



## REVIEW ARTICLE

# Regenerative Endodontics by Cell Homing: A Review of Recent Clinical trials

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## SIGNIFICANCE

Regenerative endodontics aims to regenerate the damaged "pulp-like" tissues, preserving the vitality and sensitivity of the teeth while avoiding necrosis. This article provides an overview of vital pulp tissue and various strategies to promote regeneration of damaged pulp tissue, as well as recent clinical studies on regenerative endodontics using the cell-homing technique.

## ABSTRACT

**Introduction:** The conventional treatment for irreversibly inflamed or necrotic teeth is root canal treatment or apexification. Regenerative endodontics aims to regenerate the damaged "pulp-like" tissue, which can preserve the teeth' vitality and sensitivity while avoiding necrosis. The main clinical benefit is root maturation. The "pulp-like" tissue does not refer to regenerated pulp tissue with an odontoblastic layer or the formation of pulp-dentin complexes. The cell homing technique is built on endogenous stem cells and their capacity to regenerate tissue. Cell homing refers to endogenous cells' migration or infiltration into the cite when stimulated by physiochemical or biological stimuli or by passive flow with a blood clot from the apical tissue. Its Regenerative Endodontic Procedures success criteria are defined by the American Association of Endodontists. The purpose of this article is to provide an overview of vital pulp tissue and various strategies to promote regeneration of damaged pulp tissue. The cell homing technique will be reviewed through clinical trials. **Methods:** We performed a comprehensive literature review on a total of nine clinical trials of regenerative endodontics using the cell-homing technique based on three databases and duplicate manuscripts were removed. **Results:** Regenerative endodontics using the cell-homing technique shows promising results that can be translated into clinical practice. However, a favorable result was observed in immature teeth, and the results are contradictory in mature teeth. **Conclusion:** Regeneration therapy is an attractive new alternative to conventional endodontic treatments. Preservation of vitality and continuation of root development in damaged teeth would be a clear advantage. (*J Endod* 2023;49:4–17.)

## KEY WORDS

Biomaterials; cell homing technique; growth factors; pulp tissues; regenerative endodontic; scaffolds; stem cells

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Regenerative medicine holds promise for restoring tissues and organs damaged by disease, trauma, neoplasms, and congenital deformities. Such procedures involve the combination of cells, engineering materials, and relevant biochemical factors to improve or replace biological functions to promote advances in the area of medicine<sup>1</sup>. Regenerative endodontics has emerged as a promising alternative that fits mainly into the treatment of nonvital teeth, in which new pulp tissue, but not actual pulp dentin, is developed from undifferentiated cells<sup>2</sup>. Two strategies can be applied for pulp-dentin complex regeneration: cell-based transplantation therapy or the cell homing<sup>3,4</sup>. The first therapy involves *in situ* or intravenously delivery of autogenous or allogeneic stem/progenitor cell transplants. However, such therapies still face many challenges, mainly economic and ethical concerns<sup>5,6</sup>. In the cell homing technique, the regeneration is accomplished via chemotaxis of endogenous host cells to the injured tissue via biological signaling molecules<sup>4</sup>. Stem/progenitor cell homing can be defined as the potential of stem/progenitor cells, whether endogenous or exogenous, to migrate into an environmental niche. Clinically, cell homing for pulp-dentin complex regeneration might be simpler and more economical to perform compared to the cell-based therapy and readily performed by clinicians without special training<sup>7</sup>. The aim of the present study was to provide a scoping review of the available clinical trials of cell homing techniques focusing on the current possibilities and limits of today's cell homing therapy.

## METHOD

A comprehensive literature search was undertaken using PubMed, Web of Science and Science Direct electronic databases. The specific Medical Subject Heading terms were used in combination with the Boolean operators “OR” and “AND” to create the following search strategy: “regenerative” OR “revascularization” OR “revitalization” AND ‘cell homing’ AND “endodontics”. Duplicate manuscripts were removed using Mendeley software (Elsevier, Amsterdam, the Netherlands). The search, study selection and data extraction were conducted by two independent authors (I.M.K, E.W.,) and in case of discordance after a consensus meeting, a third review author with expertise in the area made a final decision (H.H.). Inclusion criteria were articles published in the English language in 2010-2022, with original data on dental pulp regeneration using the cell homing technique, and only clinical trials with a control group. Studies examining cell transplantation, laboratory studies, reviews, and studies without original data were excluded. The initial search retrieved 35 studies and included 22 original studies. After applying the inclusion and exclusion criteria, the final selection comprised a total of 9 clinical trials.

## The Vital Pulp Tissues

The dental pulp is the soft connective tissue of the tooth; highly vascularized, innervated, and with various types of cells held together in a mesh of collagen fibres. Its functions are to produce dentin, provide nutrition to the surrounding mineralized tissue, protect and repair when damaged, and give the tooth its sensitivity. The pulp is wholly enclosed in the hard dental tissues and is organized into different zones.

The odontoblastic zone's outer layer comprises odontoblasts organized in a rim that underlies the surrounding dentin. Odontoblasts are dentin-forming cells that retain their ability to form dentin throughout life. Secondary dentin is deposited continuously and can compensate for the dentin loss caused by caries or tooth wear. Dentin comprises the bulk of tooth tissue and is organized with thousands of canals and dentin tubules arranged from the inner pulp chamber as sunrays. The number of dentin tubules is about 18,000–21,000 per mm<sup>2</sup>. The odontoblastic membrane bends outwards, creating cellular processes that infiltrate the dentin tubules<sup>8</sup>. Hence, the cells cover a large area of tooth substance and can recognize and prepare host defenses against invading bacteria. If the odontoblastic membrane is damaged, the odontoblasts may be harmed

and lose their regenerative potential. Instead, new “odontoblast-like” cells are recruited from deeper layers of the pulp that are originated and differentiated from fibroblasts, endothelial cells, pericytes, mesenchymal stem cells, or dental pulp stem cells<sup>9</sup>. In addition, the basement membrane may contribute to the dedicated pulp microenvironment and possibly influence the function of a stem cell pool dental pulp stem cells (DPSCs) that could differentiate into odontoblasts<sup>10</sup>. These “odontoblast-like” cells differ from the original and produce dentin matrix with different structures<sup>11</sup>. In addition, in these structures, dentin tubules are less frequent, and its primary goal is to protect the pulpal tissue from irritants<sup>12</sup>. The odontoblasts and surrounding dentin layer are often jointly referred to as the dentin complex, as their function is highly complementary.

It has been suggested that odontoblasts also have a neurogenic function. The cells can produce a wide range of signaling molecules and may be involved in pulp blood flow regulation and inflammatory modulation processes. However, their complete role in tissue homeostasis is still unknown<sup>13</sup>. The outer layer of the pulp also contains dendritic cells that uptake, process, and present foreign antigens to other types of adaptive immune cells<sup>14</sup>.

The underlying layer is called the cell-free zone of Weil, where no cell bodies are visible with histologic staining. Sensory nerve fibres enter the tooth through the apical foramen as myelinated nerve bundles and branch out to form the so-called plexus of Raschkow. The plexus contains both large myelinated A-delta fibres and smaller unmyelinated C-fibres. A-delta fibres initiate a rapid and sharp pain sensation in response to injury, while the C-fibres cause a slower, dull pain sensation. Neurons run alongside the odontoblastic process into the dentin tubules and give the tooth sensitivity<sup>15</sup>. Tooth sensation arises from hydrodynamic forces when the flow of fluids triggers nerve endings penetrating the dentin tubules.

The inner cell-rich zone is densely packed with cells, vessels, and nerves. Fibroblasts make up the principal cells in this tissue, making it a dense connective tissue. Undifferentiated mesenchymal cells populate the pulp core and can proliferate and differentiate into odontoblast-like cells. Immune cells are also present, with peripheral T-cells being the most common<sup>14</sup>, and a small number of macrophages, granulocytes, mast cells, and plasma cells. The pulp's major vessels and sympathetic nerves are found in the pulp core. The pulp tissue is densely vascularized and innervated tissue. The blood

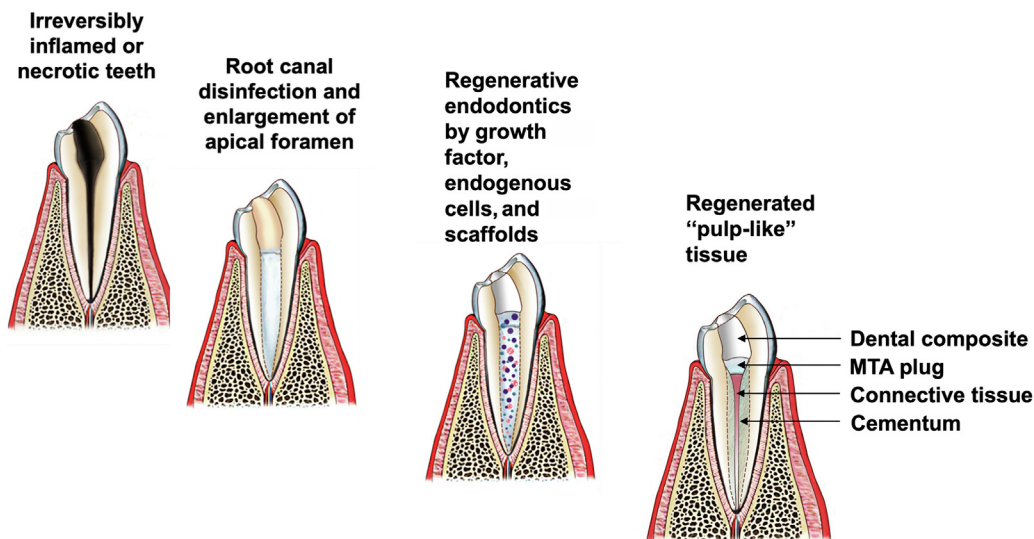
flow is relatively high compared to other tissues, estimated to be 40–50 ml/min/100g of pulp tissue<sup>16</sup>. The pulp lacks collateral blood supply and is sensitive to minor changes in circulation. Therefore, the blood flow must be strictly regulated since the pulp is confined in a small space and cannot expand. Rising intrapulpal pressure stimulates pulp nerves to register pain and threatens pulp vitality when inflamed. The C-fibres are the dominant nerve fibres in the deeper layers of the pulp, also greatly involved in inflammatory responses<sup>14,17</sup>. The vulnerability to change means that pulp inflammation often leads to hypoxia, necrosis, and pulpal damage.

The teeth are exposed to many factors that can lead to pulp inflammation and damage throughout life. Pulp disease can be caused by bacterial infection from caries lesions, fractures, or periodontal disease, resulting from trauma, earlier treatment, or chemical irritation. Tooth wear may also expose the pulp tissue to the surrounding oral environment. Vital and healthy pulp tissue is aseptic. The infection leads to inflammatory reactions in the pulp tissue, following hypoxia, tissue destruction, and possibly necrosis<sup>17</sup>.

A vital pulp is necessary for continued root formation and dentin wall thickening in immature teeth. Teeth arrested in development are susceptible to fracture and early loss. Therefore, maintaining tooth vitality and securing continued tooth development is desirable. Today's treatment of irreversibly inflamed or necrotic teeth involves removing the damaged tissue with a pulpectomy procedure. Removing pulp tissue will leave the tooth without vascularization, nerve innervation, and life. Several complications can occur, limiting the tooth life and resulting in loss of functions<sup>18,19</sup>. Regeneration of “pulp-like” tissue is a new treatment regime that can regain tooth vitality, secure further growth, and regain tooth sensitivity.

## Cell Homing as a Regenerative Therapy

Regeneration of the dental pulp tissue requires making new vital tissue in an empty and disinfected root canal space. The cell homing technique is built on the physiological aspects of normal tissue wound healing (Fig. 1). Two separate cellular processes need to occur: cell recruitment and differentiation<sup>20,21</sup>. For a successful outcome, stem cells require various growth factors and a suitable scaffold supporting tissue growth. Cell homing therapy is initiated with the creation of bleeding by over-instrumentation. The following blood clots filling the pulp space contains endogenous cells and growth factors necessary for tissue



**FIGURE 1** – Schematic illustration of the regeneration of the irreversibly diseased dental pulp tissue using cell homing technique. MTA, mineral trioxide aggregate.

engineering and provides a natural structure supporting cell activity. The three aspects necessary for tissue regeneration will be further reviewed.

### Stem Cells

Stem cells in circulation or various tissues may either remain quiescent or, proliferate and differentiate into various cells based on type of injuries and/or paracrine/endocrine signals<sup>22</sup>. These cells are responsible for tissue renewal, healing, and regeneration after injuries. Their self-renewal capacity makes stem cells an interesting cellular foundation for regenerative therapies. Dental stem cells are thought to be populations of mesenchymal origin. The most common dental stem cells include DPSC, stem cells from human exfoliated deciduous teeth, stem cells from the apical papilla (SCAP), and periodontal ligament stem cells<sup>23</sup>. DPSC are multipotent cells that can differentiate into osteoblasts, adipocytes, and neural cells and are promising from an endodontically regeneration perspective. Nakashima et al established that mobilized DPSC could be used in pulp regeneration in a clinical trial on five patients with pulpectomized teeth<sup>24</sup>. The histological analysis would affirm the pulp regeneration in a relevant non-human model. Three terms, including revascularization, revitalization, and regeneration, are commonly used in 'regenerative endodontics. Revascularization refers to the engraftment of the regenerated pulp soft tissue to host vasculature in the root canal<sup>25</sup>. Revitalization refers to the regeneration of hard and soft tissue<sup>26</sup>. Regeneration refers to both

revascularization and revitalization, including dentin and root structure as well as cells of the pulp-dentin complex<sup>27</sup>.

Clinically, most cells in a treated root canal would be surgically removed by extirpation or chemically destroyed by endodontic disinfection agents. New cells and stem cells must either be transplanted to the root canal space or recruited from the apical papilla. Cell homing techniques specialize in recruiting SCAPs for pulp regeneration<sup>28</sup>. SCAPs can migrate through the apical foramen and produce dentin and blood vessels in the canal space, bone, cementum, and a functional periodontal ligament<sup>29</sup>. They can differentiate without exogenous growth factors<sup>30</sup> and show less telomere shortening compared to other mesenchymal stem cells. SCAPs may therefore be a superior cell type for tissue regeneration<sup>31</sup>. Especially SCAPs from the apical papilla of immature teeth are highly proliferative and capable of differentiating into "odontoblast-like" cells, thus promising endodontic regeneration<sup>32</sup>.

### Growth Factors

Growth factors are polypeptides or proteins that, when bound, give rise to a broad range of cellular activities such as migration, proliferation, differentiation, and maturation<sup>33,34</sup>. Modulating specific signaling pathways makes growth factors essential in tissue reparation and regeneration. Extensive research in this field hopes to tailor these pathways in tissue engineering one day.

Cell homing growth factors can naturally occur from blood influx, from the remaining

pulp parts, stem cells, or adjacent dentin. However, recent studies have found regeneration to occur without using exogenous growth factors altogether<sup>30,35,36</sup>. Endogenous growth factors situated in the dentin wall can be released after disinfection approaches, making them available in the regenerative process<sup>37</sup>. Close to 300 proteins have been identified in human dentin<sup>38</sup>. Most proteins are involved in cell growth, communication, metabolism, and immune responses. Dentin-derived proteins induced chemotaxis and organized "pulp-like" tissue formation, extending cellular processes into dentinal tubules<sup>39</sup>. Transforming growth factor Beta 1 is produced by odontoblasts and deposited in peritubular dentin. It has been found to cause cell migration and cell proliferation<sup>40</sup>. However, its distribution does not differ between mature and immature teeth<sup>41</sup> and transforming growth factor Beta 1; therefore, it can enhance regeneration in young and old who need regeneration therapy.

Angiogenesis is essential in wound healing and repair. Dentin has also been found to be rich in angiogenic growth factors<sup>42</sup>. High concentrations of platelet-derived growth factor and lower concentrations of vascular endothelial growth factor (VEGF) and fibroblast growth factor have been identified. Platelet-derived growth factor promotes cell migration in a dose-dependent manner<sup>43</sup> and induces stem cell proliferation, differentiation, and myelin formation<sup>44</sup>. In addition, VEGF induced cell migration in an animal model<sup>45</sup>. Three weeks after root preparation, irrigation and injection with VEGF, rat somatic cells were recruited from the periapical tissue into the root



apex in a canal model without cell transplantation.

Several other growth factors have been tested for their regenerative potential. For example, injecting the root canal with stem cell factor triggered cell proliferation and differentiation into odontoblast-like cells<sup>46</sup>. The tissue formed was a mature, highly organized with hard tissue formation, revascularization, and odontoblast-like processes extending into dentin tubules.

Growth factor concentrates can be made from the recipients' own blood and injected into the root canal to further enhance regeneration. This biomaterial may contain platelets, cytokines, and a wide range of other growth factors. For example, a growth factor concentrate medium induced enhanced migration, proliferation and differentiation of dental pulp stem cells in an *in vivo* root canal model<sup>47</sup>. The

medium was inserted into immature beagle dog teeth and histologically and immunohistochemically analyses demonstrated that growth factor concentrate promoted regeneration of the dentin-pulp complex after 8 weeks. The newly formed dentine-pulp complex and the development of apical foramen were evaluated by the hematoxylin-eosin and Masson trichrome technique. The immunohistochemical staining showed that VEGF and Nestin were both moderately expressed in the regenerated "pulp-like" tissues, indicating vascularization and innervation (see Fig. 2).

### Biomaterials/Scaffolds

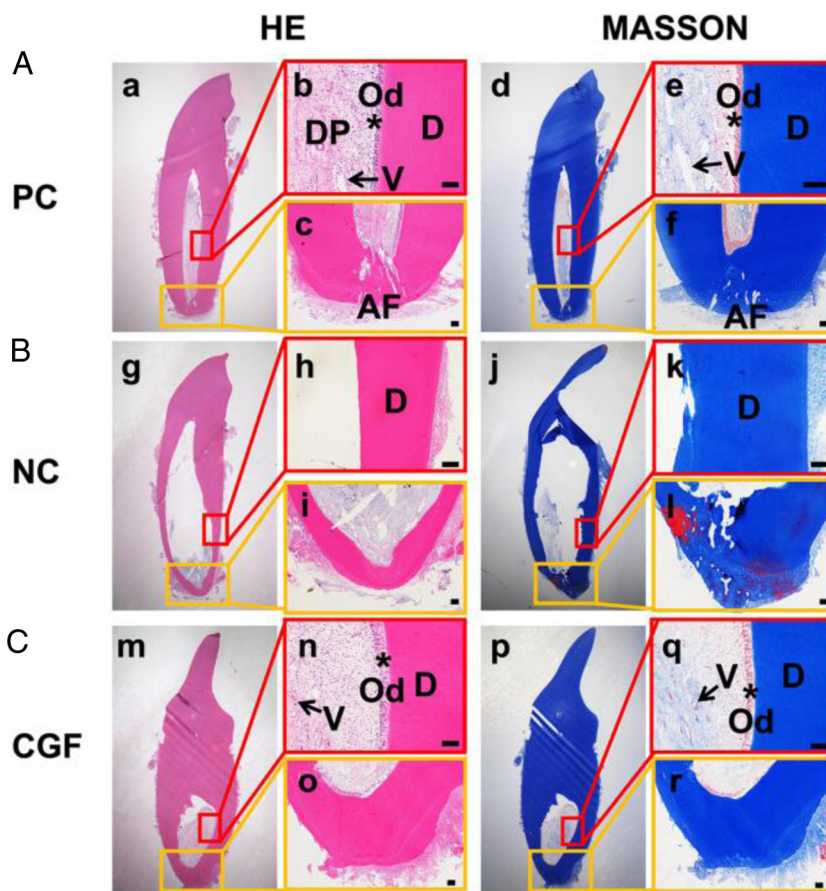
Extracellular matrixes support all living tissue and enable cells to proliferate and differentiate. A scaffold is a complex three-dimensional material with mechanical and chemical properties that mimic the native extracellular

matrix. A wide range of scaffold designs with natural and synthetic origins has been tested in regenerative therapy studies.

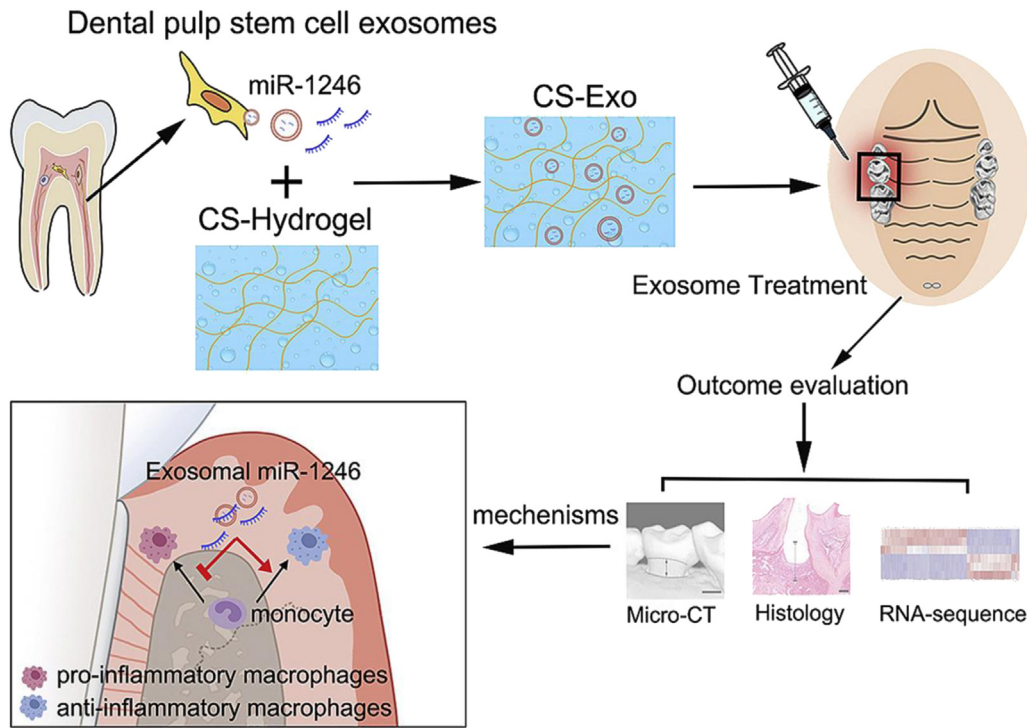
A successful scaffold should give structural support for colonizing cells, promoting cell survival, proliferation and differentiation, and promoting cell-interactions such as adhesion and deposition of extracellular matrix. The scaffold pore size, shape, and volume are essential for transporting oxygen, nutrients, and growth factors and removing waste products. As for all biomaterials, it is essential that the material is biocompatible and has adequate physical and mechanical strength. Therefore, to fully regenerate tissue, the scaffold should be degradable at a rate adapted for tissue healing<sup>49</sup>. Mixing scaffold with chemical agents, growth factors and cells will modify the scaffold and raise its conductive potential.

Fibrin is a naturally occurring biopolymer of the monomer fibrinogen. The fibrinogen molecules initiate their polymer reaction when cleaved by thrombin<sup>50</sup>. The cross-linked proteins make up a biological scaffold suitable for tissue support and cell survival. Fibrin and fibrinogen have a critical role in blood clotting, fibrinolysis, cellular and matrix interactions, inflammatory response, and wound healing. Fibrin has been a widely used scaffold design in tissue engineering and has many advantages<sup>51</sup>. Fibrin can be made from the patient's own blood, limiting the risk of foreign body reaction and infection. The material is highly versatile and can be produced in different designs, such as injectable hydrogels or microbeads. Widbiller et al conducted an animal study using 2 different fibrin scaffold designs, a custom-made fibrin scaffold and a fibrin sealant<sup>39</sup>. Root segments of human teeth filled with scaffolds and a collagen pellet with human DPSCs placed at the root tip were implanted into mice for 4 weeks. When analysed histologically, the authors found migration of stem cells into the root canal and the formation of a 'pulp-like' tissue. The 'pulp-like' tissue was a highly vascularised connective tissue with cells extending into surrounding dentin tubules. Another fibrin scaffold model showed promising results in an *in vivo* cell homing study conducted by Yang et al.<sup>52</sup>. A tooth model was implanted into immune-compromised mice for 7 weeks. Histological analysis showed that the fibrin silk scaffold supported a newly formed tissue resembling natural pulp with high cell density and organization.

Immune-orchestrating biomaterials can be designed to modulate the local immune response that promotes the clearance of the infection and tissue repair or regeneration in a tempo-controlled fashion<sup>53</sup>. Those



**FIGURE 2** – The histological results of growth factor concentrate on the generation of dentine-pulp complex and the development of apical foramen of the immature canine teeth. The hematoxylin-eosin and Masson trichrome staining results of the positive control group (A), the negative control group (B), and the growth factor concentrate filling group (C). b, c, e, f, h, i, k, l, n, o, q, r. The amplified images of the square box in a, d, g, j, m, and p. Arrowhead indicates the vessels; the asterisk indicates the odontoblasts. AF, apical foramen; CGF, concentrated growth factor; D, dentin; DP, dental pulp; Od, odontoblast; PC, positive control; NC, negative control; V, vessel. Scale bar = 100 μm Reprint with permission from<sup>47</sup> under the terms of the Creative Commons Attribution 4.0 International License, CC BY-NC-ND 4.0.



**FIGURE 3** – Dental pulp stem cells -Exosomes/CS facilitated macrophages to convert from a proinflammatory phenotype to an anti-inflammatory phenotype in the periodontium of mice with periodontitis, the mechanism of which could be associated with miR-1246 in dental pulp stem cells -exosomes. CS, chitosan hydrogels; CT, computed tomography. Reprint with permission from<sup>48</sup> under the terms of the Creative Commons Attribution 4.0 International License, CC BY-NC-ND 4.0.

biomaterials either modulate or harness the complex immune response as in the physiological conditions. Upon bacterial insult, innate immune cells alone or mobilize adaptive immune cells work together to fight against the infection. Later on, innate immune cells can change their phenotype to promote tissue healing by secreting cytokines and chemokines for tissue repairing cell homing<sup>54</sup>. For example, innate immune cell macrophages have long been recognised for their phagocytotic activity. Macrophages can also be skewed towards promoting tissue formation phenotype, away from the phagocytotic type. Shen et al reported that chitosan hydrogels incorporated with exosomes derived from DPSCs alleviate periodontitis via converting macrophages to an anti-inflammatory phenotype in mice<sup>48</sup> (see Fig. 3).

Mucins are glycoproteins expressed as transmembrane and secreted forms composing mucus gel covering the mucosal surface, offering hydration, lubrication, and protection against stress and pathogenic bacterial and viral infection. Mucins are one of the main salivary proteins, the failure of expression can lead to mucosal dryness and increased infection risk, including dental caries. As with other mucins, salivary mucins (MUC7

and MUC5B) protect against dental infections by binding to pathogens and facilitating their removal through mucus turnover<sup>55-57</sup>.

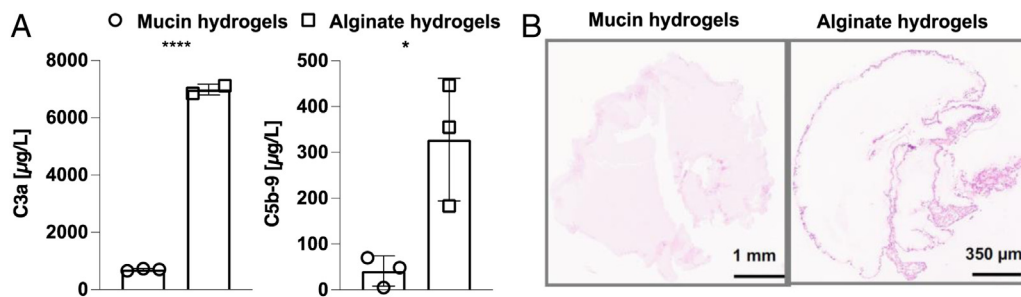
Carbohydrate groups on mucins, such as N-acetylgalactosamine, fucose, galactose, and sialic acid, which comprise 70% mass of gel-forming mucins, can be ligands for receptors on pathogens. Yan et al reported mucin-derived materials are immunologically active, as evidenced by dampening the complement activation<sup>58</sup> and evading foreign body response, as shown in Figure 4<sup>59</sup>. So far, no mucin-based biomaterials have been used for guided regeneration of pulp tissues but are promising, considering their capacity to limit infection and immunomodulation.

### Clinical Trials

In 2016 The American Association of Endodontists (AAE) published their procedure recommendations for a successful cell homing technique<sup>60,61</sup>. The procedure is recommended on necrotic teeth with an immature apex as an alternative to apexification or extraction. The procedure is undertaken over a minimum of 2 appointments. The first appointment includes irrigation with sodium hypochlorite (NaOCl) and ethylenediamine tetraacetic acid (EDTA) and a calcium hydroxide dressing. A 3–4 mm

temporary restoration ensures a tight coronally seal for 1–4 weeks. In the second appointment, regenerative therapy is initiated with bleeding by over-instrumentation. The blood coagulum fills the pulp space and is sealed with a resorbable matrix and a mineral trioxide aggregate (MTA) plug. This recommended treatment protocol gave rise to several clinical trials. The updated version of “Clinical Considerations for Regenerative Procedure” as proposed by AAE states that regenerative endodontic therapy is advocated for teeth with pulp necrosis and immature apex<sup>60,61</sup>. The advantage for a patient joining a clinical trial is access to new treatments, which may only be available as part of a clinical trial with improved regeneration. On the other hand, new treatment may not be any better than your current treatment and for validation reasons, the patient might be treated, and may not like knowing, with a placebo. From a research perspective, ethical consideration and cost of clinical trials limited the number of control and treatment groups, which may not always allow for a full scientific understanding of cell homing regeneration potential.

A small retrospective study examined 5 patients treated with regenerative therapy (Table 1)<sup>62</sup>. Patients were between 6–11 years of age and presented with pulpal necrosis or



**FIGURE 4** – Mucin hydrogels triggered mild complement system activation *in vitro* (A)<sup>58</sup> and suppressed immune immune-responses mediated foreign body response *in vivo* (B)<sup>59</sup>. C3a, C5b-9, complement activation products. Statistics analysis was determined by using GraphPad Prism 9.0, as indicated the statistical analysis and “\*”, “\*\*\*”, “\*\*\*\*”, and “\*\*\*\*\*” indicated *P* value less than .05, .01, .0005, and .0001, respectively. The figures are reprinted with permission from Wiley, copyrights (2019 and 2021).

inflammation, periapical lesion, and preoperative symptoms such as fistula, abscess, or pain. Cell homing was performed by over-instrumentation and placing a degradable bovine collagen scaffold in the root canal, which was sealed using MTA plug. Clinical and radiographic follow-ups were done over 36 months. All five teeth were functional, asymptomatic, and showed continued root development and healing of the periapical lesion. When tested with the electric pulp test, sensitivity did not return, suggesting that complete regeneration had not occurred.

A randomized control trial tested the effect of a resorbable collagen membrane compared to no scaffold using a cell homing technique (Table 1)<sup>63</sup>. In addition, 43 patients with acute apical periodontitis, apical periodontitis, or chronic pulpitis in immature teeth were included. All 46 teeth included in the study had a minimum of 2 mm root open apices. Initial signs of infection were eliminated with irrigation and antibiotic dressing for a minimum of 2 weeks before the regeneration procedure was conducted. Bleeding was induced and the teeth included in the experimental group received a collagen membrane placed over the newly formed blood clot. A tight coronally seal was made using MTA and composite restoration. The follow-up period ranged from 7–28 months and included radiographic examination. Both groups experienced complete resolution of signs and symptoms as well as dentin wall thickening and root elongation. Teeth with collagen scaffolds showed statistically significant more dentin wall thickness at the middle third of the root. The authors concluded that the collagen membrane promoted dentin wall thickening and hypothesized that this could further strengthen the root and reduce the risk of root fracture. All teeth showed an increase in root length. There was no statistically significant difference between the groups when comparing root length, apical foramen width, frequency of crown

discolouration, positive electric pulp test (7/21 in the experimental group vs 4/22 in the control group), or pulp canal calcification.

Shivashanker et al and Ulusoy et al compared the cell homing treatment using three different scaffold designs<sup>64,65</sup>. First, a platelet-rich fibrin scaffold, a platelet-rich fibrin plasma scaffold, and platelet pellet were compared to natural blood clotting in a randomized prospective trial; 88 traumatized, necrotic immature incisors were included in the study, with patients aged 8–11 years. The root canal was disinfected using chemical agents only; mechanical debridement was performed. 73 teeth were asymptomatic and received regeneration therapy. In the blood clotting group, bleeding was induced by over-instrumentation. A platelet concentrate was made from the patient’s own blood and injected into the root canal in the scaffold design groups. Patients’ follow-ups ranged from 10–49 months. Two cases showed signs and symptoms of failure, including pain. The remaining 71 teeth were asymptomatic and showed periapical healing and radiographic root development. The different groups showed similar success scores; 73.9% showed complete apical closure; 86% showed a positive response to sensitivity tests. The platelet scaffold-treated teeth regained sensitivity in earlier follow-up stages compared to the other groups. There was no statistical difference between the groups when increased root width and length was compared.

Fifty immature necrotic teeth with apical periodontitis were included in a study by Shetty et al<sup>64</sup> (Table 1). A sterile, biodegradable bovine collagen plug was used as a scaffold. Follow-ups were conducted every 6 months over 18–48 months; 26% dropped out during this period. Cone-beam computed tomography (CBCT) scans showed a significant increase in root length, decrease in pulp space diameter, and periapical translucency, as well as increased wall thickening and continued root formation

(Fig. 5). Severe calcification and root obliteration was found in 2.9%, and 2 teeth were deemed a failure as they were later diagnosed with chronic apical abscess.

One study compared regenerative cell homing to apexification on immature permanent teeth. 118 immature teeth with pulpal necrosis and apical periodontitis from trauma or *dens evaginatus* were included in a randomized control trial (Table 1)<sup>67</sup>. Regenerative treated teeth were irrigated and received 2–3 inlays with antibiotic paste until a complete revival of symptoms. Bleeding was induced in the root canal, and an absorbable collagen barrier was placed over the blood clot. A tight coronally seal was made using MTA and composite restoration. The apexification procedure was conducted using irrigation and a calcium hydroxide inlay for one week before injection of Vitapex past (Neo Dental International, Inc, Tokyo, Japan) was placed into the root canal and sealed using glass ionomer cement. Follow-ups were conducted over 12 months. All cases showed resolution of symptoms and apical healing. At the 12-month follow-up, CBCT imaging was performed to evaluate tooth length, dentin thickness, and apical foramen diameter. The regenerative group showed a significant increase in root length and thickness compared to the apexification group. However, the results differed somewhat between the 2 aetiologies. Teeth with *dens evaginatus* showed increased root thickness and length, and 91.6% healed with an additional reduction of apical foramen size. Traumatic teeth only showed a significant increase in root thickness compared to control and only 33.3% healed with a reduction in apical foramen diameter. The overall success rate was 71.4% compared to 97.9% in *dens evaginatus* teeth. The difference in success rate between the 2 subgroups was statistically significant, indicating that regenerative endodontics could be more effective on some aetiologies.

**TABLE 1** - Clinical Trials of Regenerative Endodontics by Cell Homing

Author and year	Baseline	Study design	Materials and procedure RET	Method of examination	Results
Meschi et al 2018 <sup>62</sup>	Five patients with five immature, necrotic teeth with periapical lesion.	Retrospective case study	Tooth irrigated, antibiotic paste. Second visit: bleeding induced by over instrumenting. Bovine collagen scaffold and MTA plug.	Clinical, periapical and CBCT radiograph follow ups over 36 months	All teeth functional, asymptomatic, continued root development.
Jiang et al 2017 <sup>63</sup>	Forty patents with immature teeth. Teeth with acute apical periodontitis, chronic apical periodontitis, and chronic pulpitis where included.	Randomized control trial comparing collagen scaffold to no scaffold in RET.	Tooth irrigated, calcium hydroxide paste. Second visit: Bleeding induced by over instrumentation. Bio-Gide collagen membrane used as scaffold in the experimental group. MTA plug.	Clinical and radiograph examination, follow up over 6 months.	Both groups showed complete resolution of signs and symptoms. Dentin wall thickness higher than control.
Shivashankar et al 2017 <sup>64</sup>	Sixty patients with non-vital immature teeth.	Randomized control trial where platelet- rich plasma scaffold, induced bleeding technique and platelet rich fibrin scaffold were tested against each other.	Tooth irrigated, antibiotic paste. Second visit: bleeding induced by over instrumenting. Three experimental groups with either no scaffold, platelet-rich plasma scaffold, or platelet rich fibrin scaffold used.	Clinical and radiograph examination over 12 months.	Reveal of symptoms, improved periapical index (PAI) score and continued root development was seen in all groups. 13.3%–15.8% had a positive vitality response after 12 months.
Lin et al 2017 <sup>67</sup>	118 patients with pulp-necrosis and apical periodontitis in immature teeth, either from <i>dens evaginatus</i> or trauma.	Randomized control study comparing cell homing to apexification	Tooth irrigated, antibiotic paste. Second visit: bleeding induced by over instrumenting, no scaffold used. Collagen barrier and MTA plug.	CBCT imaging was used to study tooth length, root thickness, and apical foramen size on 12 months follow up.	No difference between on resolution of symptoms and apical healing. RET showed a better outcome than apexification regarding increased root thickness and root length. <i>Dens evaginatus</i> cases showed better prognoses than trauma cases after RET.
Arslan et al 2019 <sup>68</sup>	Forty-six patients with 56 mature necrotic teeth with periapical lesion.	Randomized control trial where regenerative therapy was tested against a classic pulpectomy treatment in controls.	Tooth irrigated, antibiotic paste. Second visit: bleeding induced by over instrumenting. No scaffold or growth factors used.	Clinical and radiograph follow up in 12 months.	Not statistically significant outcomes in cf. to control, 50% of teeth not responsive to electric pup test.
Saoud et al 2016 <sup>69</sup>	Six patients with necrotic pulps and apical periodontitis in mature teeth.	Retrospective case study	Tooth irrigated, dressed with Metapaste. Second visit: Bleeding induced with over instrumentation. No scaffold or growth factors used. MTA plug.	Clinical and radiograph examinations with follow up ranging from 8–26 months.	Reduced periapical radiolucency and no clinical signs and symptoms were seen in all cases. No response to cold or electric pulp tester.
Nageh et al 2018 <sup>70</sup>	Fifteen patients with mature necrotic teeth with periapical lesion.	Retrospective case study	Tooth irrigated, antibiotic paste. Second visit: bleeding induced by over instrumenting. Platelet rich fibrin scaffold and MTA plug.	12 month follow up, pulp sensitivity measured with cold and electric pulp tests.	Statistically significant increase in sensitivity after 12 months compared to baseline. Resolution of apical periodontitis and symptoms.

CBCT, cone-beam computed tomography; MTA, mineral trioxide aggregate; RET, regenerative endodontic therapy.

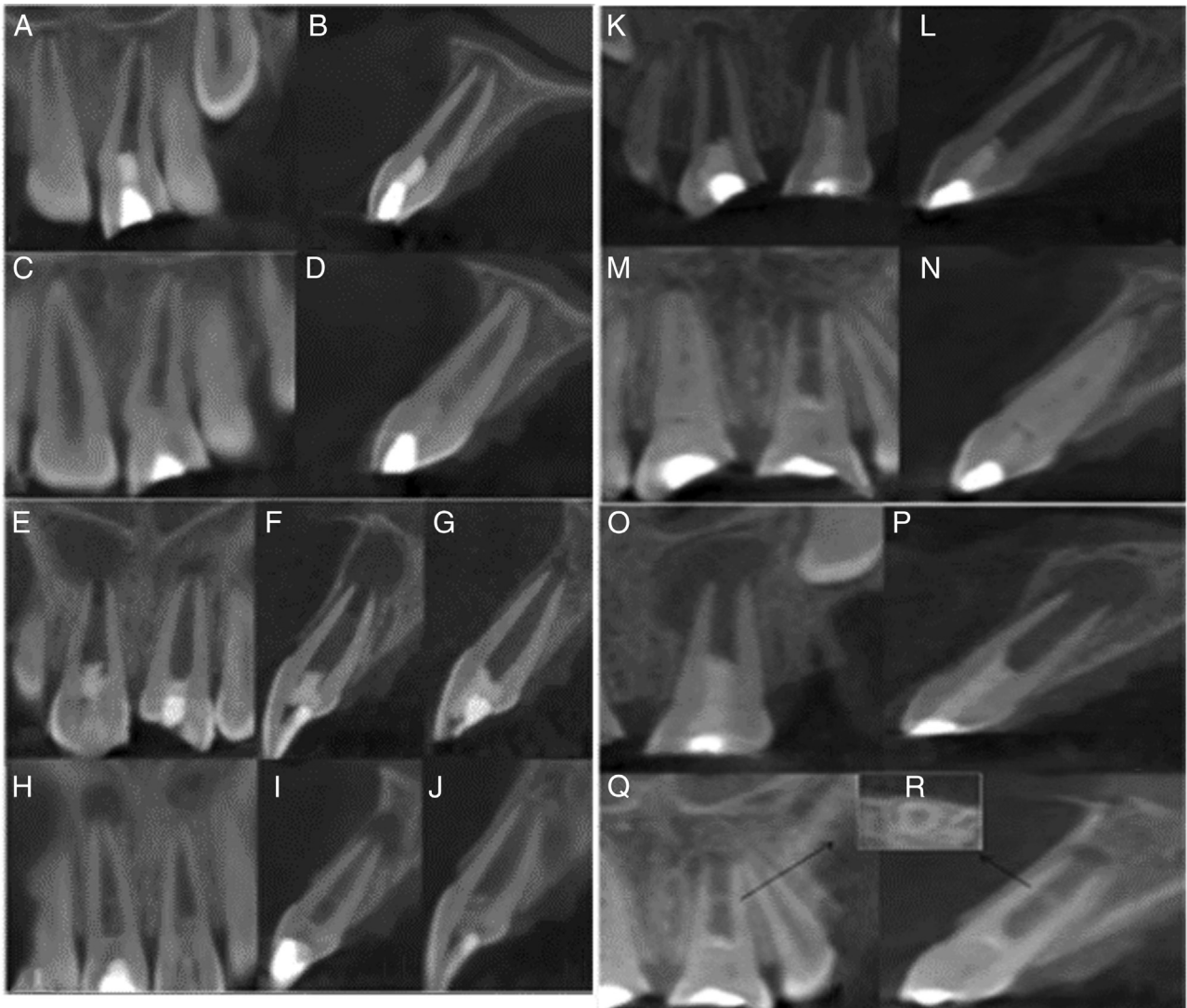
The cell homing procedure has also been tested on mature teeth as an alternative to pulpectomy or extraction. Arslan et al conducted a randomized control trial where regenerative therapy was tested against the root canal treatment (Table 1)<sup>68</sup>. The study involved 56 mature teeth in 49 patients. Patients were between 18 and 30 years of age, and all 56 teeth involved were diagnosed as necrotic with periapical lesions. The regenerative procedure was conducted without scaffolds or exogenous growth factors. A clinical and radiographic

follow-up after 12 months showed favourable outcomes in both groups. For example, 92.4% in the regenerative group achieved healing of the apical lesion, versus 80% with pulpectomy treatment. However, the difference was not statistically significant. For example, 50% of the regenerative teeth were unresponsive to electrical pulpal tests throughout the follow-up period. Ten patients were lost during follow-up.

Saoud et al found similar results in a smaller case study on 7 mature teeth

(Table 1)<sup>69</sup>. Teeth with necrotic pulp and apical periodontitis were included, with patients ranging from 8 to 21 years of age. Complete chemo-mechanical debridement and a calcium hydroxide dressing were used before over-instrumentation, which resulted in bleeding into the root canal. MTA plug and either a composite or amalgam restoration were used as sealing. Clinical and radiograph examination follow-ups ranged from 8–26 months. Radiographic imaging showed complete resolution of periapical radiolucency in 2 cases. The other 5 still underwent healing





**FIGURE 5** – A maxillary central incisor illustrating an increased thickening of the canal walls and continued root maturation: (A) and (B) are initial cone-beam computed tomography (CBCT) images in the coronal and sagittal plane, respectively, while (C) and (D) are the follow-up CBCT images in the coronal and sagittal plane; maxillary central incisors illustrating continued root development with the apical foramen remaining open: (A) initial CBCT images in the coronal plane, (B) and (C) show the initial CBCT images in the sagittal plane, (D) follow-up CBCT images in the coronal plane, (E) and (F) follow-up CBCT images in the sagittal plane; a maxillary central incisor illustrating severe calcification (obliteration) of the canal space: (A) and (B) are initial CBCT images in the coronal and sagittal plane, respectively, (C) and (D) are follow-up CBCT images in the coronal and sagittal plane, respectively; a maxillary central incisor illustrating hard tissue barrier in the canal space between the coronal plug and the root apex: (A) and (B) are initial CBCT images in the coronal and sagittal plane respectively, while (C) and (D) are follow-up CBCT images in the coronal and sagittal plane, respectively. After treatment, most cases showed an increase in root length and gain in root width, the apex remained open (E–J); in one case there was calcification/sclerosis of the pulp canal calcification/obliteration caused by hard tissue formation (K–N); and the contralateral tooth showed continued root maturation, but without apical closure (K, M, O–R). The arrow indicates the formed hard tissue barrier in the canal space. Inset showing axial plane at the level of the hard tissue barrier. Reprinted by paid license permission from Copyright Clearance Center’s RightsLink, Springer Nature Verlag from<sup>66</sup>.

at the last follow-up. All patients showed complete resolution of signs and symptoms, but none responded to cold or electric pulp tests.

In a retrospective study, 15 patients were diagnosed with necrotic teeth and periapical lesions and treated using cell homing (Table 1)<sup>70</sup>. Both symptomatic and asymptomatic mature teeth were included, and the average patient age was 25 years. Root canals were mechanically prepared up to K-file #60–80 and received an antibiotic

dressing for 3 weeks before bleeding was induced into the root canal. A platelet-rich fibrin matrix was made using the patient’s own blood and placed in the root canal. Canals were sealed off with an MTA plug, glass ionomer cement, and composite restoration. After 12 months, all teeth regained sensitivity, nine responding between 0–39 and 6 responding between 40–79 on an 80-scale electrical pulp test. The mean value decreased from 79.87 preoperative to 37.53 after 12 months. All

teeth showed resolution of apical periodontitis and symptoms. The authors concluded that sensitivity indicated the formation of a vital pulp-like tissue.

In a follow-up study, 18 necrotic immature molars and incisors were treated with the strategy of revascularization versus apexification with 9 teeth in each group<sup>71</sup>. In the revascularization treatment, no vasoconstrictor was used in local anaesthesia, but only irrigation with 1.5%–2.5% NaOCl and 17% EDTA was used. The insertion material in



the canal was a mixture of ciprofloxacin 250 mg, metronidazole 400 mg, and minocycline 50 mg in a ratio of 1:1:1. At a second visit, blood clots were induced in the root canal. A 2–3 mm thick MTA plug was applied, and the crown was restored with composite resin. The vascularization strategy significantly promoted root growth, as shown by the percental change in length (12.7% at 6 months) and dentin thickness (35.57% at 6 months) ( $P < .05$ ). However, there was no significant difference in apical healing scores between the two groups after 6 months. Similarly, another two studies showed that regenerative endodontic procedures with blood clots could regenerate “pulp-like” tissue that can respond to the electric pulp test<sup>72,73</sup>.

Some other studies have also shown that regenerative endodontic procedures are a potential treatment option for mature teeth with pulp necrosis and apical periodontitis. However, these were strategies in which exogenous cells were transplanted, so they are not discussed in detail in this review article. One of the studies, in which transplantation of autologous dental stem cells into injured teeth was even able to resemble pulp and dentin in humans<sup>74,75</sup>.

## Future Perspectives

The AAE grades a successful regenerative endodontic procedure based on 3 goals. The primary goal is to eliminate symptoms and find evidence of bony healing. Two out of 50 teeth in Shetty et al study<sup>66</sup> were deemed a failure due to chronic apical abscess, and 2 out of 73 patients in Ulusoy et al's study<sup>65</sup> experienced pain postoperatively. Overall, the elimination of symptoms was fulfilled in most cases included in this review. Clinical trials rely on radiographic imaging to evaluate bone density and the apical healing process. Apart from the 4 above cases, all teeth showed evidence of bone healing on radiographic imaging.

The secondary goal listed by the AAE has increased root wall thickness. An increased root length is also desirable. This can be achieved on immature teeth receiving regenerative endodontic treatment and is necessary for improved tooth strength. Dentin wall thickening, root elongation, and development were highly prevalent in all included studies. However, intraoral radiographs provide a two-dimensional representation of a three-dimensional object. To better analyse the changes seen on apical radiographs, some authors also included a CBCT analysis. It became evident that although dentin wall thickening was experienced, the teeth showed 5 times less hard tissue volume and 3 times less root

length than the contralateral teeth<sup>62</sup>. Some tooth strengthening can be achieved with a regenerative procedure, but the balance between dentinogenesis and root calcification is difficult to control. Therefore, obliteration of the root canal is a major and common complication that will be further discussed.

The tertiary goal in a successful regenerative endodontic procedure is a positive response to vitality testing. A positive sensitivity test is often considered indicative of an organized pulp-like tissue and tooth vitality. The number of patients that experienced regained sensitivity during follow-ups varied considerably between the clinical trials included in this paper. The differences could not be more extensive, varying from 0%–100%. For example, regained sensitivity had a higher prevalence in immature teeth receiving regenerative therapy, but the results were unpredictable even among this patient group. Different techniques, instruments and scales were used that might influence the results. For example, in the Nageh et al' clinical trial 6 out of 15 teeth tested positive with electrical pulp test intensities over 40 on a 80 scale<sup>70</sup>. The higher the intensity, the higher is the probability of responses from adjacent tissues, including the functional periodontal ligament. The test relies on patient feedback and might be misleading. Other tests indicative of tooth vitality, such as laser Doppler flowmetry, were not conducted in any of the included studies.

Only histological staining, gene expression, and polymerase chain reaction might accurately illustrate the regenerated tissue. These tests are impossible without tooth extraction. Prior *in vitro* or animal studies will not show the same complexity as human physiology and biology. The original pulp tissue is a highly organized tissue with many functions. Regeneration is when damaged tissue is replaced by new tissue growth that completely restores the tissue to its original state, fully functional<sup>76</sup>. Replacement or repair is another healing mechanism where damaged tissue is replaced with tissue that differs from the original. This new tissue consists of connective tissue that restores some of the original tissue structure, but the tissue loses its original biological function. Despite the resolution of clinical signs and symptoms and apical periodontitis, the histological analysis found that the new-formed tissue made after regeneration therapy was mineralized, similar to cementoid and fibrous connective tissue<sup>77</sup>. However, it is yet to be seen if the new tissue is able to identify external stimuli, initiate immune, and inflammatory responses and produce reparative dentin.

Another study examined five cases where regenerative endodontics had failed<sup>78</sup>. Histological examination showed that the formed tissue was less organized than healthy tissue and inflamed despite no detected residual bacteria. Biomaterial-induced calcification and reparative connective tissue were also detected. The tissue showed a vascularization, and nerve innervation significantly different from normal pulp tissue. Hence, regenerative therapy might not be the correct term. With today's results, regenerative therapy might be better characterized as reparative therapy.

Treatment complications will always be possible, and the regenerative procedure is no exception. A common complication reported from clinical trials using cell homing is calcification and obliteration of the root canal space. In addition, 22 out of 46 teeth in Jiang et al's study experienced canal calcification<sup>63</sup>. Calcification was also a major complication, occurring in 26 out of 69 cases, as reported in Lin et al's study<sup>67</sup>, while 3 out of 5 patients experienced the same in the smaller retrospective study by Meschi et al.<sup>62</sup>

In addition, to presenting itself as a failed regenerative treatment, the obliterated root canal could make later pulpectomy treatment more difficult and lower its success rate. In the future, scaffolds and signalling molecules could be essential for balancing tissue growth and avoiding unnecessary calcification and obliteration.

Another major complication seen in multiple studies was crown discoloration. In Shetty et al's clinical study, all teeth showed discoloration as early as 6 months after regeneration treatment<sup>66</sup>. Jiang et al. found similar results, where 64% in the regenerative control group and 71% in the experimental scaffold group experienced crown discoloration<sup>63</sup>. The crown discoloration is a well-known complication also found in multiple other studies<sup>79</sup>. Discoloration seen in cell homing could be related to the induced bleeding procedure, where the breakdown products of the blood clot enter the dentin tubules and create discoloration or be a result of the many chemical agents applied to the root canal<sup>80</sup>. Triple antibiotic pastes are widely used antibiotic dressings and are recommended by the AAE<sup>80</sup>. Studies have shown that triple antibiotic pastes has been associated with significant tooth discoloration<sup>81,82</sup>. In addition, MTA is another widely used material that can induce discoloration<sup>83</sup>. In addition, the invention and use of other materials could reduce tooth staining and enhance the clinical outcome. For instance, calcium hydroxide and double antibiotic paste consisting of metronidazole

and ciprofloxacin have shown no visible colour changes<sup>81</sup>. Sealing the pulp chamber walls with dentin bonding before applying antibiotic paste has also reduced coronal discolouration<sup>68,84</sup>. Dentistry aesthetics is in high demand. Therefore, a more aesthetic outcome of regeneration therapy would benefit the patient and the technician. A better-looking result could improve patient compliance and prognosis, reduce the need for additional treatment, and be more cost-efficient.

An aseptic working field is fundamental to all endodontic treatment and regenerative therapy is no exception. However, regeneration therapies are based on living cells and physiological processes. In practice, achieving the balance between bacterial death and endogenous cell survival might be challenging. The presence of prior infection could negatively affect the process of pulp tissue regeneration. This became evident in an *in vivo* experiment from 2017<sup>85</sup>. Ferret teeth were disinfected with 1.25% NaOCl and an antibiotic paste was applied prior to ferret DPSCs, encapsulated in a hydrogel scaffold, were injected, while the control group received the conventional blood clots. After 3 months, the teeth were radiographically examined, extracted, and submitted to histologic analysis. Residual bacteria resulted in significantly less mineralized tissue growth. The presence of residual bacteria was significantly associated with a lack of radiographic growth, persistent periapical radiolucency, and reduced root wall thickness ( $P < .001$ ). The oral environment is speculated to harbour over 700 microorganisms, many of which are yet to be identified<sup>86</sup>. In addition, several bacteria are coupled to dentin caries and pulpal infection. The many dentin tubules, complex root canal anatomy, lateral canals, and apical delta offer endless possibilities for bacterial growth and biofilm formation. Since the degree of bacterial infection might affect the regenerative procedure, different etiologies may yield different results. Lin et al's paper showed that regenerative endodontics had a higher success rate on teeth with *dens evaginatus* than trauma-affected teeth<sup>67</sup>. This may be linked to the degree of bacterial infection. Chemical disinfection of the root canal space is often achieved with NaOCl, EDTA, and a calcium hydroxide dressing. Strong disinfectants can damage tissue forming cells and stem cells in the periapical tissues, and, therefore, negatively affect tissue regeneration<sup>87</sup>. New ways, for example, by immunomodulation with multifunctional implants to promote eliminating infections to prepare the root canal prior to regenerative

therapies, may be advantageous compared to traditional disinfectants.

Current research and recommendations are concentrated on immature teeth under strict conditions. Exclusion criteria exclude a wide range of the average population, and strict conditions might be challenging to follow in standard practice. For mature teeth, the research is conflicting. The mature teeth included in Aslan et al's study did not show a better treatment outcome than root canal treatment<sup>68</sup>. The retrospective case study conducted by Nageh et al showed the resolution of apical periodontitis and increased sensitivity<sup>70</sup>. Even studies on mature teeth rarely include patients older than 30 years. The varied results make it hard to recommend a regenerative endodontic treatment for the adult and older populations. The regenerative potential in adults may vary significantly from younger individuals, as may the microenvironment of the canal space, Hertwig's epithelial root sheath, and apical papilla. Thus, adult patients may need other procedures to ensure success<sup>88</sup>. This may include modified stem cells, overexpression of genes, and additional growth factors.

The apical foramen is the sole entry point for recruited cells and blood supply into the dental pulp chamber. Some have speculated that the foramen on mature teeth would be too small to ensure cell recruitment and vascularization. The apical foramen on mature teeth was measured to be significantly smaller than on immature teeth,  $168 \pm 49 \mu\text{m}$  versus  $557 \pm 295 \mu\text{m}$ <sup>41</sup>. However, the cell homing technique on mature teeth has also shown cell recruitment. The typical size of human cells ranges from 10 to 100  $\mu\text{m}$ , while mesenchyme stem cells have been measured to 30  $\mu\text{m}$  *in vitro*<sup>89</sup>. Accordingly, most cells can enter the canal space for tissue regeneration.

Regeneration therapy is an attractive new alternative to traditional endodontic treatments. Retaining vitality and continuing root development in damaged teeth would be a clear advantage. However, current clinical trials show unpredictable results. For example, a pulpectomy treatment on teeth without periapical periodontitis was 92%, compared to 74% on teeth with apical periodontitis<sup>90</sup>. The treatment and treatment outcomes are well studied, and the dental professional can provide patients with solid preoperative information. Regeneration therapy studies have limited -up durations, and the long-term success rate is unknown. Before undergoing regeneration therapy, the patient must be well informed of the treatment possibilities and potential complications. In addition, the therapy might require longer follow-ups and stronger patient

motivation than other therapies. While current regeneration therapy research shows this as a promising treatment for immature teeth, this patient group is young and may not be committed to multiple dental visits and regular check-ups. For example, one clinical trial undertaken on immature teeth had a drop out of 26%<sup>64</sup>. The drop-out percentage reached as high as 20% on mature teeth after 12 months<sup>66</sup>. Therefore, it would benefit the patient to continue treatment with the same dental professional, as tissue engineering is not yet a common treatment with universal guidelines. Finally, new treatments could be initiated and mistakes made if the new dentist were uninformed of the patient treatment history.

Today, several dental professionals use regeneration techniques that include the use of scaffolds, membranes, and bioactive molecules. However, cell-based procedures are yet to be done on a larger scale. For example, transplanted cells must go through strict manufacturing processes, either from autologous sources or allogeneic biobanks, before they can have a clinical application. The use of living cells would mean the implementation of new procedures and extensive training. The cell-homing technique is based on endogenous cells and might be more clinically translatable<sup>29</sup>. Furthermore, the procedure does not require cell harvesting, culturing, and sensitive preparation. Studies have shown success even without the use of scaffolds and added growth factors, which are costly and might have a short shelf life. A clinically less challenging procedure is faster and more cost-effective and might be taken on by more than just the endodontic specialist.

Thus, regeneration therapy might be a treatment to be considered on damaged immature teeth as an alternative to pulpectomy. Several studies on immature teeth show promising results with continued root formation, increased root thickness, and regained sensitivity. However, the outcome of cell homing is not yet predictable, and the optimal procedure has not been discovered. More scientific information is needed to understand better pulp biology, including the differentiation of pulp stem cells, dentin matrix proteins, and cellular responses to stress.

## CONCLUSION

There is still no consensus on a definition of clinical regenerative endodontics, therefore its concept and terminology differ. The clinical procedure for endodontic tissue engineering differs depending on originating delivery of cells. Nevertheless, endodontics regeneration therapy is promising as therapy. Regenerative

endodontics has the potential of revitalizing necrotic teeth as well as securing continued root formation and root thickening in immature teeth. Tooth sensitivity and a remaining solid tooth substance reduce the fracture risk and tooth loss. A vital tooth also can defend itself from foreign invaders. The cell-homing approach of regenerative endodontics shows promising results that can be translated to clinical practice. Studies included in this review indicate a favourable outcome when regeneration therapy is conducted on immature teeth. For mature teeth, the results are conflicting. A substantial amount of research is published every year, bringing hope for a new therapy design in the future. For example, additional advances in tissue engineering can

give rise to new scaffold designs, antimicrobial regimes, and the use of different signaling molecules that might make the outcome more predictable in the future. Studies on larger populations and longer follow-ups are needed to secure a predictable outcome.

## CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

**Hongji Yan:** Conceptualization, Methodology, Formal analysis, Writing – original draft.

**Gustavo De Deus:** Writing – review & editing.

**Ida Marie Kristoffersen:** Conceptualization, Methodology, Formal analysis, Writing – original draft. **Elisabeth Wiig:** Conceptualization, Methodology, Formal

analysis, Writing – original draft. **Janne Elin Reseland:** Writing – review & editing. **Gaute F. Johsen:** Writing – review & editing. **Emmanuel J.N. L. Silva:** Writing – review & editing. **Håvard J. Haugen:** Conceptualization, Methodology, Formal analysis, Writing – original draft.

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