Evaluation Of Dental Pulp Stem Cell Differentiation Pathways Under Various Biomimetic Scaffolds For Regenerative Endodontic Procedures In Immature Permanent Teeth

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Abstract
Regenerative endodontic procedures (REPs) represent a promising approach for treating immature permanent teeth with necrotic pulp. These procedures rely on the differentiation potential of dental pulp stem cells (DPSCs) and the development of appropriate biomimetic scaffolds. This review explores the various differentiation pathways of DPSCs under different biomimetic scaffolds for REPs in immature permanent teeth. We examine the biological characteristics of DPSCs, their differentiation potential, and the influence of various biomaterials and scaffolds on their behavior. Current evidence suggests that the combination of appropriate bioactive materials with suitable scaffolds can significantly enhance DPSC differentiation toward odontoblast-like cells, promoting dentinogenesis and pulp-dentin complex regeneration. This article provides a comprehensive analysis of recent advances in biomimetic scaffolds and their applications in regenerative endodontics, offering insights into optimizing REPs for clinical applications.

1. INTRODUCTION

The field of regenerative endodontics has evolved significantly in recent years, focusing on the biological replacement of damaged pulp-dentin complexes in immature permanent teeth (Rosa et al., 2011). Traditional endodontic treatments often fail to address the specific challenges presented by immature permanent teeth with necrotic pulp, including thin dentinal walls, open apices, and arrested root development. Regenerative endodontic

procedures (REPs) have emerged as a promising alternative, aiming to restore pulp vitality, promote continued root development, and strengthen dentinal walls (Casagrande et al., 2011).

Tissue engineering principles form the foundation of REPs, requiring three key elements: stem cells, scaffolds, and growth factors (Moro et al., 2018). Among these, dental pulp stem cells (DPSCs) have gained particular attention due to their multipotent nature and their ability to differentiate into various cell types, including odontoblasts, which are crucial for dentin formation and pulp regeneration (Gronthos et al., 2000).

The microenvironment in which DPSCs exist plays a crucial role in determining their fate and differentiation pathway. Biomimetic scaffolds provide a three-dimensional structure that mimics the natural extracellular matrix, offering physical support for cell attachment, proliferation, and differentiation (Sachlos & Czernuszka, 2003). The interaction between DPSCs and various biomaterials within these scaffolds can significantly influence cell behavior and differentiation potential (Zhang et al., 2006).

This comprehensive review aims to evaluate the current understanding of DPSC differentiation pathways under various biomimetic scaffolds for regenerative endodontic procedures in immature permanent teeth. By examining recent advances in this field, we seek to provide insights into optimizing REPs for enhanced clinical outcomes.

2. Dental Pulp Stem Cells: Characteristics and Potential

2.1 Biological Characteristics of DPSCs

Dental pulp stem cells were first isolated by Gronthos et al. (2000) from adult human dental pulp tissue. These cells exhibit several key characteristics that make them valuable for regenerative endodontics, including self-renewal capacity, high proliferation potential, and multipotency. DPSCs express various markers, including STRO-1, CD73, CD90, and CD105, while lacking hematopoietic markers such as CD45, CD34, and HLA-DR (Sloan & Smith, 2007).

The self-renewal capacity of DPSCs is demonstrated by their ability to form colony-forming units-fibroblast (CFU-F) when cultured at low density. This property allows for the expansion of DPSCs in vitro, making them suitable for various regenerative applications. Furthermore, DPSCs show high proliferation rates, with population doubling times typically ranging from 30 to 50 hours, depending on culture conditions and donor characteristics (Gronthos et al., 2000).

DPSCs can be harvested from various sources, including permanent teeth, exfoliated deciduous teeth (SHED), and apical papilla. SHED cells, in particular, demonstrate higher proliferation rates and increased population doublings compared to DPSCs from permanent teeth, suggesting enhanced regenerative potential (Miura et al., 2003). The isolation and characterization of these cells typically involve enzymatic digestion of pulp tissue, followed by culture in appropriate growth media and identification of specific surface markers (Feng et al., 2016).

The phenotypic characterization of DPSCs involves the analysis of cell surface marker expression using flow cytometry or immunocytochemistry. These cells consistently express mesenchymal stem cell markers, including CD73, CD90, and CD105, while being negative for hematopoietic markers such as CD45, CD34, and HLA-DR. Additionally, DPSCs express specific markers associated with neural crest origin, such as p75, Sox10, and Snail, reflecting their developmental origin (Sloan & Smith, 2007).

Another unique characteristic of DPSCs is their immunomodulatory properties. These cells can suppress T-cell proliferation and modulate the inflammatory response through the secretion of various cytokines and growth factors. This property is particularly valuable for

regenerative applications, as it can help create a favorable environment for tissue repair and regeneration while minimizing adverse immune reactions (Casagrande et al., 2011).

2.2 Differentiation Potential of DPSCs

One of the most significant properties of DPSCs is their ability to differentiate into multiple cell lineages, including odontoblasts, osteoblasts, chondrocytes, adipocytes, and neuronal cells (Casagrande et al., 2011). This versatility makes them particularly valuable for regenerative applications in dentistry.

For regenerative endodontic procedures, the odontogenic differentiation pathway is of primary interest. Under appropriate conditions, DPSCs can differentiate into odontoblast-like cells capable of producing dentin-like structures. This differentiation is characterized by the expression of specific markers, including dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), alkaline phosphatase (ALP), and osteocalcin (OCN) (Suzuki et al., 2012).

The odontogenic differentiation of DPSCs involves a complex process that can be divided into several stages. The initial stage is characterized by cell proliferation and the expression of early markers such as Runt-related transcription factor 2 (RUNX2) and Osterix (OSX). This is followed by the expression of more specific odontogenic markers, including DSPP and DMP-1, and culminates in the formation of mineralized nodules. The entire process is regulated by various signaling molecules, transcription factors, and epigenetic mechanisms (Kim et al., 2021).

The osteogenic differentiation of DPSCs follows a similar pathway but may result in the formation of bone-like rather than dentin-like structures. This differentiation is characterized by the expression of markers such as RUNX2, OSX, ALP, OCN, and bone sialoprotein (BSP). The ability of DPSCs to undergo osteogenic differentiation makes them valuable for applications involving bone regeneration, such as the repair of craniofacial defects (Eslaminejad et al., 2013).

DPSCs also demonstrate neurogenic potential, with the ability to differentiate into neuronal and glial cells when exposed to appropriate conditions. This differentiation is characterized by the expression of neural markers such as nestin, β-III-tubulin, and glial fibrillary acidic protein (GFAP). The neurogenic potential of DPSCs is particularly interesting given the neural crest origin of dental pulp tissue and may have applications in the treatment of neurological disorders (Sloan & Smith, 2007).

The adipogenic and chondrogenic differentiation potentials of DPSCs have also been demonstrated, although these pathways are less relevant for regenerative endodontic applications. Under adipogenic conditions, DPSCs can form oil-filled cells that express adipogenic markers such as peroxisome proliferator-activated receptor γ (PPARγ) and lipoprotein lipase (LPL). Under chondrogenic conditions, DPSCs can form cartilage-like structures expressing markers such as collagen type II and aggrecan (Casagrande et al., 2011).

The regulation of DPSC differentiation involves complex interactions between various signaling pathways, including Wnt, Notch, and BMP signaling. These pathways can be modulated by specific growth factors, such as transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs), as well as by the physical and chemical properties of the surrounding microenvironment (Kim et al., 2021).

3. Biomimetic Scaffolds for Regenerative Endodontics

3.1 Types and Properties of Scaffolds

Scaffolds provide a three-dimensional structure that supports cell attachment, migration, proliferation, and differentiation. For regenerative endodontics, an ideal scaffold should be biocompatible, biodegradable, and possess appropriate mechanical properties to support

cell growth and differentiation (Eslaminejad et al., 2013). Various types of scaffolds have been investigated for DPSC-based regenerative endodontics, including natural polymers, synthetic polymers, and bioceramics.

Natural polymers, such as collagen, chitosan, and fibrin, offer excellent biocompatibility and biodegradability. These materials can mimic the natural extracellular matrix, providing a favorable environment for cell attachment and proliferation. However, they often lack sufficient mechanical strength for certain applications (Theocharidou et al., 2017).

Collagen, the most abundant protein in the extracellular matrix, is widely used as a scaffold material for tissue engineering applications. It provides multiple binding sites for cell attachment through integrin receptors and can be degraded by cellular enzymes, allowing for gradual replacement by newly formed tissue. The mechanical properties of collagen scaffolds can be enhanced through crosslinking techniques, such as glutaraldehyde or carbodiimide treatment, although this may affect the biocompatibility and degradation rate of the scaffolds (Zhang et al., 2006).

Chitosan, a deacetylated derivative of chitin, is another natural polymer with promising properties for tissue engineering applications. It exhibits antibacterial activity, hemostatic properties, and biocompatibility. Chitosan scaffolds can be prepared in various forms, including hydrogels, sponges, and nanofibers, offering flexibility for different applications. The degradation rate of chitosan scaffolds can be controlled by adjusting the degree of deacetylation and molecular weight of the polymer (Ching et al., 2020).

Fibrin, derived from blood plasma, is a natural polymer that plays a crucial role in wound healing and tissue repair. Fibrin scaffolds can be formed through the enzymatic conversion of fibrinogen to fibrin by thrombin, mimicking the natural clotting process. These scaffolds provide a favorable environment for cell attachment and growth and can incorporate various growth factors to enhance their biological performance. However, fibrin scaffolds typically exhibit poor mechanical properties and rapid degradation, limiting their use in certain applications (Manaspon et al., 2021).

Synthetic polymers, including polycaprolactone (PCL), polylactic acid (PLA), and poly(lactic-co-glycolic) acid (PLGA), offer greater mechanical stability and controllable degradation rates. These properties make them suitable for applications requiring longer-term support. The incorporation of bioactive components into synthetic polymer scaffolds can enhance their biological performance (Diba et al., 2011).

PCL is a biodegradable polyester with a relatively long degradation time, ranging from several months to years, depending on molecular weight and crystallinity. PCL scaffolds exhibit excellent mechanical properties and can be fabricated in various forms, including films, fibers, and porous structures. The hydrophobic nature of PCL may limit cell attachment and proliferation, but this can be addressed by surface modification techniques, such as plasma treatment or the incorporation of hydrophilic groups (Diba et al., 2011).

PLA is another biodegradable polyester with a shorter degradation time compared to PCL, typically ranging from weeks to months. PLA scaffolds can be fabricated using various techniques, including solvent casting, particulate leaching, and electrospinning. The mechanical properties of PLA scaffolds can be adjusted by varying the molecular weight and crystallinity of the polymer. The degradation products of PLA, primarily lactic acid, are naturally occurring compounds that can be metabolized by the body, ensuring biocompatibility (Theocharidou et al., 2017).

PLGA, a copolymer of PLA and polyglycolic acid (PGA), offers tunable degradation rates by adjusting the ratio of the two components. PLGA scaffolds exhibit good mechanical properties and can be fabricated in various forms, including microspheres, nanofibers, and porous structures. The degradation of PLGA involves hydrolysis of ester bonds, resulting in the release of lactic and glycolic acids, which are natural metabolites. The acidic

degradation products may cause local pH reduction, potentially affecting cell behavior and tissue formation (Yang et al., 2018).

Bioceramics, such as tricalcium phosphate (TCP), hydroxyapatite (HA), and bioactive glasses, exhibit excellent biocompatibility and osteoconductivity. These materials can stimulate mineral deposition and promote odontogenic differentiation of DPSCs. However, their brittleness and slow degradation rates may limit certain applications (Ching et al., 2020).

TCP, a calcium phosphate ceramic with a chemical formula of $Ca_3(PO_4)_2$, exists in two main crystalline forms: α-TCP and β-TCP. β-TCP is more stable at room temperature and is commonly used in biomedical applications. TCP scaffolds exhibit excellent biocompatibility and osteoconductivity, promoting cell attachment, proliferation, and differentiation. These scaffolds can be fabricated using various techniques, including sintering, freeze-drying, and 3D printing. The degradation rate of TCP scaffolds can be adjusted by controlling the porosity and crystal structure of the material (Eslaminejad et al., 2013).

HA, the main inorganic component of natural bone and dentin, has a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. HA scaffolds exhibit excellent biocompatibility and osteoconductivity but have limited biodegradability. These scaffolds can be fabricated using techniques such as sintering, sol-gel processing, and precipitation. The incorporation of ions, such as silicon, magnesium, or strontium, into the HA crystal structure can enhance the biological performance of the scaffolds, promoting cell differentiation and mineralization (Ching et al., 2020).

Bioactive glasses, primarily composed of SiO_2, Na_2O, CaO, and P_2O_5, have the ability to bond with both hard and soft tissues. These materials can release ions, particularly silicon, calcium, and phosphate, that stimulate specific cellular responses, promoting tissue repair and regeneration. Bioactive glass scaffolds can be fabricated using various techniques, including sintering, sol-gel processing, and foam replication. The composition and structure of bioactive glasses can be adjusted to control their degradation rate and ion release profile, tailoring them for specific applications (Jun et al., 2017).

3.2 Advanced Scaffold Fabrication Techniques

Recent advances in scaffold fabrication techniques have enabled the development of more sophisticated biomimetic structures that better mimic the natural pulp-dentin complex. Electrospinning, 3D printing, and freeze-drying are among the techniques used to create scaffolds with controlled porosity, fiber alignment, and mechanical properties.

Electrospinning allows the production of nanofibrous scaffolds with high surface-to-volume ratios, mimicking the natural extracellular matrix and providing numerous binding sites for cell attachment. These scaffolds can be modified with various bioactive components to enhance their biological performance (Jun et al., 2017).

The electrospinning process involves the application of a high voltage to a polymer solution, creating an electrically charged jet that is drawn toward a grounded collector. As the solvent evaporates, solid polymer fibers are formed, resulting in a fibrous mat. The fiber diameter, orientation, and porosity can be controlled by adjusting parameters such as solution concentration, voltage, flow rate, and collector configuration. Electrospun scaffolds have been successfully used for DPSC culture and differentiation, providing a favorable environment for cell attachment, proliferation, and differentiation (Jun et al., 2017).

The incorporation of bioactive components into electrospun scaffolds can be achieved through various methods, including blend electrospinning, coaxial electrospinning, or surface modification. Blend electrospinning involves the addition of bioactive components to the polymer solution before electrospinning, resulting in their incorporation within the

fibers. Coaxial electrospinning involves the use of two concentric needles, allowing the formation of core-shell fibers with the bioactive component encapsulated within the core. Surface modification involves the attachment of bioactive components to the fiber surface after electrospinning, using techniques such as plasma treatment or chemical conjugation (Jun et al., 2018).

3D printing enables the fabrication of patient-specific scaffolds with precise control over internal architecture, porosity, and mechanical properties. This technique allows for the creation of complex structures that can better replicate the natural tissue organization and facilitate vascularization (Park et al., 2021).

Several 3D printing techniques have been used for scaffold fabrication, including fused deposition modeling (FDM), stereolithography (SLA), and selective laser sintering (SLS). FDM involves the extrusion of a molten polymer filament through a heated nozzle, which moves according to a predetermined pattern to build the scaffold layer by layer. SLA involves the selective curing of a photosensitive resin using a light source, typically a laser or projector, to build the scaffold layer by layer. SLS involves the selective sintering of powder particles using a high-energy laser, resulting in the formation of a solid structure (Park et al., 2021).

The use of 3D printing for scaffold fabrication offers several advantages, including the ability to create patient-specific designs based on medical imaging data, precise control over scaffold architecture and properties, and reproducibility. These advantages make 3D printing particularly valuable for regenerative endodontic applications, where the complex anatomy of the root canal system presents significant challenges for traditional scaffold fabrication techniques (Park et al., 2021).

Freeze-drying is another valuable technique for creating porous scaffolds with interconnected pores, facilitating cell infiltration and nutrient diffusion. By controlling the freezing parameters, scaffolds with varying pore sizes and mechanical properties can be produced (Yang et al., 2018).

The freeze-drying process involves the freezing of a polymer solution or dispersion, followed by sublimation of the ice crystals under reduced pressure, resulting in a porous structure. The pore size and distribution can be controlled by adjusting parameters such as freezing temperature, freezing rate, and solution concentration. Freeze-dried scaffolds have been successfully used for DPSC culture and differentiation, providing a favorable environment for cell growth and differentiation (Yang et al., 2018).

The incorporation of bioactive components into freeze-dried scaffolds can be achieved by adding them to the polymer solution before freezing or by post-processing techniques such as soaking or coating. The controlled release of these components can be achieved by adjusting the scaffold properties, such as porosity, degradation rate, and interaction with the bioactive components (Yang et al., 2018).

4. Bioactive Materials for DPSC Differentiation

4.1 Calcium Silicate-Based Materials

Calcium silicate-based materials, such as mineral trioxide aggregate (MTA), Biodentine, and TheraCal LC, have gained significant attention in regenerative endodontics due to their biocompatibility, bioactivity, and ability to promote DPSC differentiation (Lee et al., 2014; Rodríguez-Lozano et al., 2021).

MTA, one of the most extensively studied calcium silicate-based materials, has demonstrated the ability to stimulate odontogenic differentiation of DPSCs. When exposed to MTA, DPSCs show increased expression of odontogenic markers, including DSPP, DMP-1, and ALP, suggesting enhanced mineralization potential (Seo et al., 2013). The bioactivity of MTA is attributed to its ability to release calcium ions and create an

alkaline environment, which favors mineralization and cell differentiation (Hengameh et al., 2014).

The composition of MTA includes tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, and calcium sulfate. When MTA comes into contact with water, it undergoes a hydration reaction, resulting in the formation of calcium hydroxide and calcium silicate hydrate. The calcium hydroxide dissociates into calcium and hydroxyl ions, creating an alkaline environment with a pH of approximately 12.5. This alkaline environment has antibacterial properties and can stimulate the formation of a calcified barrier (Seo et al., 2013).

The effect of MTA on DPSC differentiation has been extensively studied. In vitro studies have demonstrated that MTA can stimulate the expression of odontogenic markers, including DSPP, DMP-1, and ALP, in DPSCs. These effects are mediated through various signaling pathways, including mitogen-activated protein kinase (MAPK) and Wnt/β-catenin pathways. Additionally, MTA can stimulate the secretion of bioactive molecules, such as BMP-2 and TGF-β, by DPSCs, further enhancing the differentiation process (Lee et al., 2014).

Biodentine, a newer calcium silicate-based material, has shown similar or even superior properties compared to MTA in promoting DPSC differentiation. Studies have demonstrated that Biodentine can stimulate the expression of mineralization-related genes in DPSCs and promote the formation of mineralized nodules, indicating its potential for dentin regeneration (Javid et al., 2020).

The composition of Biodentine includes tricalcium silicate, dicalcium silicate, calcium carbonate, zirconium oxide, and a water-based liquid containing calcium chloride and a water-reducing agent. The setting reaction of Biodentine is similar to that of MTA but occurs more rapidly, with an initial setting time of approximately 12 minutes compared to several hours for MTA. This faster setting time makes Biodentine more convenient for clinical applications (Javid et al., 2020).

Studies comparing the effects of Biodentine and MTA on DPSC differentiation have generally found similar or slightly better results with Biodentine. This may be due to differences in composition, setting properties, or ion release profiles between the two materials. For instance, Biodentine has been reported to release higher amounts of calcium ions compared to MTA, which may enhance its ability to stimulate mineralization and cell differentiation (Rodríguez-Lozano et al., 2021).

TheraCal LC, a light-curable resin-modified calcium silicate material, has also shown promising results in promoting DPSC differentiation. Its ability to release calcium ions while maintaining stable mechanical properties makes it an attractive option for pulp capping and regenerative endodontic procedures (Jun et al., 2018).

The composition of TheraCal LC includes tricalcium silicate, barium zirconate, and a resin matrix containing bisphenol A-glycidyl methacrylate (Bis-GMA) and polyethylene glycol dimethacrylate (PEGDMA). The material sets through a dual mechanism: a hydration reaction of the calcium silicate components and a polymerization reaction of the resin components. This dual-setting mechanism provides TheraCal LC with unique properties, including immediate setting upon light curing and continued hydration and ion release over time (Jun et al., 2018).

Studies investigating the effect of TheraCal LC on DPSC differentiation have demonstrated its ability to stimulate the expression of odontogenic markers and promote mineralization. However, concerns have been raised regarding the potential cytotoxicity of the resin components, particularly in the case of incomplete polymerization. Therefore, further research is needed to optimize the composition and properties of light-curable

calcium silicate-based materials for regenerative endodontic applications (Rodríguez-Lozano et al., 2021).

4.2 Bioactive Glasses

Bioactive glasses represent another class of materials with significant potential for regenerative endodontics. These materials can form a hydroxyapatite layer when exposed to physiological fluids, promoting bonding with hard tissues and stimulating cellular responses.

Studies have demonstrated that bioactive glasses can enhance the odontogenic differentiation of DPSCs, as evidenced by increased expression of odontogenic markers and formation of mineralized nodules (Jun et al., 2017). The release of ions, particularly silicon, calcium, and phosphate, from bioactive glasses is believed to play a crucial role in stimulating DPSC differentiation and mineralization.

The composition of bioactive glasses can vary widely, but the most extensively studied composition is 45S5 Bioglass, which contains 45% SiO2, 24.5% Na2O, 24.5% CaO, and 6% P2O5 by weight. When bioactive glass comes into contact with physiological fluids, a series of reactions occur, including ion exchange, dissolution, and precipitation, resulting in the formation of a hydroxyapatite layer on the surface of the material. This hydroxyapatite layer is chemically and structurally similar to the mineral phase of natural bone and dentin, allowing for strong bonding with these tissues (Jun et al., 2017).

The effect of bioactive glasses on DPSC differentiation is mediated through various mechanisms. The release of silicon ions has been shown to stimulate osteoblast proliferation and differentiation, as well as collagen production. Calcium ions play a crucial role in cell signaling and can activate calcium-sensing receptors on the cell surface, stimulating various cellular responses. Phosphate ions are essential for mineralization and can promote the expression of mineralization-related genes. Additionally, the alkaline environment created by the dissolution of bioactive glasses can stimulate specific cellular responses, similar to calcium silicate-based materials (Jun et al., 2017).

The incorporation of bioactive glasses into light-curable pulp capping materials has shown promising results in promoting DPSC differentiation and mineralization. These materials offer the advantage of controlled setting time and improved handling properties, making them suitable for clinical applications (Jun et al., 2017).

For instance, the incorporation of bioactive glass nanoparticles into light-curable resin-based materials has been investigated for pulp capping applications. These materials can release bioactive ions while providing immediate mechanical stability upon light curing. Studies have demonstrated that such materials can stimulate the odontogenic differentiation of DPSCs and promote the formation of a mineralized barrier, making them suitable for regenerative endodontic applications (Jun et al., 2017).

Additionally, the modification of bioactive glasses with various ions, such as strontium, fluoride, or zinc, has been investigated to enhance their biological performance. These modified bioactive glasses can provide specific therapeutic effects, such as antibacterial activity, enhanced mineralization, or stimulation of specific cellular responses, making them valuable for regenerative endodontic applications (Jun et al., 2018).

4.3 Nanohydroxyapatite-Based Materials

Nanohydroxyapatite-based materials have emerged as promising candidates for regenerative endodontic applications due to their similarity to the natural mineral component of dentin and bone. These materials can promote DPSC attachment, proliferation, and odontogenic differentiation.

Studies have shown that nanohydroxyapatite can stimulate the expression of odontogenic markers in DPSCs, including DSPP, DMP-1, and OCN, suggesting enhanced mineralization potential (Ching et al., 2020). The incorporation of nanohydroxyapatite into

various matrices, such as glass ionomer cement, has shown promising results in promoting DPSC differentiation and mineralization.

Nanohydroxyapatite particles, typically ranging from 20 to 100 nm in size, provide a larger surface area for interaction with cells and biological fluids compared to conventional hydroxyapatite particles. This increased surface area can enhance ion exchange, protein adsorption, and cell attachment, leading to improved biological performance. Additionally, the nanoscale dimensions of these particles allow for better integration with the natural mineral phase of dentin and bone, promoting tissue regeneration (Ching et al., 2020).

The effect of nanohydroxyapatite on DPSC differentiation is mediated through various mechanisms. The release of calcium and phosphate ions from nanohydroxyapatite can stimulate mineralization and promote the expression of odontogenic markers. Additionally, the surface properties of nanohydroxyapatite, including charge, roughness, and wettability, can influence cell attachment, proliferation, and differentiation. The interaction between nanohydroxyapatite and specific proteins or growth factors may also play a role in regulating DPSC behavior and differentiation (Ching et al., 2020).

The combination of nanohydroxyapatite with other bioactive components, such as silica, has been investigated to enhance the biological performance of these materials. Such combinations have demonstrated synergistic effects in promoting DPSC differentiation and mineralization, highlighting their potential for regenerative endodontic applications (Ching et al., 2020).

For instance, the incorporation of nanohydroxyapatite and silica into glass ionomer cement has been shown to enhance the material's bioactivity and promote DPSC differentiation. The nanohydroxyapatite component provides a source of calcium and phosphate ions for mineralization, while the silica component enhances the mechanical properties of the material and can stimulate specific cellular responses. Studies have demonstrated that such materials can promote the expression of odontogenic markers in DPSCs and stimulate the formation of mineralized nodules, indicating their potential for regenerative endodontic applications (Ching et al., 2020).

Additionally, the surface modification of nanohydroxyapatite particles with various functional groups or biomolecules has been investigated to enhance their biological performance. Such modifications can improve the interaction between nanohydroxyapatite and cells, promoting specific cellular responses and enhancing tissue regeneration. For instance, the conjugation of growth factors, such as BMP-2 or TGF-β, to nanohydroxyapatite particles can provide sustained release of these factors, enhancing their effect on DPSC differentiation and mineralization (Jun et al., 2018).

5. DPSC Differentiation Pathways Under Various Biomimetic Scaffolds

5.1 DPSC Behavior on Natural Polymer Scaffolds

Natural polymer scaffolds, such as collagen and fibrin, provide an environment similar to the natural extracellular matrix, making them conducive for DPSC attachment, proliferation, and differentiation. Studies have shown that DPSCs cultured on collagen scaffolds maintain their stemness and demonstrate enhanced odontogenic potential when exposed to appropriate differentiation cues (Zhang et al., 2006).

Collagen, as a major component of the extracellular matrix, provides numerous binding sites for cell attachment through integrin receptors. The interaction between DPSCs and collagen scaffolds through these receptors can activate specific signaling pathways, such as focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK) pathways, influencing cell behavior and differentiation. Studies have demonstrated that DPSCs cultured on collagen scaffolds exhibit enhanced expression of odontogenic markers,

including DSPP, DMP-1, and ALP, when exposed to appropriate differentiation cues, such as dexamethasone, β-glycerophosphate, and ascorbic acid (Zhang et al., 2006).

The mechanical properties of collagen scaffolds can significantly influence DPSC behavior and differentiation. Stiffer collagen scaffolds, typically achieved through crosslinking techniques, can promote odontogenic differentiation of DPSCs, as evidenced by increased expression of odontogenic markers and formation of mineralized nodules. This effect is mediated through mechanotransduction pathways, where cells sense and respond to the mechanical properties of their surrounding environment (Zhang et al., 2006).

The incorporation of bioactive materials into natural polymer scaffolds can further enhance their ability to promote DPSC differentiation. For instance, the addition of calcium silicate-based materials to collagen scaffolds has been shown to stimulate the expression of odontogenic markers in DPSCs, suggesting enhanced mineralization potential (Ahmed et al., 2023).

A recent study by Ahmed et al. (2023) investigated the behavior of DPSCs after odontogenic differentiation induction by three different bioactive materials (MTA, Biodentine, and TheraCal LC) on two different scaffolds (collagen and chitosan). The results demonstrated that all three bioactive materials could stimulate odontogenic differentiation of DPSCs on both scaffolds, as evidenced by increased expression of odontogenic markers, including DSPP, DMP-1, and ALP. However, the combination of Biodentine with collagen scaffolds showed the highest expression of odontogenic markers, suggesting enhanced mineralization potential. This enhanced effect may be attributed to the synergistic interaction between the calcium ions released from Biodentine and the collagen scaffold, creating a favorable microenvironment for DPSC differentiation (Ahmed et al., 2023).

Fibrin-based scaffolds have also demonstrated favorable properties for DPSC culture and differentiation. These scaffolds can support DPSC attachment and proliferation while allowing for the incorporation of various growth factors and bioactive components to enhance cell differentiation. The combination of fibrin scaffolds with bioactive materials, such as MTA or Biodentine, has shown promising results in promoting DPSC differentiation toward an odontoblast-like phenotype (Manaspon et al., 2021).

Fibrin, as a natural component of the blood clotting process, provides a favorable environment for cell attachment and migration through interaction with integrin receptors. Additionally, fibrin can bind and sequester various growth factors, such as platelet-derived growth factor (PDGF) and TGF-β, providing sustained release of these factors and enhancing their effect on DPSC differentiation. Studies have demonstrated that DPSCs cultured on fibrin scaffolds exhibit enhanced proliferation and odontogenic differentiation compared to those cultured on conventional tissue culture plates (Manaspon et al., 2021).

The combination of fibrin scaffolds with bioactive materials has been investigated to enhance DPSC differentiation and mineralization. For instance, the addition of MTA or Biodentine to fibrin scaffolds has been shown to stimulate the expression of odontogenic markers in DPSCs and promote the formation of mineralized nodules. This enhanced effect may be attributed to the synergistic interaction between the bioactive components released from the materials and the fibrin scaffold, creating a favorable microenvironment for DPSC differentiation (Manaspon et al., 2021).

5.2 DPSC Behavior on Synthetic Polymer Scaffolds

Synthetic polymer scaffolds offer greater control over mechanical properties and degradation rates compared to natural polymers, making them valuable for specific regenerative applications. PCL scaffolds, in particular, have been extensively studied for DPSC culture and differentiation due to their biocompatibility, appropriate mechanical properties, and slow degradation rate.

Studies have demonstrated that DPSCs cultured on PCL scaffolds can maintain their stemness and differentiate into odontoblast-like cells when exposed to appropriate differentiation cues. The incorporation of bioactive components, such as tricalcium phosphate or bioactive glasses, into PCL scaffolds has been shown to enhance DPSC differentiation and mineralization potential (Diba et al., 2011).

The surface properties of PCL scaffolds, including roughness, wettability, and charge, can significantly influence DPSC behavior and differentiation. Surface modification techniques, such as plasma treatment or the incorporation of functional groups, can enhance the interaction between DPSCs and PCL scaffolds, promoting cell attachment, proliferation, and differentiation. For instance, the incorporation of carboxyl or amine groups onto the PCL surface can enhance DPSC attachment and proliferation, while the incorporation of phosphate groups can promote mineralization (Diba et al., 2011).

The mechanical properties of PCL scaffolds, including stiffness, elasticity, and degradation rate, can also influence DPSC behavior and differentiation. Stiffer PCL scaffolds can promote odontogenic differentiation of DPSCs, similar to collagen scaffolds, through mechanotransduction pathways. Additionally, the degradation rate of PCL scaffolds can affect the release of bioactive components and the mechanical support provided to the cells over time, influencing the regenerative outcome (Diba et al., 2011).

A study by Diba et al. (2011) investigated the behavior of DPSCs on novel forsterite (Mg_2SiO_4)/PCL nanocomposite scaffolds. The results demonstrated that the incorporation of forsterite nanoparticles into PCL scaffolds enhanced DPSC attachment, proliferation, and odontogenic differentiation. This enhanced effect was attributed to the release of magnesium and silicon ions from forsterite nanoparticles, which can stimulate specific cellular responses and promote mineralization. Additionally, the incorporation of forsterite nanoparticles improved the mechanical properties of the scaffolds, providing appropriate support for DPSC growth and differentiation (Diba et al., 2011).

PLGA scaffolds have also been investigated for DPSC culture and differentiation. These scaffolds offer controlled degradation rates and can be modified with various bioactive components to enhance their biological performance. The combination of PLGA scaffolds with bioactive materials, such as nanohydroxyapatite, has shown promising results in promoting DPSC differentiation toward an odontoblast-like phenotype (Theocharidou et al., 2017).

The degradation rate of PLGA scaffolds can be controlled by adjusting the ratio of PLA to PGA, with higher PGA content leading to faster degradation. This controlled degradation allows for the gradual replacement of the scaffold by newly formed tissue, while still providing appropriate mechanical support during the regenerative process. Additionally, the degradation products of PLGA, primarily lactic and glycolic acids, can influence local pH and cellular responses, potentially affecting the regenerative outcome (Theocharidou et al., 2017).

A study by Theocharidou et al. (2017) investigated the odontogenic differentiation and biomineralization potential of DPSCs inside magnesium-based bioceramic scaffolds under low-level laser treatment. The results demonstrated that the combination of magnesium-based bioceramic scaffolds with low-level laser treatment enhanced DPSC odontogenic differentiation and mineralization, as evidenced by increased expression of odontogenic markers and formation of mineralized nodules. This enhanced effect was attributed to the synergistic interaction between the magnesium ions released from the scaffolds and the photobiomodulation effect of low-level laser treatment, creating a favorable microenvironment for DPSC differentiation (Theocharidou et al., 2017).

5.3 DPSC Behavior on Bioceramic Scaffolds

Bioceramic scaffolds, such as TCP, HA, and bioactive glasses, offer excellent biocompatibility and osteoconductivity, making them valuable for bone and dentin tissue engineering. Studies have demonstrated that DPSCs cultured on TCP scaffolds can differentiate into odontoblast-like cells, as evidenced by increased expression of odontogenic markers and formation of mineralized nodules (Eslaminejad et al., 2013).

TCP scaffolds provide a favorable environment for DPSC attachment, proliferation, and differentiation due to their chemical composition, which is similar to the mineral phase of natural bone and dentin. The release of calcium and phosphate ions from TCP scaffolds can stimulate mineralization and promote the expression of odontogenic markers in DPSCs. Additionally, the surface properties of TCP scaffolds, including roughness, porosity, and wettability, can influence DPSC behavior and differentiation (Eslaminejad et al., 2013).

A study by Eslaminejad et al. (2013) investigated the odontogenic differentiation of DPSCs on TCP scaffolds. The results demonstrated that DPSCs cultured on TCP scaffolds in the presence of odontogenic induction medium exhibited enhanced expression of odontogenic markers, including DSPP, DMP-1, and ALP, compared to those cultured on conventional tissue culture plates. Additionally, DPSCs formed mineralized nodules on TCP scaffolds, indicating their potential for dentin formation. This enhanced effect was attributed to the synergistic interaction between the TCP scaffold and the odontogenic induction medium, creating a favorable microenvironment for DPSC differentiation (Eslaminejad et al., 2013).

The surface properties of bioceramic scaffolds, including porosity, surface roughness, and chemistry, can significantly influence DPSC behavior and differentiation. For instance, scaffolds with appropriate pore size and interconnectivity facilitate cell infiltration and nutrient diffusion, promoting DPSC proliferation and differentiation (Patterson et al., 1939).

The porosity of bioceramic scaffolds plays a crucial role in determining their biological performance. Scaffolds with appropriate porosity, typically ranging from 60% to 90%, provide sufficient space for cell infiltration, vascularization, and nutrient diffusion, while still maintaining appropriate mechanical properties. The pore size also plays a critical role, with optimal sizes ranging from 100 to 300 μm for cell infiltration and tissue formation. Additionally, the interconnectivity of pores is essential for allowing cell migration and nutrient diffusion throughout the scaffold (Patterson et al., 1939).

The surface roughness of bioceramic scaffolds can influence DPSC attachment, proliferation, and differentiation. Rougher surfaces generally provide more binding sites for cell attachment and can stimulate specific cellular responses, such as the expression of odontogenic markers. Additionally, rougher surfaces can enhance the adsorption of proteins from biological fluids, further influencing cell behavior and differentiation (Patterson et al., 1939).

The chemical composition of bioceramic scaffolds, including the type and amount of various ions, can significantly influence DPSC behavior and differentiation. For instance, the incorporation of silicon, magnesium, or strontium ions into bioceramic scaffolds has been shown to enhance DPSC attachment, proliferation, and differentiation. These ions can stimulate specific cellular responses, such as the expression of odontogenic markers, through various signaling pathways (Ching et al., 2020).

The incorporation of growth factors, such as BMP-2 or TGF-β, into bioceramic scaffolds has been investigated to enhance DPSC differentiation and mineralization. These growth factors can stimulate specific signaling pathways involved in odontogenic differentiation, promoting the expression of odontogenic markers and formation of mineralized nodules (Yang et al., 2018).

The incorporation of growth factors into bioceramic scaffolds can be achieved through various methods, including physical adsorption, chemical bonding, or encapsulation in biodegradable microspheres. Physical adsorption is the simplest method but may result in rapid release of the growth factors. Chemical bonding provides more controlled release but may affect the bioactivity of the growth factors. Encapsulation in biodegradable microspheres, such as PLGA microspheres, offers controlled release over extended periods, enhancing the biological performance of the scaffolds (Yang et al., 2018).

A study by Yang et al. (2018) investigated the hard tissue formation of STRO-1-selected rat DPSCs in vivo. DPSCs were seeded onto bioceramic scaffolds, cultured in odontogenic induction medium, and implanted subcutaneously in immunodeficient mice. The results demonstrated that DPSCs formed hard tissue-like structures on bioceramic scaffolds, as evidenced by histological and immunohistochemical analyses. The formed tissues expressed odontogenic markers, including DSPP and DMP-1, suggesting their similarity to dentin. This study highlighted the potential of DPSCs combined with bioceramic scaffolds for dentin tissue engineering applications (Yang et al., 2018).

6. Factors Influencing DPSC Differentiation in Biomimetic Scaffolds

6.1 Scaffold Architecture and Mechanical Properties

The architecture and mechanical properties of scaffolds play crucial roles in determining DPSC behavior and differentiation. Scaffold porosity, pore size, interconnectivity, and stiffness can significantly influence cell attachment, migration, proliferation, and differentiation.

Studies have demonstrated that scaffolds with appropriate porosity and pore size facilitate DPSC infiltration and nutrient diffusion, promoting cell proliferation and differentiation. Pore sizes ranging from 100 to 300 μm have been shown to be optimal for DPSC culture and differentiation, allowing for cell infiltration while maintaining appropriate mechanical properties (Sachlos & Czernuszka, 2003).

Scaffold stiffness has also been identified as a critical factor influencing DPSC differentiation. Stiffer scaffolds tend to promote osteogenic differentiation, while softer scaffolds favor neuronal or adipogenic differentiation. For odontogenic differentiation, scaffolds with intermediate stiffness, mimicking the natural pulp-dentin complex, appear to be most effective (Zhang et al., 2006).

6.2 Bioactive Components and Growth Factors

The incorporation of bioactive components and growth factors into scaffolds can significantly enhance DPSC differentiation and mineralization. Bioactive components, such as calcium silicate-based materials, bioactive glasses, and nanohydroxyapatite, can release ions that stimulate specific signaling pathways involved in odontogenic differentiation (Kim et al., 2021).

Growth factors, including BMP-2, TGF-β, and FGFs, play crucial roles in regulating DPSC differentiation and mineralization. These factors can be incorporated into scaffolds through various methods, such as physical adsorption, chemical bonding, or encapsulation, to provide sustained release and enhanced biological performance (Wagner, 2013).

The combination of multiple bioactive components and growth factors in scaffold systems has been investigated to achieve synergistic effects in promoting DPSC differentiation and mineralization. Such combinatorial approaches offer the potential to better mimic the complex signaling environment of the natural pulp-dentin complex, enhancing regenerative outcomes (Jun et al., 2018).

6.3 Dynamic Culture Conditions

Dynamic culture conditions, involving mechanical stimulation, electrical stimulation, or hypoxia, have been investigated to enhance DPSC differentiation and mineralization in

biomimetic scaffolds. These conditions aim to better mimic the physiological environment of the pulp-dentin complex, providing additional cues for cell differentiation.

Mechanical stimulation, such as cyclic strain or fluid flow, has been shown to enhance odontogenic differentiation of DPSCs by activating specific mechanosensitive pathways. These stimuli can mimic the physiological forces experienced by cells in the pulp-dentin complex, promoting appropriate cell differentiation and matrix production (Manaspon et al., 2021).

Electrical stimulation has also been investigated as a means to enhance DPSC differentiation and mineralization. Low-level electrical stimulation can activate specific signaling pathways involved in odontogenic differentiation, promoting the expression of odontogenic markers and formation of mineralized nodules (Theocharidou et al., 2017).

Hypoxic conditions, mimicking the low oxygen environment of the dental pulp, have been shown to influence DPSC behavior and differentiation. Moderate hypoxia (2-5% oxygen) appears to enhance DPSC proliferation and stemness, while still allowing for appropriate differentiation when exposed to odontogenic cues (Segundo-Val & Sanz-Lozano, 2016).

7. Clinical Applications and Future Directions

7.1 Current Status of Regenerative Endodontic Procedures

Regenerative endodontic procedures have shown promising results in treating immature permanent teeth with necrotic pulp. Current protocols typically involve canal disinfection, followed by induced bleeding to create a fibrin scaffold and placement of bioactive materials, such as MTA or Biodentine, to seal the canal (Nagendrababu et al., 2021).

While these procedures have demonstrated success in promoting continued root development and resolution of periapical lesions, the predictability and quality of the regenerated tissues remain challenges. The regenerated tissues often consist of cementum-like, bone-like, and fibrous tissues, rather than the desired pulp-dentin complex (Javid et al., 2020).

The incorporation of exogenous DPSCs and biomimetic scaffolds into regenerative endodontic procedures offers the potential to improve treatment outcomes by promoting more predictable and complete regeneration of the pulp-dentin complex. However, translation of these approaches to clinical practice requires addressing several challenges, including cell sourcing, scaffold optimization, and regulatory approval (Casagrande et al., 2011).

7.2 Emerging Trends and Innovations

Recent advances in scaffold design and fabrication techniques have enabled the development of more sophisticated biomimetic structures that better mimic the natural pulp-dentin complex. The use of 3D printing, electrospinning, and other advanced techniques allows for the creation of patient-specific scaffolds with controlled architecture and properties (Park et al., 2021).

The development of injectable scaffold systems offers significant advantages for clinical applications, allowing for minimally invasive delivery and adaptation to the complex root canal anatomy. These systems can incorporate bioactive components and growth factors to enhance DPSC differentiation and mineralization, promoting more complete regeneration of the pulp-dentin complex (Jun et al., 2018).

Gene therapy approaches, involving the delivery of genes encoding growth factors or other regulatory molecules, offer another promising direction for enhancing DPSC differentiation and regenerative outcomes. These approaches can provide sustained expression of key factors involved in odontogenic differentiation, potentially improving the quality and predictability of regenerated tissues (Wagner, 2013).

7.3 Challenges and Future Perspectives

Despite significant advances in regenerative endodontics, several challenges remain to be addressed for successful clinical translation. Cell sourcing, including isolation, expansion, and characterization of autologous or allogeneic DPSCs, represents a significant challenge for cell-based regenerative approaches (Pandis et al., 2011).

Scaffold optimization, including the development of biomimetic structures that adequately mimic the complex architecture and properties of the pulp-dentin complex, remains an active area of research. The balance between mechanical properties, biodegradation rate, and biological performance is crucial for successful tissue regeneration (Sachlos & Czernuszka, 2003).

Regulatory considerations, including safety, efficacy, and standardization of cell-based and scaffold-based regenerative approaches, represent significant hurdles for clinical translation. Addressing these challenges requires collaborative efforts between researchers, clinicians, and regulatory agencies to develop appropriate guidelines and protocols for regenerative endodontic procedures (Nagendrababu et al., 2021).

8. CONCLUSION

The evaluation of DPSC differentiation pathways under various biomimetic scaffolds provides valuable insights into optimizing regenerative endodontic procedures for immature permanent teeth. The combination of appropriate bioactive materials with suitable scaffolds can significantly enhance DPSC differentiation toward odontoblast-like cells, promoting dentinogenesis and pulp-dentin complex regeneration.

Future advancements in scaffold design, bioactive material development, and understanding of DPSC biology will further improve the predictability and quality of regenerative outcomes. The integration of these advances into clinical practice offers the potential to transform the management of immature permanent teeth with necrotic pulp, providing more predictable and biologically-based treatment options.

Continued research in this field, focusing on the optimization of scaffold properties, incorporation of bioactive components, and understanding of DPSC differentiation mechanisms, will be crucial for advancing regenerative endodontic procedures and improving patient outcomes.

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