SYSTEMATIC REVIEW

Clinical applications and novel approaches in stem cell: an insight to dental pulp regeneration

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Abstract

Objectives: The aim of the review is to present the currently applied tissue regeneration through stem cells technology in regenerative endodontics. This review also summarises the various preclinical models used for the evaluation of stem cell-based therapies, their limitations, recent advances and challenges related to clinical applications of human stem cells.

Methodology: A literature survey from 2010 to August 2022 was carried out in various electronic databases to identify the articles required for review on Pulp Regeneration through Stem Cells Technology. MeSH terms/keywords such as "Pulp regeneration," "Pulp Revascularization," "Pulp revitalization," "Regenerative Endodontics" were used to search in the electronic databases comprised of PubMed database, SCOPUS, COCHRANRE library, EMBASE, CINAHL, ICTRP, Science Direct and a manual search was also done using the cross references and textbooks.

Results: The searches revealed 299 articles. After reading the full text articles and applying the inclusion and exclusion criteria 15 articles were selected for the review fulfilling the criteria of the study.

Conclusion: Currently in regenerative endodontics, there is a broad consent that the final tissue acquired is more likely to bone-like tissue mixed with connective tissue rather than the pulp-dentin complex. Moreover, re-innervation from sensory axons in regenerated tissue, is still to date, difficult to achieve.

Keywords: Endodontics, Biocompatible, Pulp, Stem Cells, Tissue, Dentin, Axons

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Introduction

Advances in gene-based knowledge on the stem cells within the somatognathic tissues has contributed to the variety of pioneering treatment modalities.[1,2] Ensuing the ground-breaking innovation of DPSCs discovery virtually 20 years ago, stem cell technology has been paving the way for the significant advancement in molecular dentistry and tissue engineering.[2]

In advanced molecular dentistry, focus has been directed towards multidisciplinary approaches exploiting combination of advanced tissue engineering, stem cells, scaffolds, biomaterials, and digital technology, that exhibit significant potentials for regeneration.[3] Stem cells are multipotent, undifferentiated or unspecialized cells capable of self-renewal and multilineage differentiation under permissive conditions and controlled by variety of cell markers.[1] These cells have the ability to replicate, creating a pool of stem cells with high capacity to differentiate and develop along various different lineages (in vivo and in vitro). This ability is governed by an array of cell processes, their inherent multipotency and sensitivity to indigenous paracrine signalling, which are in turn regulated by the niche environment provided.[1,4]

Regenerative endodontic procedures (REP) are biologically based techniques intended to anticipate recruitment stem cell progenitors, indicated for the need to optimize regeneration of dental pulp, whether recruited locally from periapex within the tissue or transplanted.[5] It was originated by the pivotal work done by Dr. Ostby who proposed in the early 1960s that the presence of a blood clot within the root canal stimulates pulp healing, hence preserving pulp vitality.6 A first case report published by Banchs and Trope put forward a novel therapeutic approach called 'revascularization', by locally recruiting stem cells from SCAP in an immature permanent teeth with apical periodontitis, referred to as regenerative endodontic treatment.[7]

Although most applications are tested on animals, stem cell-based regeneration of the pulp is accepted to be theoretically achievable, its therapeutic outcomes are uncertain and remain an elusive goal. This review sought to outline the currently employed tissue regeneration approaches, their limitations, and the potential of stem cell- based therapies as regeneration tools in endodontics. This review also highlights the various

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sources of dental stem cells, their potential for differentiation, the cell markers, and the contemporary status of these approaches in dental pulp regeneration. Moreover it summarises the various pre-clinical prototypes and precursors employed for pulp regeneration and reports the recent advances and future challenges associated to their clinical application.

Methodology

Search Strategy and eligibility criteria: A literature survey in August 2022 was carried out in different electronic databases to requisite the articles for review on Pulp Regeneration through Stem Cells Technology. MeSH terms/keywords such as "Regenerative Endodontics," "Pulp regeneration," "Pulp revitalization" and "Pulp Revascularization" were employed to search in. Articles that fulfilled the study criteria and published in English language between 2010 and August 2022 were included.

Inclusion criteria: The articles required for the review were selected based on the following eligibility criteria.

1. Clinical trials, randomized controlled studies, Investigative reports, and systematic reviews.
2. In-vitro/ in-vivo studies on Regenerative Endodontics on immature/ mature teeth using stem cells.

Screening and Data Collection

The comprehensive review process was carried out by primary authors (SAT and ZH). The screening and selection of articles was divided into two stages: an initial screening of titles and abstracts, followed by a final screening of full text publications. Articles were initially included if they reported the above mentioned keywords in the title or abstract, and this was followed by full text publication screening. During this stage, articles were evaluated and selected based on predetermined inclusion and exclusion criteria. During the screening procedure, there was no disagreement amongst the authors.

Results

The initial search displayed 299 articles that met the inclusion criteria through MeSH terms/keywords in the electronic databases including PubMed database (48), SCOPUS (5), COCHRANRE library (162), EMBASE(42), CINAHL (1), ICTRP (41), Science Direct and a manual search was also done using the cross references and textbooks. Following the evaluation of abstract, 96 duplicated articles were removed. Remaining articles were further re-evaluated and scrutinized on the basis of language, relevant object and full text availability. A total of 15 studies were included as summarized in PRISMA flowchart [figure 1]. The results and outcomes from the studies were evaluated to determine the insights regarding the role of stem cells in regenerative endodontics.

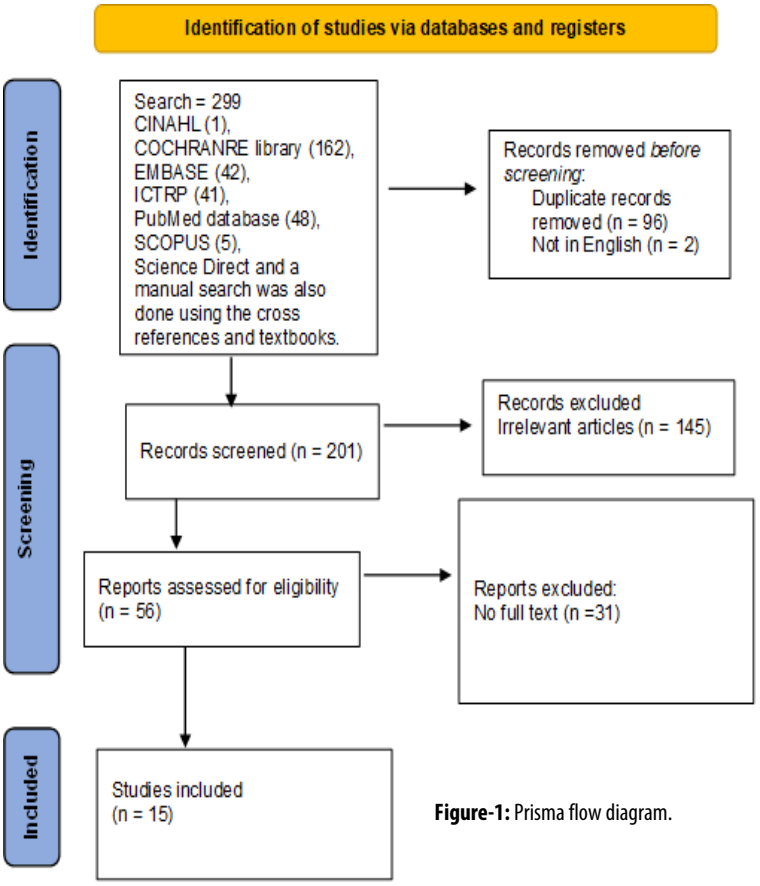

Figure-1: Prisma flow diagram.

Discussion

Stem Cells with potential for Pulp Regeneration:

Dental pulp stem cells were the first discovered dental mesenchymal stem cells (MSC).[5] Following their discovery, MSCs were explored and brought into light in the dental follicle, gingiva, apical papilla stem cells, periodontal ligament stem cells, and exfoliated deciduous teeth.[3] The capacity for growth and differentiation along with the overall patterns of gene and protein expression vary across each of these MSC populations.[2] Stem cells are used as one of the three most critical components (cells, scaffolds, and signalling molecules) critical to

Table-1: Summary of Biomedical approaches in regenerative endodontics in animal model.

Authors	Year	Stem Cells	Methods	Scaffold	Results
Galler *et al.*[35]	2011		In-vitro	GF–laden peptide with VEGF, TGFβ-1, and FGF-2	VEGF, TGF-β1, and FGF2 promoted odontoblast-like cell differentiation nto pulp-like tissue formation
Galler *et al*.[23]	2012	DPSCs	In-vivo	Self-assembling MDP (FGF, TGFβ-1, VEGF)	Led to the formation of a vascularized soft connective tissue similar to dental pulp.
Aksel and Huang.[24]	2017		In-vitro	VEGF and BMP-2	VEGF and BMP-2 enhances differentiation of DPSCs in the presence of odonto/osteogenic medium in 21 days.
Almeida *et al.*[22]	2014	SCAPs	In-vitro	BDNF	SCAP release concentration-dependent BDNF responsible for the chemical signal driving axons to target regenerated tissue.
Coyac *et al.*[36]	2013		In-vitro	Col Type I	Collagen scaffold promoted SHED osteo/odontogenic cell differentiation and mineralization.
Rosa *et al.*[16]	2013	SHED	In-vitro and In-vivo	Col Type I, Peptide	Promoted formation of Pulp-like tissues with odontoblasts capable of generating new tubular dentin throughout the root canals.
Feng *et al.*[37]Wang *et al.*[38]	2014 2012	DPSCs SCAPs	In-vitro	IGF-1	IGF-1 promoted proliferation and osteogenic differentiation of DPSCs and SCAPs.
Huang *et al.*[19]	2010	DPSCs & SCAPs	In-vitro and In-vivo	Poly-D,L-lactide/glycolide	Pulp-like tissue formation with vascularity and dentin-like structure.
Galler *et al*.[20]	2011	DPSCs, SCAPs, PDLSCs, and BMSSCs		PEGylated fibrin gel	Fibrin allows for the growth and differentiation of dental stem cells, can be inserted into small defects and thus appears to be a promising biomaterial for tissue regeneration in the oral cavity.
Kim *et al*.[26]	2010		In-vivo induced Cell homing	FGF, VEGF, PDGF, BMP and NGF	FGF, VEGF, or PDGF with NGF and BMP-7 has potent re-cellularization and revascularization effect on native dentinal wall in root canal.
Bottino *et al.*[25]	2015	No Stem Cells	In-vitro	NF PDS II-HNTs	Potential bioactive scaffold for the attachment and proliferation of human-derived pulp fibroblast cells.

GF: VEGF: Vascular endothelial growth factor. TGFβ-1: Transforming growth factor FGF: Fibroblast growth factors, MDP: multidomain peptides BMP: bone morphogenetic protein NGF: nanofibrous growth factor NF: nanofibrous, PDS: nanocomposite scaffold composed of polydioxanone HNT: halloysite nanotubes IGF: Insulin-like growth factors BDNF: Brain-derived neurotrophic factor DMP1:dentin matrix protein 1. PG: polyethylene glycol

regeneration and tissue engineering.[8] These cells from different origins have been investigated for their potential to be employed in tissue engineering and stem cell-based pulp regeneration in different species of animals, and in humans due to their peculiar bio-characteristics [Figure 2] [Table 1].

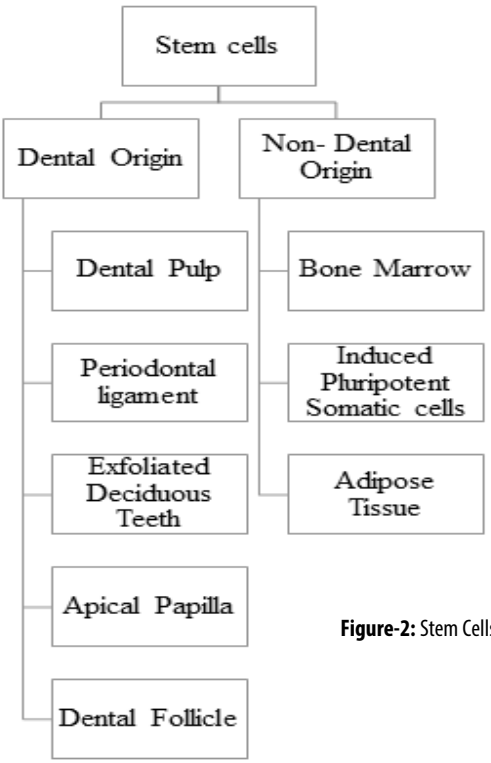

Figure-2: Stem Cells Origin

Stem Cells of Dental Origin:

Dental Pulp Stem cells (DPSCs): Subpopulations of DPSCs with multilineage differentiation potential have odontoblast-like cells, osteogenic, chondrogenic, myogenic, neurogenic, and adipogenic differentiation capacity by demonstrating morphologies of neuronal- and adipocyte-like cells and expression of relevant gene and cell markers. DPSCs develop vascularized pulp-like tissue, surrounded by an odontoblast-like layer that expresses dentin sialophosphoprotein (DSPP), which in turn promote formation of dentin along with dentinal tubules representing natural dentin. Surface Antigens

and cell markers included are CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146, STRO-1. Immunomodulatory Functions comprised of release of prostaglandin E2 (PGE2), interleukin-6 (IL-6) and transforming, growth factor beta (TGF-β).

Periodontal ligament Stem Cells (PDLSCs): PDLSCs exhibit a significant potential to develop into cementum/ PDL-like structures, as well as cement oblasts, osteoblasts, fibroblasts and adipocytes indicating a high regeneration capability. The evidence to date indicates the impact on pulpal regeneration of these cell population is limited and inconclusive. Surface Antigens and cell markers included are CD105, CD73, CD44, CD29, CD10. Immunomodulatory Functions comprised of down-regulation of tumour necrosis factor-α (TNF-α), suppression of production and proliferation of IL-1β and peripheral blood mononuclear cells (PBMNCs).

Stem Cells from Human exfoliated Deciduous Teeth (SHED): SHED exhibited the capacity of rapid differentiation into osteogenic and adipogenic tissues, and expression of numerous cell markers specific to neural cells. Upon being triggered by a neurogenic medium, expression of βIII-tubulin, nestin, Glutamic acid decarboxylase (GAD), and Neuronal nuclei (NeuN) is increased.9 This shows great potential for use in future neuronal replacement. Surface Antigens and cell markers included were CD166, CD146, CD90, CD73, CD29. Immunomodulatory Functions comprised of repression of M2 macrophages, lymphocytes; T helper 17 (Th17) upregulation of CD206+.

Stem Cells from Apical Papilla (SCAP): Significant levels of telomerase activity and the capability to differentiate into odontoblasts and adipocytes indicate that these cells have a high proliferation potential. After transplantation in vivo, SCAP can also develop normal dentin structures, which may be a viable route for cell-based interventions for tissue repair and tissue engineering.[8] Surface Antigens and cell markers included are CD146, CD90, CD44, CD24, STRO-1. Immunomodulatory Functions comprised of suppression of T cell proliferation

Dental Follicle Stem Cells (DFSC): These cells possess therapeutic immunomodulatory potential by rescuing the regeneration mechanism of inflamed dental pulp.[10] Surface Antigens and cell markers included are CD13, CD29, CD44, CD49d, CD56, CD59, CD90, CD105, CD106, CD166, STRO-1. Immunomodulatory Functions comprised of suppression of mononuclear cells, proliferation. upregulation of TGF-β and IL-6 secretion.

Stem Cells of Non-Dental Origin

Bone Marrow Stromal Stem Cells (BMSSCs): These cells have a high proliferative and rapid expansion potential in vitro and can develop into a variety of cell lineages. BMSSC have been demonstrated in vivo to have a high capacity for differentiation into alveolar bone osteoblasts, cementoblasts, and periodontal ligament fibroblasts when transplanted into periodontal defects.[11] However, evidences to date indicates the impact on pulpal regeneration of these cell population is scarce.

Induced/ Reprogrammed Pluripotent Somatic Cells (iPSC): These stem cells can be originated from the cells derived from any tissue, and due to their infinite growth capacity, offer an abundant array of stem cells. They are recruited from a variety of dental-derived cells, including pulpal, gingival, and periodontal ligament cells. Other forms of stem cells derived from dental origin have also been used in dental pulp regeneration, but their regeneration efficacy varies, which is owing in part to the lack of a stem cell marker and scaffold that can identify the pluripotency of tooth MSCs.[12] For this, the identification and characterisation of iPSCs provide an alternative cell source for the very effective regeneration of dentin-pulp complex-like tissues.

Adipose Tissue Stem Cells: These stem cells share many characteristics with BMSSCs but difference in protein and function. They have neither ethical nor immune-reactive contemplation and might be an alternative cell source for pulp regeneration.[13] However studies reported lower proliferator and migratory effects as compare to other MSCs.[14]

Scaffolds for Dental Tissue Engineering

Collagen Scaffold: The ideal biomaterials for tissue engineering are recognised to be collagens that are animal derived and recombinant, particularly type I. Studies has shown that collagen scaffold cross-linking may aid in the growth, adhesion, and differentiation of dental pulp in humans.[15,16]

Blood Derived Scaffolds: PRP: Increased platelets concentrations, growth factors and cytokines found in an autologous supply of blood enhance wound healing, stimulate SCAP proliferation and draw in pulp stem cells. Comparing PRP to blood clot scaffold, clinical studies observed that PRP allows for a greater proportion of hard tissue deposition.[17,18]

PRF: A second-generation platelet concentrate includes a plethora of growth factors, has cell differentiation characteristics, and degrades swiftly. PRF is considered to be a growth factor for dental pulp cells as well as a scaffold for cell adhesion and migration.[17]

Synthetic Polymers: Synthetic polymers are easily manufactured from a wide range of biodegradable polymers and are modifiable, with easy control over degradation. PEGylated fibrin gel and poly-D,L-lactide/glycolide enhanced proliferation, migration, and adhesion of the pulp stem cells. [19,20]

Inorganic Scaffolds: Calcium alginate, Demineralized bone matrix, and primarily calcium phosphate are examples of inorganic regenerative scaffold materials. Calcium phosphate scaffolds have been extensively and effectively employed in bone tissue regeneration with osteoblast cells. The biological properties of scaffolds formed a porous structure that are determined by pore size and shape, percentage of porosity, and the interconnection pathways between pores.[21]

Bioactive Molecules: These bioactive molecules are released from demineralized dentin matrix or delivered exogenously. They have been discovered to play a crucial role in pulp revascularization by generating favourable microenvironments. They include PDGF, TGF, BMP, IGF, FGF, VEGF, NGF, and BDNF. [22–24]

Electro spun Nanofibers for Dental Growth: Due to simplicity in manufacture, tailor ability of pore size, scaffold form, and control over fibre alignment and thickness, electro spun nanofibers are excellent materials in the field of tissue engineering since their architectures behave like natural extracellular matrix. Human DPSCs have displayed odontogenic differentiation and development on mineralized PCL electro spun nanofibrous scaffolds..[25] However, these scaffolds lack mechanical and bifunctional properties that are required for clinical use.

Recent Approaches in Stem Cell Technology

Frequent clinical trials employing regenerative approaches without cells have been conducted since long.[26] Recent preclinical studies and trials using adult stem cells and scaffolds have shown encouraging results, and the groundwork is now established for more innovation in clinical use.[27]

Stem cells through Inflamed Dental Pulp: Chen et al. reported that proliferation capacity of dental pulp stem cells through extracted teeth with deep carious lesions encroaching dentin revealed significantly greater colony-forming units. The fact that cDPSCs had a higher in vitro angiogenesis capability than those produced from normal teeth suggested that they had a larger propensity for proliferation. Angiogenesis is mostly regulated by cDPSC marker expressions and angiogenic-related molecules, particularly VEGF. [28]

Umbilical Cord mesenchymal cells: These cells have an unlimited supply and a significant proliferative capacity as well as the potential for multilineage differentiation.[29] More significantly, the placental barrier's protection reduces the possibility of virus contamination as compared to other sources of adult stem cells.[30] Additionally, research has demonstrated that they can develop into osteoblasts, chondrocytes, cardiomyocytes and neurons. As a result, they have been used for bone and cartilage tissue regeneration.[31,32] Study by Brizuela et al. reported successful dentin-pulp complex regeneration through encapsulated umbilical stem cells in plasma derived scaffolds.[27] However, whether these cells can be employed for dental pulp regeneration remains unprecedented with negligible evidences to date.

Cell Free Regeneration: Leukocytes, cytokines, and glycoproteins like thrombospondin are packed inside the fibrin and proteins that constitute PRP and PRF. Leukocytes provide a biocondensed scaffold and play a crucial role in both the release of growth factors and the immune response. Infection and inflammatory cascades are inhibited by leukocytes, cytokines, and lymphocytes. Pulp regeneration is achieved due to this concentrated suspension of platelets rich in growth factors, TGF-β promotes reactionary dentin formation by stimulated by remaining or differentiating odontoblasts and VEGF aids in angiogenesis which is critical in revascularization. In a recent clinical trial by Ulusoy et al[17] and Ali et al.[33] hypothesized that without preceding apical bleeding and with considerably lower risks of root canal obliteration than BLC, PRP, PRF, and PP can produce results that are comparable to those of BLC in terms of clinical and radiographic outcomes.

Cryopreservation of Stem Cells: Cell banking has gained popularity due to its ease of use and promising therapeutic uses. Clinically acquired teeth and harvested intact dental pulp isolated can be cryopreserved for years, providing a stored reservoir of stem cells for future use.[34]

Limitations: Even though stem cells appear to be the optimal therapy, there are still various challenges to be addressed. Concern over ethics remains one of the initial issues. Furthermore, tumorigenicity is one of the grounds against using IPSCs. When cells are reprogrammed, there is a chance that the expression of oncogenes may rise. Another issue is the process's low efficiency, which is decreasing with each passing year.

Stem cells of different origin exhibit differentiating capacities due to gene variations. For instance, quantitative findings of adipogenesis and osteogenesis showed that SHED are more capable of differentiation

and, DPSCs show more neurosphere development.

Moreover, it was discovered that the tissue created by REPs in the canal of a human revascularized tooth was hard tissue that resembled cementum or bone and connective tissue similar to that found in the periodontal ligament, suggesting that the process was more like repair rather than regeneration.

Conclusion and Future Challenges

There is currently a board consensus in regenerative endodontics that the ultimate tissue acquired, rather than the dentin-pulp complex, is more likely to be bone-like tissue mixed with connective tissue, stirring up controversy over terminology such as "pulpal repair" and "wound healing" rather than "pulp-dentin regeneration". However, the angiogenesis and neurogenic potentials of the stem cells might shift the paradigm towards regeneration rather than just repair. Moreover, re-innervation from sensory axons in regenerated tissue is still to date are difficult to conquer. Traditional therapies are still employed in routine practices, because most of the recently proposed treatment modalities are still in the experimental stage. The limitations such as unpredictability in bleeding induction, numbers of stem/progenitor cell and their viability, and imbalance between complete disinfection remains elusive. For data to endorse the use of stem cells and scaffolds for pulp regeneration, large-scale, high-quality, randomised clinical studies must be conducted with longer follow-ups.

Abbreviations:

Blood count (BLC); Vascular endothelial growth factor (VEGF); Fibroblast growth factors (FGF); Transforming growth factor (TGF); Insulin-like growth factors (IGF); multidomain peptides (MDP); Brain-derived neurotrophic factor (BDNF), dentin matrix protein 1 (DMP1); polyethylene glycol (PEG); bone marrow stromal stem cells (BMSSCs); nanofibrous (NF); nanocomposite scaffold composed of polydioxanone (PDS-II); halloysite nanotubes (HNT); bone morphogenetic protein (BMP).

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