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Review Article

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Update on pulp tissue regeneration using cell homing and cell-based approaches: a literature review

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Abstract

Objective: The purpose of this literature review is to analyze available scientific data on stem cell related aspects and its involvement in current endodontic regeneration concepts.

Materials and Methods: A bibliographic search of relevant articles on the different approaches to regenerative endodontics was performed using the following database searches: PubMed, Google Scholar, ScienceDirect. This search identified articles published between 2015 and 2023 in two languages (French and English).

Results: 15 articles were identified on the basis of the inclusion and exclusion criteria.

Discussion: Regenerative endodontics can be considered as an application of the tissue engineering concept and relies on the triad: stem cells, growth factors and scaffolding. For functional regeneration, two regenerative strategies have been proposed: Cell Based (transplantation of stem cells into the canal) and Cell Homing (active recruitment of endogenous stem cells into the canal). Each technique has its own advantages, disadvantages and clinical results. However, in-depth studies with long term follow-up will be necessary in order to reach a clear consensus on the application of these techniques.

Conclusion: Regenerative therapy can be considered a promising alternative to conventional endodontic therapy. it shows encouraging results with potential for revitalization of necrotic teeth and continuation of root development in damaged teeth.

Keywords: Regenerative endodontics, Tissue engineering, Stem cells, Scaffolds, Growth factors, Cell Based, Cell Homing

Introduction

Dental pulp infection caused by caries or trauma often requires endodontic treatment. Despite reported clinical success, studies evaluating endodontic treatments show very high failure rates, with about 14% of unsatisfactory treatments due to lack of sealing, bacterial contamination, or chronic pathologies involving infection or inflammation of pulp tissue.[1] The anatomical complexity of the root canal creates non-instrumentable zones, which disinfection cannot easily access. Furthermore, pulp removal reduces the mechanical strength of the tooth, as it requires the creation of an often extensive access cavity, as well as the loss of tissue to the caries process and a complete loss of sensory and immune functions. A devitalized tooth is less resistant to infection due to odontoblast necrosis, and disappearance of pulp pressure that opposes the diffusion of pathogenic agents. Thus, current endodontic research aims to create biological alternatives to conventional endodontic treatment and to develop "regenerative endodontics. "This approach aims to replace infected or necrotic pulp tissues with new tissues similar to the original tissues, with the restoration of physiological functions.

Regenerative endodontics is based on tissue engineering, defined as the science of designing and manufacturing new tissues to replace weakened or damaged tissues. The application of tissue engineering in regenerative endodontics combines the use of three components: stem cells, growth factors, and scaffold. Stem cells are undifferentiated cells with the ability of self-renewal and differentiation, which cannot be expressed without the presence of growth factors, and a biological matrix called scaffold, providing cells with a favorable environment for development. Two regenerative strategies have been introduced for functional regeneration, namely the Cell Based strategy based on autogenous stem cell transplantation inside the canal system and the Cell Homing strategy based on active recruitment of endogenous stem cells within the canal. Each technique presents advantages, disadvantages, and different clinical outcomes.

The aim of this article is to describe the various components of tissue engineering and their implications for endodontic regeneration, as well as to explain the various current approaches to endodontic regeneration, their advantages and limitations.

Materials and methods

Bibliographic research strategy:

Two types of literature search were performed: a computerized search and a manual search.

A systematic electronic search of Pubmed, Google scholar and Science Direct was conducted to identify potentially eligible studies.

The search strategy included three Mesh (Medical Subject Heading) terms: "regenerative endodontic," "pulp," or "stem cells" and "tissue engineering".

Inclusion and exclusion criteria

Inclusion and exclusion criteria were agreed by consensus between all authors, taking into account the research question and study objectives, while trying to obtain a wide range of results from the research strategy.

Inclusion Criteria:

- Articles in English or French.

- Articles from 2015 to 2023

- Articles about :

- Regenerative endodontics.
- Mature and immature permanent teeth
- Cell based.
- Cell homing.
- Cell free.
- Dental stem cells of pulpal origin.

Exclusion Criteria:

- Articles not available in full text.
- Articles published in other language than French or English.

Boolean search equations

Boolean operators ("OR" and "AND") were used to append search terms related to the search question.

1. Pulp stem cells AND tissue engeneeing OR regenerative endodontics
2. Pulp stem cells AND regenerative endodontics procedures
3. "Stem cells" OR "stem cell therapy" OR "cell-based therapy" OR "cell therapy" OR "cell transplantation" OR "cell grafting" AND "Regenerative endodontic" OR "Endodontic tissue engineering".

Results

Our bibliographic search revealed 158,558 articles, 96,153 of which complied with the periodical restriction.

The titles and abstracts of the articles were read and duplicates eliminated using the zotéro software. 94 articles were selected on the basis of this sorting.

The full text of the articles was then read. The most relevant articles for our issue were selected.

15 articles remained for the synthesis of our topic at the end of our search.

Table 1 Studies Included Based on Search Strategy

	Year	Author	Title	Type of study	Journal	Population	Principal results
1	2015	Narang I et coll.[2]	A comparative evolution of the blood clot, platelet-rich plasma, and platelet rich fibrin in regeneration of necrotic immature permanent teeth : a clinical study.	Clinical trial	Contemporary clinical dentistry	20 patients divided into four groups. Group I (control group) apexification with MTA was performed, group II: blood clot was used as scaffold, group III: platelet-rich fibrin and group IV: platelet-rich plasma.	Group III showed better results than the other groups. Platelet-rich fibrin has significant regenerative potential.
2	2017	Chufang Peng et coll.[3]	Histological findings of a human immature vascularized/ regenerated tooth with symptomatic irreversible pulpitis	Case report	Journal of endodontics	A case presenting an immature premolar affected by irreversible pulpitis treated by an endodontic regeneration procedure, after 24 months the tooth was extracted and a histological examination was performed .	Histological examination revealed the formation of fibrous connective tissue, the presence of odontoblast-like cells lining the newly-formed mineral tissue, and the formation of dentin-like tissue with tubilis in the apical third of the root.
3	2017	Nakashima M et coll.[4]	Pulp Regeneration by Transplantation of Dental Pulp Stem Cells in Pulpitis: A Pilot Clinical Study .	Case series	Stem Cell Research & Therapy	Five patients followed for 24 months after transplantation of autologous dental pulp stem cells into teeth with irreversible pulpitis.	Safety and efficacy of dental pulp stem cell transplantation
4	2018	Shetty et coll.[5]	Cone beam computed tomographic and histological investigation of regenerative endodontic procedure in an immature mandibular second premolar with chronic apical abscess	Case report	J Investig Clin Dent	A case presenting an immature premolar affected by irreversible pulpitis and chronic apical periodontitis treated by an endodontic regeneration procedure. After 3 years, the tooth was extracted and histological and volumetric examinations using CBCT were carried out.	CBCT showed a reduction in the size of the apical radiolucency, apical closure and thickening of the walls. Histological examination revealed the presence of inflamed fibrous tissue, a cement-like mineral tissue on the walls and a thickening of the apices.

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Table 1 Continued...

	Year	Author	Title	Type of study	Journal	Population	Principal results
5	2018	Nageh et al.[6]	Assessment of Regaining Pulp Sensibility in Mature Necrotic Teeth Using a Modified Revascularization Technique with Platelet-rich Fibrin: A Clinical Study	Retrospective case study	Journal of Endodontics	Fifteen patients with mature necrotic teeth with periapical lesions. Tooth irrigated, antibiotic paste applied. Second visit: bleeding due to over-instrumentation. Platelet-rich fibrin scaffold and MTA plug.	Statistically significant increase in sensitivity at 12 months in comparison with baseline. Resolution of apical periodontitis and associated symptoms.
6	2018	Kun Xuan et al.[7]	Deciduous Autologous Tooth Stem Cells Regenerate Dental Pulp after Implantation into Injured Teeth	Randomized clinical trial.	Science Translational Medicine	40 patients with pulpal necrosis were randomly assigned: 30 patients to the human deciduous pulp stem cell (hDPSC) implantation group and 10 patients to the traditional root canal treatment group.	The efficacy of human deciduous pulp stem cells (hDPSC) in pulp regeneration
7	2018	Y.Itoh et al.[8]	Pulp Regeneration by 3-dimensional Dental Pulp Stem Cell Constructs.	In vitro study In vivo study	Journal of dental research	In vitro and in vivo studies were carried out to assess the viability of three-dimensional constructs of transplanted pulp stem cells without the use of scaffolds.	In the in vitro study, dental pulp stem cells remained viable even after prolonged culture. In the in vivo study, blood vessel-rich tissue was formed, and the transplanted cells differentiated into odontoblast-like cells.
8	2019	Arslan H et coll.[9]	Regenerative Endodontic Procedures in Necrotic Mature Teeth with Periapical Radiolucencies: A Preliminary Randomized Clinical Study.	Randomized clinical trial	Journal of endodontics	58 patients were divided into two groups; traditional treatment for the first group and a regenerative endodontic treatment for the second group.	50% of grp 2 teeth had a positive response to the electric pulp test
9	2019	Gastón Meza et al.[10]	Personalized Cell Therapy for Pulpitis Using Autologous Dental Pulp Stem Cells and Leukocyte Platelet-Rich Fibrin: A Case Report .	Case report	Journal of Endodontics	A patient followed after dental pulp stem cell transplantation combined with platelet-rich fibrin in a monoradicular teeth with irreversible pulpitis.	The potential effectiveness of the combination of pulp stem cells and platelet- and leukocyte-rich fibrin.
10	2020	Chrepa V et coll.[11]	Clinical Outcomes of Immature Teeth Treated with Regenerative Endodontic Procedures-A San Antonio Study.	Retrospective study	Journal of endodontics.	51 necrotic immature permanent teeth treated with regenerative endodontic treatment and followed for at least one year were included.	The results of regenerative endodontic procedures depend on several factors related to the patient and the clinical protocol followed
11	2021	Victor Pinheiro Feitosa et coll.[12]	Dental pulp autotransplantation : A New Modality of Endodontic Regenerative Therapy Follow-Up of 3 clinical Cases	Case series	Journal of Endodontics	Autotransplantation of a wisdom tooth pulp into a monoradicular tooth with irreversible pulpitis was performed in three patients.	A 12-month follow-up showed positive results.
12	2022	Nakashima et al.[13]	Pulp Regenerative Cell Therapy for Mature Molars: A Report of 2 Cases.	Case series	Journal of Endodontics	Two patients, a 26-year-old and a 29-year-old, were referred for maxillary molar pulp regeneration. Autologous DPSCs were isolated from extracted third molars and cultured according to good manufacturing practice. The DPSCs were seeded into the prepared root canals with granulocyte colony stimulating factor in atelocollagen.	The usefulness of DPSCs and the potential for regenerative pulp cell therapy in molars are demonstrated in this report of 2 cases.

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Table 1 Continued...

	Year	Author	Title	Type of study	Journal	Population	Principal results
13	2023	Yassmin Elsayed Ahmed et al.[14]	Evaluation of postoperative pain and healing following regenerative endodontics using platelet-rich plasma versus conventional endodontic treatment in necrotic mature mandibular molars with chronic periapical periodontitis. A randomized clinical trial	A randomized clinical trial	Int Endod J	To evaluate the post-operative pain and the healing of necrotic mature tooth, twenty-eight patients were included. The patients were randomized either to control group where standard endodontic treatment or assigned to intervention group where PRP revascularization technique.	PRP revascularization could be an alternative treatment to root canal treatment
14	2023	Hongji Yan et al.[15]	Regenerative Endodontics by Cell Homing: A Review of Recent Clinical trials	literature review	J Endod.	A total of nine clinical trials of regenerative endodontics based on the cell-homing technique were reviewed.	Regenerative endodontics by means of the cell-homing technique shows promising results that can be translated into the clinical practice. However, favorable results were observed in immature teeth and conflicting results in mature teeth.
15	2021	Sahng G Kim et al.[16]	A Cell-Based Approach to Dental Pulp Regeneration Using Mesenchymal Stem Cells: A Scoping Review	Scoping Review	Int J Mol Sci	A total of 17 studies, including two clinical trials and 15 animal studies using orthotopic pulp regeneration models, were included to evaluate the efficacy of mesenchymal stem cell transplantation for pulp regeneration.	In animal models, the stem cell transplantation strategy resulted in the regeneration of vascularized pulp-like tissue. Autologous cell transplantation has also been successfully used to regenerate vascularized vital tissue in two clinical trials. The most commonly used stem cell subpopulations for pulp regeneration have been mobilized pulp stem cells, injectable scaffolds such as atelocollagen, and a granulocyte colony-forming factor.

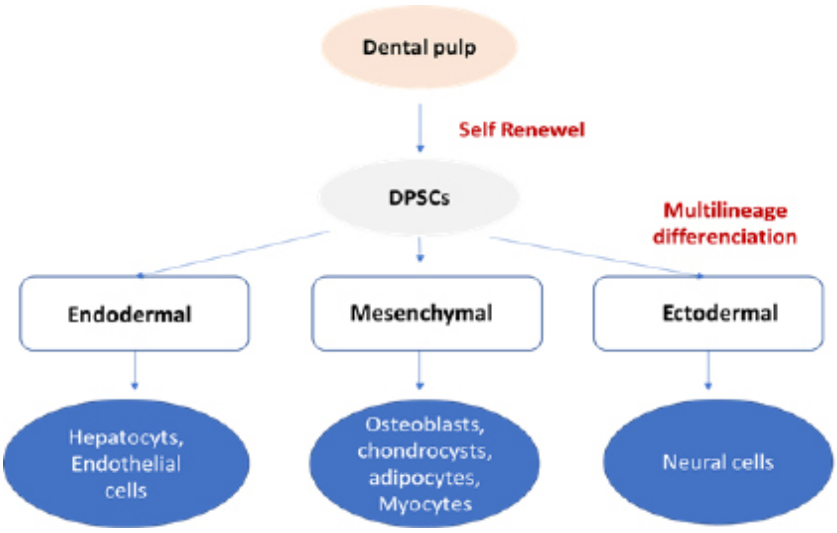

Figure 1 Characteristics of Dental Pulp Stem Cells (DPSCs). DPSCs can be obtained from dental pulp tissue and possess self-renewal and multilineage differentiation potential. DPSCs express mesenchymal stem cell (MSC) markers similar to those of bone marrow-derived MSCs (BMMSCs).

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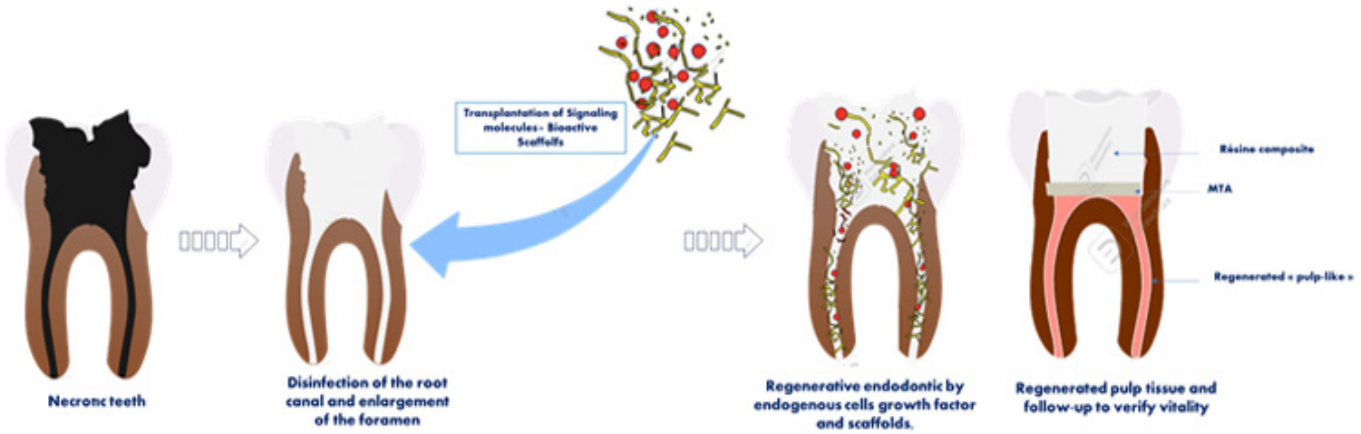

Figure 2 Steps of the cell homing technique.[43]

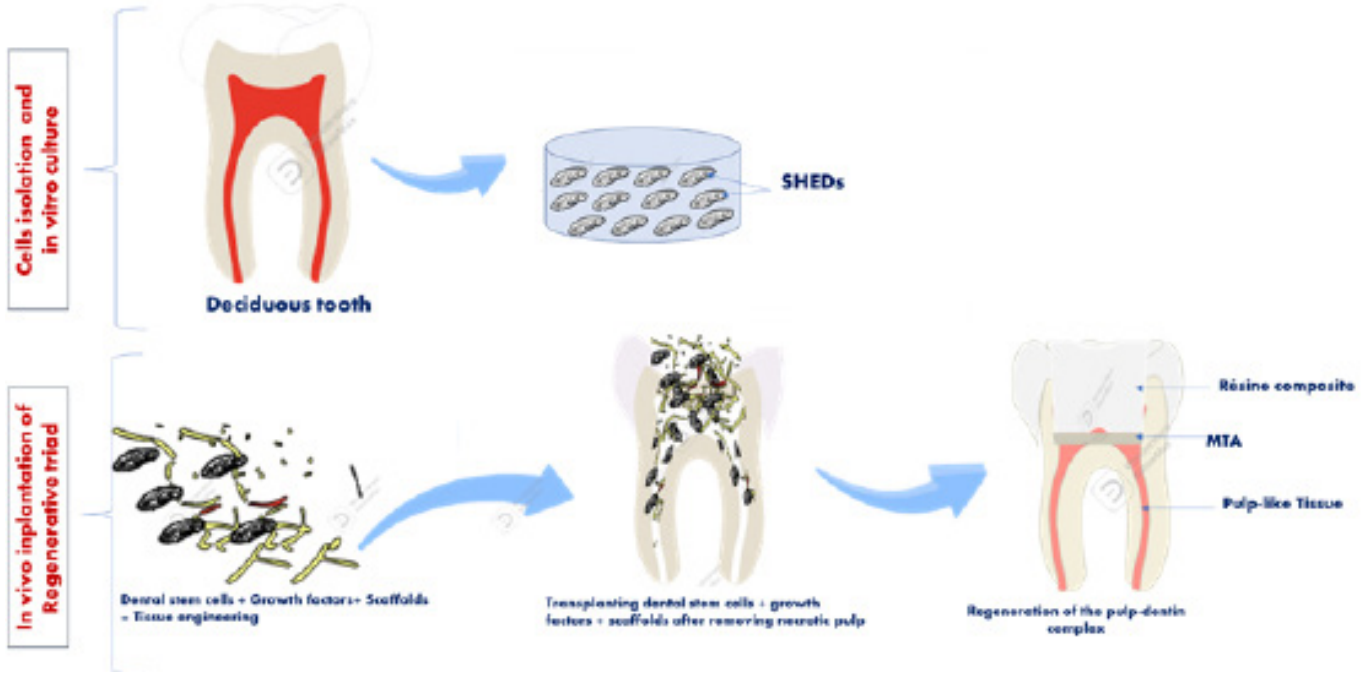

Figure 3 Steps of the Cell Based technique.

Discussion

Tissue engineering involves the creation of new tissue to replace damaged tissue. Endodontic regeneration aims to form new pulp and dentin tissues with the same structure and function as the original tissue. Tissue engineering in endodontics requires the use of stem cells, growth factors, and a biological matrix acting as a scaffold. The scaffold provides chemical stability and mechanical resistance for the newly formed tissue, while stem cells are responsible for tissue renewal, healing, and regeneration after injury, and growth factors control cellular activities such as proliferation and differentiation. Each component plays a crucial role in pulp tissue regeneration.

Stem cells

A stem cell is an undifferentiated cell. It can self-renew and differentiate into specialized cells. There are two main categories of stem cells: Embryonic stem cells (which are pluripotent and can give rise to all types of tissues in the body) and adult stem cells (which are multipotent and can differentiate into a limited number of cell types). These cells are compartmentalized within mesenchymal tissues in "stem cell niches."[17] For example, dental pulp and alveolar bone are rich in mesenchymal stem cells (MSCs).

These niches correspond to a specific microenvironment that contains these cells to maintain tissue homeostasis. They regulate the balance between quiescence, self-renewal, mobilization and differentiation.[17]

The first Dental Pulp Stem Cells (DPSCs) were discovered in the 2000s by Gronthos et al. Stem Cells From Human Exfoliated Deciduous Teeth (SHEDs) were collected later by Miura et al.in 2002.[18,19]

Other stem cell populations of dental origin have been identified in the oral cavity: Stem Cells of Apical Papilla (SCAPs), Dental Follicle Stem Cells (DFSCs), Periodontal Ligament Stem Cells (PDLSCs), Bone Marrow Mesenchymal Stem Cells (BMSCs).[17,20]

DPSCs and SHEDs are the most widely used because of their multipotency, the expression of markers associated with mesenchymal

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cells, their therapeutic potential and the simplicity of their isolation, which is minimally invasive compared with mesenchymal stem cells from other tissues.[21]

These cells have several properties:

- Self-renewal and development of DPSCs and SHEDs

In response to signals, the stem cell starts mitosis. One of the stem cell's progeny will remain undifferentiated; this is known as a self-renewal capacity. The second will irreversibly initiate differentiation.

A stem cell is capable of two types of cell division: asymmetrical, producing a cell destined to differentiate, and symmetrical, producing an undifferentiated stem cell.[22]

- Multipotency of DPSCs and SHEDs

Several in vivo studies have demonstrated that DPSCs and SHEDs can differentiate into odontoblast-like cells and form dentin/pulp complexes. This was demonstrated when transplanted subcutaneously into the dorsal surface of immunocompromised mouse models.[22-25]

- Differentiation of DPSCs

The processes of stem cell proliferation and differentiation are enabled by gene transcription activity, which is regulated by transcription factors. Gene transcription is mediated by the activity of RNA polymerase. Differentiation requires an undifferentiated state, maintained by growth factors, cytokines, intercellular contacts and extracellular matrix.[26] All these elements come together to form what is known as a"stem cell niche", keeping them undifferentiated. This local microenvironment is a physical support as well as a dynamic interface that enables exchanges with the surrounding cells and molecules.

To induce the differentiation of a stem cell into one cell type or another, certain factors, such as signaling molecules are necessary to mimic the molecular signals received during the development of the organism.[27,28]

Other types of stem cells can be mentioned:

- Stem cells of the apical papilla (SCAP)

These types of stem cells are isolated from the dental papilla, an embryonic-type tissue derived from the dental pulp during the formation of the dental tooth crown and located at the apex of the developing dental roots.[18]

Third molars could provide SCAPs for potential future use. Indeed, although human SCAPs are inflamed, for example during apical periodontitis, they retain their vitality and stemness and can undergo osteogenic and angiogenic differentiation.[29]

- Periodontal ligament stem cells (PDLSCs)

PDLSCs are a postnatal stem cell population distinct from mesenchymal stem cells derived from dental pulp or bone marrow. This group of stem cells consists of multipotent cells with osteogenic, adipogenic and chondrogenic differentiation potential.[18]

- Dental follicle stem cells (DFSCs)

They are found in a dental follicle or in the connective tissue surrounding developing teeth, but also in impacted teeth. These are usually extracted and discarded, so there are no controversial ethical issues associated with obtaining stem cells from the dental follicle.

Thus, dental pulp stem cells (DPSCs, SHEDs) and perivascular stem cells (SCAPs, PDLSCs, DFSCs) possess these essential characteristics: mutipotency, the ability to differentiate into different cell types, proliferation, growth and self-renewal, which favor their application in regenerative medicine. However, these properties cannot be optimally expressed without the presence of a growth matrix (scaffold) that provides a favorable environment for the cells to develop.

Scaffolds

The "scaffold" is a three-dimensional (3D) porous solid support designed to provide a favorable environment for tissue development.

Scaffolds must meet the following requirements

1. Promote the optimal spatial location of cells.
2. Facilitate the transport of oxygen, nutrients, bioactive factors, and waste that contribute to cell survival, proliferation, and differentiation;
3. Biodegradable to a degree compatible with the formation of repaired tissue ;
4. Physical and mechanical strength;[30]
5. Anti-inflammatory and non-toxic to surrounding tissue.[31]

Depending on the origin of the scaffolds, there are 2 types:

natural matrices (PRP, collagen, chitosan, glycosaminoglycan, dentin matrix, etc.) and artificial matrices (bioceramics, polymers..)

The most important natural scaffolds are:

- Platelet-rich plasma (PRP)

Platelet-rich plasma (PRP) is an autologous platelet concentrate enriched with growth factors. It is easy to prepare and forms a three-dimensional fibrin matrix that helps retain growth factors.[32,29]

- Platelet-rich fibrin (PRF)

This is a fibrin matrix containing platelet cytokines and growth factors, which acts as a biodegradable scaffold. PRF has been shown to be more clinically effective than PRP.[29]

- Collagen

Collagen is the most abundant fibrous protein in the extracellular matrix. Collagen ensures tensile strength, regulates cell adhesion and promotes cell chemotaxis. A combination of collagen matrix and growth factor SDF-1α promotes vessel formation in the pulp space by attracting surrounding tissue cells via the apex.[32] The disadvantage of collagen is its rapid degradation.[29]

- Hyaluronic acid (HA)

Hyaluronic acid (HA) is one of the best known GAGs. It plays an important role in maintaining morphologic structure. An in vivo study showed that when a hyaluronic acid sponge was implanted at the level of a dentinal defect, a greater number of cell-rich reorganizing tissues (dental pulp, blood vessels, etc.) were observed than when a collagen sponge was implanted.[29]

The disadvantage of hyaluronic acid is its high water solubility, leading to rapid degradation by enzymes such as hyaluronidase.[33]

While for the synthetic scaffold we can cite the following:

- Polymers

Many synthetic polymers such as polylactic acid (PLA), poly-l-lactic acid (PLLA), polyglycolic acid (PGA) and poly-epsilon-caprolactone (PCL) have been used as scaffold materials for pulp regeneration.[31]

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Synthetic polymers are non-toxic, biodegradable and allow accurate control of physicochemical properties such as mechanical rigidity, degradation rate, porosity and microstructure,[34] whereas the main drawback of polymers is their longer degradation time compared with most scaffolds of natural origin.[29]

Growth factors:

Growth factors are released from the demineralized dentin matrix or delivered exogenously.[35] They modulate each key cellular event in pulp homeostasis and dental morphogenesis. Growth factors also play an essential role in functional pulp regeneration.[36] (Table. II) There are several families of growth factors, including.

Table 2 Several families of growth factors[36]

Abbreviation	Factor	Source	Role
BMP	Bone morphogenetic proteins	Bone matrix	Mineral matrix synthesized and secreted by stem cells
PDGF	Platelet-derived growth factor	Platelets, endothelial cells, placenta	Increased number of stem cells
TGF - α	Transforming growth factor α	Macrophages	Induces development of epithelium and tissue structure
TGF - β	Transforming growth factor β	Dentin matrix, activated TH1 cells (T-helper) and Natural killer (NK) cells	Anti-inflammatory, promotes wound healing, inhibits proliferation of macrophages and lymphocytes
FGF	Fibroblast Growth Factor	Large selection of cells	Increased number of stem cells
NGF	Nerve growth factor	Protein secreted by neuronal tissue	Promotes neuronal growth and cell survival

Regeneration techniques

The "Cell Homing" technique

In tissue regeneration, "Cell Homing" is defined as the active recruitment of endogenous cells, including stem cells, into an anatomical compartment.

The concept of cell homing is to achieve tissue repair/regeneration through the chemotaxis of endogenous host cells by means of biological signaling molecules. Cytokines are critical signaling molecules involved in pulp regeneration as they mobilize endogenous cells and regulate stem cell proliferation and differentiation.[29,37]

The use of biological signaling molecules for cell recruitment makes pulp regeneration more easily transposable to the clinic, since administration of growth factors is not as complex and costly as cell transplantation.[38]

Cellular homing consists of two distinct cellular processes: recruitment and differentiation. Recruitment refers to the directional migration of cells, including stem cells, to the site of tissue defects. Pulp/dentine regeneration requires the recruitment of mesenchymal stem cells into the root canal, where they can differentiate into the multiple cell lineages that form pulp and dentin.

Differentiation is the process of transformation of stem cells into mature cells. In other words, pulp and dentin regeneration requires stem cells to differentiate into odontoblasts, neural, endothelial and other angiogenesis-related cells.[38]

The scaffold is a three-dimensional environment for tissue formation, and can also serve as a reservoir for growth factors. The scaffolds most commonly used in the literature are blood clot, PRP and PRF.

Endogenous growth factors can be released from dentin by demineralization. In vitro experiments have demonstrated that concentrations of growth factors harvested from a root canal can be sufficient to induce chemotaxis, proliferation, migration and differentiation of dental stem cells.[39]

Clinical considerations of the Cell Homing technique:

- Effective canal disinfection

Chemical debridement by irrigation is the most important factor in eliminating endodontic infection. However, the preservation of the stem cells is important in RET. It is advisable to use lower concentrations of sodium hypochlorite NaOCI (1.5%) (20 mL/canal, 5 min) followed by EDTA (20 mL/canal, 5 min) with the irrigation needle placed approximately 1 mm from the root tip to minimize cytotoxicity to stem cells in apical tissues.[40]

Indeed, a concentration of 1.5% NaOCl had minimal destructive effects on stem cells in the apical papilla SCAPs. EDTA conditioning of dentin promoted the adhesion, migration and differentiation of stem cells from the dental pulp.[41] Furthermore, the use of 17% EDTA increased the survival of SCAPs.

In addition, complete mechanical debridement is required to eliminate infection and remove necrotic tissue in mature permanent teeth,[42] whereas minimal debridement is required in immature permanent teeth.

- Intra-canal medicament placement

The AAE (American Association of Endodontists) recommends the use of calcium hydroxide. Calcium hydroxide has been shown to promote the survival and proliferation of SCAPs. In addition, a study has shown that SCAPs had the highest survival rate when cultured on dentin exposed to calcium hydroxide compared to dentin exposed to concentrations of 1 mg/mL or higher of tri-antibiotic paste (ciprofloxacin/metronidazole/minocycline).[43,44]

Clinically, an analysis of case reports using calcium hydroxide as an intracanal medicament showed further root maturation.[45,41]

- Creating a blood clot or protein scafold in the canal

After canal disinfection, regenerative endodontic procedures typically involve laceration of periapical tissue to induce bleeding or the use of platelet-rich plasma (PRP) or platelet-rich fibrin (PRF).[41]

- Efficient crown seal

Current protocols recommend that when a blood clot is formed, a pre-measured collagen sponge is carefully placed over the blood clot to serve as an internal matrix for the placement of approximately 3 mm of white MTA followed by a 3-4 mm layer of glass ionomer.

The glass ionomer is then covered with a bond-reinforced composite restoration.

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- Immature permanent teeth

The stem cells most likely to be recruited for cell homing are SCAPs (stem cells of the apical papilla). Periodontal ligament stem cells (PDLSCs) are another potential source of undifferentiated stem cells.

Histologic evidence from a systematic review[46] indicates that rootcanal tissue formed on immature human teeth following REP indicates repair or a combination of repair and regeneration. Residual pulp and healthy periapical tissue appear to enhance regeneration.[47]

- For mature permanent teeth

The diameter of the foramen and the stem cell population present at the periapical level are two important and clinically relevant variants of the mature permanent tooth.

The foramen is the access through which blood vessels, nerves and cells within the dental pulp communicate with the surrounding tissues. If the apical foramen is too small, there will be an impact not only on endogenous cell migration, but also on neovascularization and reinnervation during regeneration.[43]

Currently, there is no consensus on the optimal diameters for apical preparation to promote pulp regeneration, whether in permanent immature teeth or permanent mature teeth.

Additionally, Yang et al. showed in 2016 that apical diameters <1mm achieved clinical success after regenerative endodontic treatment, with highest clinical success in teeth with apical diameters between 0.5-1.0mm.[43]

In contrast to immature permanent teeth, there are no SCAPs apical papilla stem cells to ensure pluripotency in mature permanent teeth, so cell homing in mature permanent teeth relies on chemotaxis of resident cells around the apex, including alveolar bone stem cells, periodontal ligament stem cells, and other cells that can populate a periapical cell niche.[48]

A study was carried out by Arslan et al. 2019 on a population of 58 patients divided into two groups. A conventional root canal treatment was performed for group 1 and a cell free technique for group 2, all cases presenting a necrotic mature teeth with periapical radiolucencies.

The clinical protocol for group 2 included disinfection with triantibiotic paste for 3 weeks, followed by induction of intracanal bleeding, blood clot formation and MTA placement; no use of exogenous growth factors was mentioned. With a 12-month follow-up rate of approximately 73.4% of the total patients, favorable clinical and radiographic results were found in 92.3% and 80% of the REPs and CRCT groups, respectively, and the difference was not statistically significant ($P > .05$). The electric pulp test was positive in 50% of teeth in group 2.[9]

A positive pulp test response does not necessarily mean pulp tissue regeneration. In fact, endodontic regeneration requires dentin-pulp complex formation, presence of odontoblast layer, blood vessels and nerve fibers.

Histological examination of the tissue formed in the mature tooth after the Cell Free technique showed no significant differences from the immature tooth.[49]

"Cell Based" technique

Cell Based or Cell Transplantation is an endodontic regeneration strategy characterized by the transplantation of stem cells (allogenic or autologous) seeded on a scaffold and with growth factors.

The procedure begins with a biopsy of a pulp tissue section containing the stem cells. Then, the stem cells are cultured to multiply and retain their function in the laboratory, followed by inoculation onto a scaffold which is finally transplanted into a disinfected and prepared root canal of the host. After transplantation, the scaffold is degraded and/or remodeled by the host and transplanted cells, resulting in a completely natural tissue.

The cell-based strategy is in the experimental stage. The AAE (American Association of Endodontics) and the ESE (European Society of Endodontology) have not yet recommended autologous stem cell transplantation in clinical regenerative endodontics, as it involves a number of difficulties, such as stem cell isolation, ex vivo expansion, installation of Good Manufacturing Practices (GMPs), stem cell banking, government regulatory issues and comparatively high cost.[50]

A systematic review of 15 animal studies showed that the most widely used scaffold was Collagen and its derivatives (9 studies), while Chitosan hydrogel, Hyaluronic Acid and PRP were also used, however, two animal studies did not use scaffolds, and instead used cellular aggregates containing extra-cellular matrix which acted as a scaffold.[16]

For human studies, Nakashima et al.2017 used Atelo collagen in their Clinical trial.[4] Meza et al.2019 used leukocyte-platelet-rich fibrin (L-PRF) in combination with blood clot.[10] Xuan et al.2018 did not require a scaffold as they used pulp stem cell-derived cellular aggregates (DPSCs) that already contain extracellular matrix.[10]

While the administration of exogenous growth factors requires FDA approval; consequently, their clinical use is currently limited. In human studies, Nakashima et al.2017 were the only ones to use growth factors, using G-CSF.[4]

The Cell Based approach aims to regenerate tissues. Transplantation of exogenous stem cells is necessary to initiate tissue formation and regeneration as early as possible. Regarding exogenous stem cell types, autologous pulp stem cells DPSCs or allogeneic mesenchymal stem cells from the umbilical cord have been tested and found to be effective and safe. Other cell types, such as apical papilla stem cells or mesenchymal stem cells from other tissues, could also be useful.[50]

The pilot clinical trial by Nakashima et al.2017 demonstrated the safety of DPSCs transplantation and the efficacy of the combination of DPSCs and G-CSF for detine-pulp regeneration. It demonstrated no secondary effects on general health during 24-week follow-up, no post-operative pain on percussion and a positive pulpal response 4 weeks after DPSCs transplantation in 4 patients. In addition, magnetic resonance imaging (MRI) signal intensity of regenerated tissue after 24 weeks was similar to that of normal pulp. For cone beam, it showed dentin formation in three patients.[4] Regarding pulp stem cells from deciduous teeth, they are capable of regenerating the entire dental pulp and may be useful for treating traumatized teeth, and this has been confirmed in a randomized clinical trial by Xuan et al. 2018.[7] Another randomized clinical trial has revealed the efficacy of the potential use of DPSC and L-PRF in an adult patient as an alternative procedure to endodontic treatment of mature permanent teeth, and also paves the way for the design of personalized cell-based clinical trials.[10]

An alternative technique is called "pulp implantation", which enables pulp regeneration to be achieved through clinical procedures in the practice without the need for in vitro expansion of pulp stem cells in the laboratory. This is achieved by autotransplantation of the intact pulp of a healthy donor tooth (a wisdom tooth) into the root canal of

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a recipient tooth (requiring root canal treatment).[12] This innovation may be promising because transplantation of the whole intact pulp provides the optimal scaffold for differentiation of pulp stem cells in their environment without the need to add platelet concentrates such as PRP, PRF and L-PRF, and endodontic regeneration will be possible faster because the connective tissue is mature and most of the nerves and vessels are already formed.

Challenges of the cell transplant approach:

- The efficiency of disinfection

Bacteria are likely to remain in the canal even after disinfection, especially in the dentin tubules. In addition, conventional disinfection techniques may prove inadequate. It may therefore be necessary to resort to improved strategies for root canal disinfection such as, using irrigation-activating techniques which can maximize canal disinfection. (ENDOVAC, Sonic System, Ultrasonic, Photonic Activation.[27]

- Regeneration of tubular dentin

The formation of dentin by odontoblasts is unique because it creates dentinal tubules, enabling dentinal sensitivity. This process is difficult to recreate, as shown by the pulp regeneration experiments carried out using various study models. Extensive studies by Gt et al.2021 using cell-based animal models have shown that tubular dentin is not always produced, and when it is produced, the tubules are not organized and compacted as they are in natural dentin.[10]

- Cell Banking

Stem cell collection for long-term storage for therapeutic use is a service that is now available in several countries. Specifically, the dentist collects teeth and "dental banking" services extract and store stem cells in the pulp for the future benefit of the patient. While banks of stem cells collected from bone marrow and placental cord blood have been operating for decades, banks specializing in stem cells isolated from teeth are relatively new.[51]

- Cryopreservation

Once pulp stem cells have been isolated, they need to be successfully cryopreserved. Cells are suspended in a preservation medium, possibly containing growth factors and a cryoprotectant, usually dimethylsulfoxide (DMSO), which inhibits the growth of ice crystals likely to disrupt the cell membrane and thus reduce overall viability. Samples are frozen and placed in low-temperature storage containers filled with liquid nitrogen.[51]

Conclusion

Today, there is still no consensus on a clear definition of regenerative endodontics, nor consensus on the clinical steps to follow for cell-based and cell homing techniques. However, the results of all selected studies are promising and show that these therapies can be considered as a future alternative to conventional endodontics. Nevertheless, studies in larger populations and longer follow-ups are needed to ensure clinically predictable results.

Likewise, although current protocols repair rather than truly regenerate, it is hoped that further research into stem cell pulp engineering will allow for true regeneration and better treatment outcomes.

Acknowledgments

None.

Conflicts of Interest

None.

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