GEORGIAN BIOMEDICAL NEWS

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994 VOLUME 1. ISSUE 4. OCT-DEC 2023

Cell Engineering Potential in Regenerative Endodontics

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DOI: 10.52340/GBMN.2023.01.01.51

ABSTRACT

Dental loss is intricately linked to masticatory and digestive functions, impacting individuals' overall quality of life. The onset of dental caries, explicitly affecting the pulp, gives rise to a prevalent dental pathology recognized as carious lesions, leading to pulpitis and necrosis directly associated with morpho-functional disruptions of the pulp. Devitalized teeth exhibit a decline in trophic, protective, and proprioceptive functions. Conventional endodontic interventions carry a notable risk of complications. With a substantial loss of dental tissues, tooth fracture has an elevated likelihood, consequently diminishing the functional lifespan within the oral cavity. Contemporary dentistry aims to preserve compromised pulp tissue's vitality, facilitate the pulp-dentin complex's functional restoration, and ensure the morpho-functional unit's prolonged functional efficacy. A relatively recent avenue in endodontic therapy, Regenerative Endodontic Procedures (REPs), aligns with the interdisciplinary principles of tissue engineering. This therapeutic approach is grounded in the synergistic combination of three key elements: stem cells, growth factors, and scaffolds.

Keywords: Mesenchymal stem cells; pulp-dentin complex; regenerative endodontic procedures.

INTRODUCTION

The American Association of Endodontists (AAE) defines regenerative endodontics as "biology-based procedures to replace damaged structures, including the dentinal structure, the tooth root, and the dentin-pulp complex.[1] Nowadays, two main ways of REPs (regenerative endodontic procedures) are considered: First includes revascularization of pulp tissue by targeted migration of stem cells in the body (cell homing/cell-free REPs), and the second - pulp restoration by mesenchymal stem cell transplantation (stem cell transplantation/cell-based REPs).

REVIEW

Cell-homing REPs revascularization

The emergence of revascularization in regenerative endodontics as a pioneering field traces its roots back to 1960, marked by Nygaard-Østby's initial endeavors in pulp regeneration.[2] A significant resurgence occurred in 2001 when Iwaya revisited the concept, seeking to rejuvenate the dentin-pulp complex within the periodontal tissue by harnessing circulating stem cells, a process known as cell homing. His successful revascularization of a young permanent tooth diagnosed with periodontitis led to complete apexification and root wall thickening.[3] Further refining the approach, in 2004, Banchs F. and Trope M. introduced a modified protocol for pulp revascularization.[4]

From 2001 to the present, numerous authors have proposed diverse protocols and presented various clinical cases of pulp revascularization. Presently, clinicians widely adhere to two principal guidelines: firstly, the clinical guideline for Regenerative Endodontic Procedures (REPs) published by The American Association of Endodontists (AAE) in 2013, last updated in 2021.[5] Secondly, The European Society of Endodontology (ESE) introduced a protocol in 2016, which outlines success criteria for ESE REPs, including the resolution of inflammation, thickening of root walls, longitudinal growth of the root, restoration of periapically damaged bone tissue, positive pulp electrical excitability test, and absence of tooth discoloration.[6] These success criteria are predominantly centered on improvements in the clinical presentation and aim to achieve three primary objectives: first and foremost, alleviation of clinical symptoms and restoration of bone health; secondly, the desired outcome of increased root length and wall thickness; and thirdly, a positive pulp test of more organized pulp tissue.[5]

REPs/Revascularization with a blood clot

The American Association of Endodontists (AAE) and the European Association of Endodontists (ESE) recognize blood clots as a natural scaffold formed in the root canal due to bleeding resulting from mechanical irritation of periapical tissues. This fibrin network, linked by cross-links, contains vital growth factors facilitating stem cell migration, proliferation, and differentiation. Notably, in young permanent teeth, where the periapical growth zone remains fixed, diverse types of mesenchymal stem cells follow the blood flow into the canal space, holding the potential for restoring the dentin-pulp complex.[7-11]

In 2022, Xi Wei and fellow researchers extensively investigated and presented the cell-free treatment of REPs as an expert consensus. The analysis indicated that this method is deemed ineffective in cases of periodontitis, traumatic tooth loss, permanent teeth with a small apex (<0.5), and stable loss of dental tissue (teeth requiring restoration on fiberglass). The guidelines of AAE and ESE are considered ideal but challenging to achieve and unpredictable.[12] Observational research from 2014 to 2017 indicates that alleviating infection symptoms and restoring periapical tissue is achievable in 91-94%,[13,14] while indicators of root length growth and wall thickening range from 2.70% to 71.43% and 4% to 72.67%, respectively, showcasing some instability.[15,16]

Protocols for canal irrigation vary; AAE recommends final root canal irrigation with 17% EDTA to release growth factors from dentin and activate stem cells. However, studies suggest EDTA in the canal may prevent blood clot formation.[17] Uncontrolled infection is identified as the main reason for clinical failure, with root resorption, pain, and tooth discoloration as major complications. Obtaining the apical clot poses a significant technical challenge.[12] In cases where apical bleeding is problematic, autotransplantation of platelet concentrates like PRP or PRF is employed, although this requires venous blood collection centrifugation and adds to treatment costs. However, studies have not confirmed the superiority of REPs with PRP and PRF, and these matrices may carry a higher risk of introducing infection into the canal compared to clots induced by apical bleeding.[18,19]

Clinical results, while impressive in some cases, must be interpreted cautiously. A 12-month follow-up in 2017 showed asymptomatic and periapical tissue recovery in 100% of cases. However, histological studies revealed fibrous connective, abscess-like, or bone-like tissues instead of the dentin-pulp complex.[20,21]

Revascularization with a blood clot between REPs has several positive aspects. This method is readily available, does not require additional equipment or complex laboratory manipulations, and does not have a high financial cost.[11] However, unpredictable treatment results and false regeneration of the dentin-pulp complex are noteworthy.

Cell-based REPs

Cell-based REPs involve the implantation of endogenous or exogenous mesenchymal stem cells (MSCs) into the root canal space, where they undergo proliferation and differentiation, giving rise to various phenotypic cells within the dental pulp.[22] Extensive studies have established that MSCs are highly promising for restoring the pulp-dentin complex among diverse tissue stem cells. This heterogeneous subset of progenitor stromal cells exhibits significant proliferative capacity and tissue regenerative potential. MSCs can be harvested from different human and animal tissues, including but not limited to bone marrow, epidermal and muscle tissue, liver, placenta, amniotic fluid, umbilical blood (umbilical cord blood), menstrual blood, fatty tissue, and dental pulp. Apart from their reparative capabilities, MSCs can modulate the inflammatory response by down-regulating pro-inflammatory cytokines and activating anti-inflammatory factors. Additionally, MSCs exhibit remarkable immunosuppressive properties, suppressing T cells (T-cells) and NK cells (Natural killer cells) function and regulating the activity of dendritic cells.[23]

For Cell-based Regenerative Endodontic Procedures (REPs) in dentistry, oral mesenchymal stem cells are predominantly utilized with extracellular matrices and signaling molecules. However, some researchers still prefer Human Dental Pulp Stem Cells (DPSCs) and Human Deciduous Tooth Pulp Derived Stem Cells (SHED) due to their distinctive angiogenic, neurogenic, and odontogenic capabilities.

Oral Mesenchymal Stem Cells serve as a valuable source, encompassing five types of Dental Stem Cells (DSCs): DPSCs, Human Dental Peripheral Ligament Stem Cells (PDLSCs), SHED, Apical Papilla, and Follicular Stem Cells. DSCs prove to be not only beneficial for regenerative endodontics but also hold promise for broader applications in regenerative medicine. Their potential to differentiate into mesodermal and ectodermal cells, coupled with immunomodulatory properties demonstrated through the secretion of cytokines and immune receptors, opens avenues for treating various inflammatory, autoimmune, allergic, and other diseases.

DSCs exhibit high potency, long-term genetic stability, and compatibility with scaffolds and growth factors. While using DSCs does not raise moral or ethical concerns, there are still limitations on their clinical exploitation. Achieving complete safety and efficacy requires further clinical observations and an in-depth study of the regulatory mechanisms governing DSC use.[24]

STEM cells from the apical papilla (SCAP)

Like DPSCs and SHED cells, SCAP exhibits a notable capacity for odontogenic differentiation.[25] An optimal source for autotransplantation of SCAP involves the extraction of unerupted third molars at an early age and subsequent cell banking. However, the impact of freeze-thaw processes on the viability of these high-quality stem cells is an ongoing area of investigation. Given that SCAP originates from developing tissue, it represents a population of progenitor cells encompassing differentiating cells, such as stem cells, that have yet to demonstrate self-renewal ability. This characteristic raises the prospect that, beyond its role in restoring the dentin-pulp complex, SCAP may emerge as an excellent source for regenerating other tissues as well.[26]

Periodontal ligament stem cells (PDLSCs)

Periodontal ligament stem cells (PDLSCs) derived from the human periodontal ligament constitute a distinctive subset of mesenchymal stem cells renowned for generating

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

ligament-like and fibrous tissues in vivo. A well-functioning peritoneum encompasses both progenitor and specialized cells. Specialized cells are implicated in synthesizing cancellous, fibrous, connective tissue, and alveolar bone.[27] Among these, osteoblasts, fibroblasts, and cementoblasts serve as ligament synthetic cells (forming cells), while osteoclasts, fibroblasts, and cementoclasts function as resorbing cells. Synthesizing and reabsorbing stem cells originate from adjacent lymphoid and hematopoietic stem cells. A comprehensive study conducted in 2014 explored the tendon's potential as a promising reservoir of stem cells, focusing on alterations in collagen levels within the tendon.[28] Subsequent research in rats confirmed the metabolic activity of molar PDLSCs. Notably, collagen turnover in the mucosal layer proper of the periodontal gingiva was identified to be five times faster than that of fibroblasts and fifteen times faster than that of fibroblasts in the skin.[29]

Human dental pulp stem cells (hDPSCs)

In 2000, Gronthos substantiated the presence of stem cells within the human dental pulp. The isolation of perivascular markers such as STRO-1, VCAM-1, MUC-18, and smooth muscle actin from the dental pulp by Gronthos suggested that dental pulp stem cells (DPSCs) represent a heterogeneous group of mesenchymal stem cells likely residing within the perivascular niche of the pulp. In 2002, the same investigator extracted dentin sialoprotein (DSPP) from a structure resembling dentin. Analogous to bone marrow skeletal stem cells (BMSSCs), human DPSCs (hDPSCs) exhibit characteristics of self-renewal and the ability to generate diverse cell phenotypes under in vivo conditions. Distinct cell subpopulations of DPSCs demonstrate variable proliferative rates and developmental potential, mirroring characteristics observed in BMSSCs, thereby belonging to a novel category of postnatal somatic stem cells. In light of these distinctive attributes, human dental pulp stem cells emerge as a compelling research model for in vitro differentiation studies and hold promise as a model for in vivo tissue regeneration.[25]

Stem cells from human exfoliated deciduous teeth (SHED)

Stem cells derived from human deciduous teeth, known as stem cells from human exfoliated deciduous teeth (SHED), originate from the neural crest within the pulp tissue of deciduous teeth. These cells exhibit notable proliferative capabilities and possess the capacity to differentiate into osteoblasts, adipocytes, and nerve cells. Given SHED's extensive proliferative and versatile differentiation potential, investigations have been undertaken to explore their regenerative potential in dentin, bone, muscle, cartilage, cornea, hair, and nerve tissues.[30] In 2003, Miura and colleagues isolated stem cells from mammary sheep, demonstrating their differentiation into new postnatal stem cells capable of giving rise to neural, odontogenic, and adipocyte cells. The study revealed that SHED secretes various osteoblastic markers, including alkaline phosphatase (ALP), extracellular phosphoglycoprotein (MEPE), bone sialoprotein (BSP), and human dentin sialoprotein (DSPP). Subsequently, the authors transplanted SHED, differentiated into odontoblast-like cells, along with a hydroxyapatite-tricalcium phosphate (HA/TCP) carrier, into (immunocompromised) mice, resulting in the formation of a small dentin-like structure.[31] Furthermore, evidence supports the in vivo differentiation of SHED into odontoblasts. With its potential applications in restoring damaged tooth structures, bone regeneration, and potential treatment of damaged nerve tissue or degenerative diseases, SHED stands out as an ideal source for therapeutic interventions.[32]

Identification of stem cell markers

Selecting the appropriate cell population and achieving therapeutic success necessitates the identification of stem cell markers. DPSC and SHED, sharing phenotypic profiles with MSCs, secrete various MSC-specific markers, including CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166, CD271, Stro-1, and Stro-3. They should be negative for markers such as CD3, CD8, CD11b (or CD14), CD15, CD19 (or CD79α), CD33, CD34, CD45, CD71, CD117, and HLA-DR.[33,34]

Undifferentiated MSCs contain osteogenic markers (osteonectin, osteocalcin, osteopontin, BMP-2, BMP-4, Runx2, collagen type I), chondrogenic markers (collagen type II, SRY-Box 9), adipogenic markers (leptin, adipophilin), and myogenic markers (desmin, myogenin, myosin IIa, αSMA).[35] PSCs have a substantial neurogenic potential and express such markers as c-fos, γ-enolase, nestin, βIII tubulin, A2B5, musashi-1, neurofilament heavy and light, microtubule-associated protein 2, glial fibrillary acidic protein, and oligodendrocyte-associated CNPase.[35,36] Differences in embryonic and neurogenic markers between DPSC and SHED align with the distinct age categories of the pulp tissues from which they are isolated.[37]

Cell-based REPs experimental and clinical studies

Over the past decade, a wealth of data has been amassed regarding the odontogenic potential of mesenchymal stem cells. Numerous studies substantiate the efficacy of transplanting human dental pulp stem cell (hDPSC) cultures to restore the dentin-pulp complex. Pioneering efforts in big animal tooth restoration were undertaken by Iohara and colleagues in 2009, marking the initial endeavor to regenerate the dentin-pulp complex. In this groundbreaking study, sheep pulp stem cells were transplanted into the pulp chamber of a dog tooth following pulpotomy, leading to the observed regeneration of capillaries and nerve cells within 14 days of control.[38] Building upon this success, the same

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

research group reported promising outcomes in 2011 following the transplantation of CD105(+) and SDF-1 positive pulp stem cells into the pulp chamber of an animal (dog) tooth.[39]

There is currently not much data regarding the safety of clinical use of mesenchymal stem cells. However, clinical studies on pulpectomized canine teeth have confirmed DPSC's regenerative potential, safety, and non-toxicity. In 2013, an animal (mouse) experiment (in vitro and in vivo) with SHED and injectable extracellular matrices, collagen type I (human recombinant BD™ Fibrinogen Collagen Type I; BD Biosciences) or Puramatrix™ (BD Biosciences, Bedford, MA, USA), revealed differentiated, functional odontoblasts and vascularization similar to human dental pulp throughout the length of the root canal.[40]

In 2017, a cohort of researchers disseminated the outcomes of a clinical pilot study involving five patients diagnosed with post-traumatic irreversible pulpitis. Following pulp extraction, the investigators conducted autotransplantation of mobilized dental stem cells (MDPSC) and the administration of granulocyte colony-stimulating factor (G-CSF). Remarkably, within four weeks, a positive pulp test was achieved, and subsequent cone beam computed tomography (CT) scans after 24 weeks revealed the development of functional dentin in three out of the five patients.[41] In a parallel development, a randomized clinical trial focused on traumatic pulp necrosis cases showcased pulp regeneration characterized by the formation of blood vessels, neural markers, connective tissue, and the odontoblast layer after dental pulp stem cell (DPSC) transplantation.[42]

In 2020, they published in vivo animal studies to show scaffolds' crucial role in cell engineering. The investigation revealed that a discernible dentin layer was successfully generated through tissue engineering principles, specifically regenerative endodontic procedures (REPs) involving cell transplantation. This dentin formation was achieved through the synergistic interaction of dental pulp stem cells (DPSC) and hydroxyapatite/tricalcium phosphate (HA/TCP). Furthermore, the newly synthesized dentin layer exhibited the secretion of dentin sialoprotein (DSPP), indicative of cellular differentiation into odontoblasts, as verified by specific markers.[43]

In 2021, a group of authors conducted a comprehensive meta-analysis, reviewing 247 articles to assess the potential of dental pulp stem cells (DPSC).[44] This analysis encompassed the outcomes of xenotransplantation, allotransplantation, and autotransplantation of stem cells isolated from dental pulp. Xenotransplantation cases involved the transplantation of DPSCs into various animal models, including dogs, rabbits, pigs, mice, and rats. A clinical review of four cases of autotransplantation in humans highlighted the multi-proliferative characteristics of dental stem cells, observing their potential for new tissue formation across all graft types. Animal studies demonstrated the versatile regenerative capabilities of mesenchymal stem cells, contributing to the regeneration of diverse tissues such as bone, the dentin-pulp complex, abscesses, blood vessels, nerves, spinal cord, cartilage, muscles, pancreas, and kidneys. Four clinical cases were reviewed, with two involving the transplantation of DPSCs for intrabone defect regeneration and two for pulp regeneration. These findings underscore the broad spectrum of applications for DPSCs in regenerative medicine across both preclinical and clinical settings.

Most human dental pulp stem cells (DPSCs) have been isolated from extracted third molars or premolars with orthodontic relevance.[45-57] The age range of patients from whom teeth were sourced for stem cell isolation spanned from 6 to 39 years. In xenotransplantation, stem cells extracted from human teeth pulp were introduced into animal subjects, primarily rats and pigs. DPSCs for transplantation studies in rats were sourced from extracted incisors and molars,[58-62] while in pigs, DPSCs were exclusively derived from molars.[63] A total of 58 clinical cases detailing DPSC autotransplantation in humans were documented. Among these cases, stem cells were procured from deciduous teeth in 41 cases, inflamed pulp (diagnosed with irreversible pulpitis) in 2 cases, and permanent teeth extracted for complex or orthodontic reasons in the rest of the cases.

Stem cell transplantation is aimed at the restoration of the pulp-dentin complex.[37,39] Subsequent pulp extraction from mature teeth in dogs has demonstrated remarkable achievements in achieving complete pulp regeneration neurogenesis and vasculogenesis.[40] In the context of autotransplantation, the infusion of pulp progenitor cells (CD105(+)) was orchestrated alongside stromal cell-derived factor-1 (SDF-1) and a collagen scaffold, leveraging its heightened biocompatibility. This strategic approach yielded the observation of a novel pulp-like tissue within the canal, featuring integrated blood vessels, nerves, and the development of freshly formed dentin along the canal walls. The regenerative potential of human dental pulp stem cells (DPSCs) in autotransplantation has been substantiated in cases of irreversible pulpitis and post-traumatic pulp necrosis.[40]

Recent pilot studies involving humans have promising results, evident in both clinical and radiographic assessments, showcasing the potential to restore the pulp-dentin complex effectively.[21]

Limitations of cell-based REPs

The primary challenges in Cell-based Regenerative Endodontic Procedures (REPs) are isolation, expansion/proliferation, and subsequent storage of stem cells in a cell bank. Moreover, obtaining sufficient Mesenchymal Stem Cells (MSCs) in 2D and 3D cultures is

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

complicated, costly, and requires highly specialized laboratory conditions. Of particular concern is the observed short survival span of implanted MSCs.[64] Transplantation of MSCs into inflamed tissue has demonstrated a reduced likelihood of their survival, with a significant decrease of implanted MSCs within the initial 24 hours.[65] These challenges underscore the imperative for further exploration and refinement of strategies to optimize the isolation, proliferation, and survival of MSCs in Cell-based REPs, aiming to enhance the overall efficacy of regenerative therapies

CONCLUSIONS

Based on the sources above, it is evident that the variations and unpredictable outcomes associated with the dental revascularization protocol using the cell-free approach in Regenerative Endodontic Procedures (REPs) introduce significant uncertainty among clinicians. Furthermore, the blood clot, often considered an extracellular matrix in REPs, falls short of an ideal substrate for dentin-pulp complex restoration. In the best-case scenario, it yields pulp-like connective tissue within the root canal space.

In contrast, Cell-based REPs, a method based on cell engineering, have promising potential for pulp regeneration. Despite the considerable financial investment and the need for high multidisciplinary competence on the practitioner's part, the studies suggested that only the cell-based treatment pathway truly offers prospects for restoring the authentic pulp-dentin complex. It is crucial to note that cell-based REPs are currently in the pilot study phase. The safety of their clinical application and the potential of transition into clinical practice depends on accumulating clinical data and technological refinements and simplifying and enhancing accessibility.

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Georgian Biomedical News
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994