (DFPCs)	dental follicle
Tooth germ progenitor cells (TGPCs)	tooth germ

Potential dental stem cell sources are presented in Table 2. The stem cells most frequently used in regenerative endodontics are DPSCs and SCAPs, as well as stem cells from human exfoliated deciduous teeth (SHED) and periodontal ligament stem cells. At present, regenerative endodontic strategies either induce host cells to differentiate into new tissues or transplant stem cells into the tooth cavity [4, 74, 75, 76].

Stem cell induction

Stem cell homing is a method that incorporates growth factors into a scaffold to promote migration and proliferation of host stem cells [4, 5, 57]. Potential cell sources include DPSCs, SCAPs, SHED, and bone marrow stem cells (BMSCs) [67]. Although animal studies are still underway, this method is already used in human dentistry as a pulp revitalization method [4, 5, 78].

Pulp revitalization (revascularization) belongs in the category of stem cell induction therapies [79, 80]. This approach is used to treat permanent teeth with incomplete root apex development and necrotic pulp with simultaneous changes in periapical tissues [60, 81, 82]. Moreover, research is being conducted on applying the same procedure to mature permanent teeth [16, 83]. In contrast to transplantation, revitalization does not require isolation of stem cells and is associated with lower costs and no risk of immunological response. Stem cell induction is based on chemotactic migration of host cells to specific tissue compartments [77, 84].

The revitalization method takes advantage of the regenerative potential of the stem cells derived from dental papilla located in the periapical region. Furthermore, SCAPs can generate tissue that is comparable to dental pulp but must first penetrate the root canal. The canal is disinfected and then a thin sterile endodontic instrument is passed through the apical foramen causing periapical bleeding. The clot that forms in the canal is covered with a biocompatible material, e.g., Pro Root MTA (Dentsply, Tulsa, OK, USA) or Biodentine (Septodont, Saint Maur des Fosses, France) and the cavity is sealed [85, 86]. The newly produced pulp-like connective tissue is responsible for further apexogenesis [19, 79, 81]. Any inflammation of periapical tissues that occurs at the root of the treated tooth will typically heal after 6–12 months [25, 28, 79].

The pulp revitalization procedure, as defined by the American Association of Endodontists and the European Society of Endodontology begins with the administration of a local anesthetic, followed by isolation of the surgical field using a rubber dam [28]. Once access to the tooth cavity is established, the following procedure is carried out: the canal is rinsed with 1.5% sodium hypochlorite and EDTA (both in the amount of 20 mL/canal) using a needle inserted to its working length and reduced by 1 mm; calcium hydroxide or triple antibiotic paste (ciprofloxacin, metronidazole, minocycline) are introduced into the canal and the cavity is covered with a 3–4 mm layer of material, e.g., glass ionomer. The next visit takes place 1–4 weeks later. During the subsequent procedure, the patient is anaesthetized without vasoconstrictors, the surgical field is isolated with a rubber dam, and the canal is rinsed with a 17% EDTA solution (20 mL/canal) and dried with paper points [87]. Next, blood from the periapical tissue is produced by passing a thin tool (K-file) outside the apical foramen. After a clot forms in the canal, resorbed matrix, e.g., an absorbable collagen wound dressing for dental surgery (CallaCote) is applied and the canal is secured with biocompatible MTA (a 3–4 mm layer). Finally, the biocompatible matrix is covered with light-cured glass-ionomer cement and the cavity is filled [13, 16, 23, 79, 82]. Due to discoloration of mineralized teeth after MTA, biomaterials that do not cause discoloration, such as Biodentine or EndoSequence BC RRM-Fast Set Putty (Brasseler, USA) are recommended for anterior sections [28, 88, 89].

Stem cell transplantation

Stem cells can be seeded onto a scaffold along with growth factors and transplanted into the canal to facilitate pulp regeneration [4, 5, 90, 91]. Transplanted cells are either taken from the host (autologous stem cells) or from another donor (allogeneic stem cells). From a clinical perspective, this method is

more difficult than revitalization since it involves extracting the tooth, pulp extirpation, and cell culture.

In 2017, Nakashima et al. presented a novel protocol for human dental pulp regeneration therapy by transplanting autologous DPSCs into teeth subjected to pulpectomy [6]. Five otherwise healthy patients (aged 20–55 years) with irreversible pulpitis took part in the study. Before tooth extraction and DPSC isolation, blood was collected from each patient and autologous plasma was isolated, which was subsequently used for expanding the stem cells while maintaining their differentiation ability. After 14 days, the teeth were removed and autologous DPSCs were isolated. Fetal bovine serum is commonly used as a supplement, however, a plasma-based medium was used instead to keep the cell expansion protocol free of xenogeneic products and to avoid potential pathogen contamination. Stem cell sets were produced and the teeth with irreversible pulpitis were subjected to pulpectomy. Briefly, the apex region was widened to the ISO diameter of 45–55, and root canals were rinsed alternately with 3 mL 6% NaOCl and 3% hydrogen peroxide (H_2O_2). After drying the root canals with paper points, a paper point soaked in 10 mg/mL minocycline or 0.5% levofloxacin was introduced into each canal and the canal was temporarily closed with Caviton (GC, Tokyo, Japan) and composite resin (Clearfil Megabond, Kuarary Medical). On the next visit, the canal was again alternately rinsed with 3 mL of 6% NaOCl and 3% H_2O_2, followed by 5 mL of saline, 2 mL of 3% EDTA solution for 2 min and finally, another 5 mL of saline. The canal was dried with paper points without causing bleeding. The previously prepared DPSC sets were then introduced into the root canal using a cannula and protected with a gelatin sponge. The canal was covered with a layer of glass ionomer cement and the treated tooth was covered with a protective layer of a composite resin. Patient safety and the efficacy of the therapy was evaluated at 1, 2, 4, 12, 24, 28, and 32-weeks post-transplantation. No side effects were detected in the patients based on radiological imaging. In 3 of the patients, no periapical lesions were found, however, a small periapical lesion was observed in 1 patient and widening of the dentitional space and brightening of the periapical area in another. The electric pulp sensitivity test conducted after 4 weeks revealed a positive response in 4 of the patients. The last patient demonstrated a positive reaction to the pulp sensitivity test after 36 weeks.

Xuan et al. evaluated the effects of dental pulp regeneration of human deciduous pulp stem cells (hDPSC) after implementing them into 26 injured immature permanent teeth. The participants of the study were patients aged 7–12 years old, who had a diagnosis of a traumatized permanent incisor tooth, showing (secondary to trauma) pulp necrosis. The authors concluded that hDPSCs are able to regenerate whole dental pulp and are a promising method for treating injuries to teeth due to trauma [92].

A personalized cell therapy for pulpitis in a mature permanent tooth (28) using autologous DPSCs and leucocyte-platelet-rich fibrin (L-PRF) was presented in 2019 by Meza et al. [93]. The presented case study reveals the potential use of a patient's own stem cells (DPSCs) and L-PRF as an interesting alternative therapy for the treatment of pulpitis in permanent mature teeth. However, the authors highlight the possible limitations of this treatment, which are the high cost associated with autologous therapy and difficult, complicated laboratory procedures.

Although the proposed autologous cell transplantation strategy is very promising and could significantly change current treatment methods, many more human studies are required.

CONCLUSIONS

The oral cavity seems to be a plenteous and virtually unlimited source of stem cells. However, the success of stem cells in regenerative endodontic procedures depends on suitable scaffolds, growth factors and local conditions, including the age and health of the patient. Tissue engineering strategies create unlimited possibilities and are expected to realize a high success rate of dentin-pulp complex regeneration.

Although the presented strategies show an excellent view for future clinical regenerative endodontic procedures, further clinical studies and easier novel protocols with higher levels of evidence are required.

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