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BIOMARKERS OF DENTAL PULP STEM CELLS

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ABSTRACT

Regenerative medicine and stem cell-based tissue engineering offer promising solutions for various tissue and organ defects. Adult stem cells, particularly those from dental pulp, are crucial due to their versatility and adaptability. Dental pulp is an abundant source of mesenchymal stem cells, Dental pulp stem cells (DPSCs), containing undifferentiated cells and blood vessels essential for tooth function. Biomarker analysis can determine regenerative properties, facilitating clinical integration and unlocking therapeutic potential. Selected biomarkers reflect mesenchymal, multi-lineage potency, and various differentiation capacities. DPSCs exhibit self-renewal, rapid proliferation, colony formation, and multi-lineage differentiation, making dental pulp an attractive reservoir for advancing tissue regeneration.

KEYWORDS: Biomarkers, Dental pulp stem cells, Regenerative medicine, Stem cells, Tissue engineering, Dental pulp.

INTRODUCTION

The identification of dental stem cells, coupled with advancements in cellular and molecular biology, has led to the emergence of innovative therapeutic approaches focused on regenerating oral tissues damaged by disease or injury. Tissue engineering is inherently multidisciplinary by nature, bringing together biology, engineering, and clinical sciences with the goal of creating new tissues and organs.[1]

Over the past decade, stem cells have emerged as a leading candidate for regenerative medicine applications, with a particular spotlight on mesenchymal stem cells (MSCs). These cells have garnered attention as a promising therapeutic avenue in regenerative medicine and tissue engineering, due to their regenerative and protective abilities.[2]

Mesenchymal stem cells (MSCs) are abundant in various adult tissues, spanning from skin and adipose tissues to peripheral blood, bone marrow, pancreas, intestine, brain, hair follicles, and notably in the dental pulp cells.[3] DPSCs have remarkable potential in regenerating dentin/pulp-like complexes and have shown effectiveness in treating a wide array of conditions, including spinal cord injuries (SCIs), neurogenesis, myocardial infarction, muscular dystrophy, diabetes, liver diseases, eye diseases, immune diseases, and various oral diseases.[4] They also showed a difference in the structures formed after transplantation compared with the MSCs.[5]

MATERIALS AND METHODS

A electronic research was done on Pubmed, Scopus, and Research gate with key word search of Stem cells, Dental pulp stem cells, Dental pulp stem cells application in Regenerative medicine and Dentistry. Out of 200 articles only 30 articles have been chosen which were directly related to the present review.

DENTAL PULP STEM CELLS BIOMARKERS

S no.	Biomarkers	Interpretation
1	Nestin,Notch1,CD271, musashi-1, βIII tubulin glial fibrillary acidic protein (GFAP), and neuronal nuclei (NeuN)	Neural crest cells[9]
2	CD73,CD90,CD105,CD44	Stromal associated markers[10]
3	CD 34 ,CD45,CD11BCD73, CD90 and CD105, but not CD11b or CD14, CD19 or CD79α, CD45, CD34 and HLA Class II surface markers	Hematopoietic, differentiate into osteoblasts, chondroblasts and adipocyte [2]
4	CD146, STRO-1, CD73 and CD166	Perivascular markers[10]
5	OCT4, NANOG, SOX 2, SSEA, C-myc	Pluripotent[2]
6	MAP1B	Generate neural components[6]
7	CD 31, CD 146, CD 34, CD 40, CD 117, CD 133, CD 105+	Neurogenic regeneration[11]
8	Neural markers like nestin, beta- III tubulin, neurofilament, S100, synaptophysin, Vimentin	Capable of deriving neural structures[6]
9	Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and GDNF	Huge potential to treat neurological disease[6]
10	Dentin sialophospoprotein Dentin matrix protein1 Osterix Osteocalcinin Osteopointin Alkaline phosphates Type 1 collagen RUNX2, ALP, SP7, OSX,STRO-1,CD34,FLK-1,VEGFR2,CD90+,CD13+	Osteogenic regeneration[2]
11	Presence of OCT4, CD 117 and various other hepatocytic growth factors	Osteogenic and hepatocytic potential[6]
12	BMP1,BMP2,BMPR-1A ,TGF-B2,IGF-1R,SMAD-4,SMAD-5,LOL10AL,ITGA1,ITGA2,ITG-B,ITG-A,ALP	Odontoblastic regeneration[12]
13	CD3, CD4, CD13, CD14, CD29, CD34, CD44, CD45, CD73, CD90, CD106, CD117, CD146, CD166, HLA-DR, and HLA-ABC	Ability to derive from all stem cell lines[6]
14	Cytokeratin 18 and 19	Odontoblast differentiation and dentine repair[6]
15	Ppar-2, LPL	Adipogenic potential[13]
16	Nanog, Oct4, Nucleostemin, Slain-1, Jmjd1, Jmjd2, and Cyclin D1	Ability to regenerate myogenic tissues[6]
17	STRO-1, CD90, CD105, CD146	Inflammed[14]
18	PAX6, Atoh7, BRN3D	Retional ganglion regeneration[11]

DENTAL STEM CELLS

Sources of dental stem cells are the Dental pulp which contains the DPSCS, Periodontal ligament, which houses Periodontal ligament stem cells (PDLSCS), and the dental follicle which contains Dental follicle progenitor cells (DFPCS).[3]

The dental pulp presents a distinctive form of connective tissue, constrained by its placement within the rigid chamber of tooth. Comprising cellular and noncellular elements, as well as collagen and fibrillin fibres, it possesses a distinct anatomical architecture shaped by its dental enclosure.[6]

Dental pulp lodges mesenchymal stem cells called as Dental pulp stem cells (DPSCS). DPSCS are also obtained from human permanent and primary teeth.[3]

DENTAL PULP STEM CELLS (DPSCS)

DPSCs stand out as the initial orofacial stem cells to be isolated and recognized, possess typical characteristics as mesenchymal stem cells (MSCs). They exhibit distinctive features such as plastic adherence and clonogenicity, along with the ability to differentiate into multiple lineages. DPSCs are high proliferative capacity and can differentiate into odontoblasts, facilitating the formation of an ectopic dentin-pulp complex post-transplantation.[6]

The inner region of the dental pulp chamber contains a population of highly proliferative stem/progenitor cells possessing a self-renewal and differentiation properties. Compared to other somatic cells, DPSCs necessitate a lengthier duration for the initiation of colony formation.[3]

Regenerative capacity is explained on the basis of presence of various cellular constituents of the dental pulp, which contains odontoblasts, fibroblasts, undifferentiated cells.[6] Compared with MSCs derived from bone marrow, DPSCs are highly proliferative and able to differentiate into odontoblasts, generating an ectopic dentin-pulp complex after transplantation.[7]

DPSCs, or dental pulp stem cells, have shown promising applications in regenerating tissues not only within the oral cavity but also in other areas of the body. They hold potential for both soft tissue and mineralized structure regeneration, making them valuable in various medical contexts.[8]

Hence based on the aforementioned facts, literature review in DPSC was undertaken, which includes various

biomarkers having regenerative potential and lineage, and probable clinical applications were identified.

DENTAL PULP STEM CELLS ISOLATION

Based on many experiments and investigations, the stem cells were isolated from lumen of dental pulp by several methods. Some of these methods were explained in this review.

Raoof et al.2014 has done different methods for isolation of DPSCs from dental pulp tissue: Dental pulp tissue undergoes enzymatic digestion using collagenase or dispase enzymes. Following digestion, trypsin is used to isolate individual cells, which are then plated onto culture dishes for further growth and study. They directly explanted small tissue pieces of dental pulp onto petri dishes without undergoing enzymatic digestion. They began by subjecting dental pulp tissues to trypsinization, followed by explanting small tissue fragments onto petri dishes to encourage outgrowth. These cultures were nurtured in Minimum Essential Medium (MEM) supplemented with 20% Fetal Bovine Serum (FBS) under standard cell culture conditions, including a temperature of 37°C, 5% CO2, and 90% humidity within a CO2 incubator. The third method yields superior cell outgrowth, reaching a confluency of 60% within just 2 days of culture. As a result, they advocate for the third method as the preferred approach for isolating DPSCs from dental pulp.[15]

Lindemann et al. 2014 They isolated dental pulp cells from both non-cryopreserved and cryopreserved human deciduous teeth, all of which were 7 days old, and cultured them concurrently. Both non-cryopreserved and cryopreserved dental pulp cells exhibited no variation in their differentiating and immunophenotype properties. However, there was a noticeable difference in the morphology and proliferative capacity of cryopreserved cells compared to non-cryopreserved cells.[16]

Lin et al. 2014 Human DPSCs were isolated from extracted teeth that underwent freezing and were subsequently stored at -196°C for 24 hours. During the freezing process, the cells were suspended in freezing media comprising 10% dimethyl sulfoxide (DMSO). When the freezing medium is DMSO free, the survival rates of revived DPSCs increase by 2 to 2.5 folds.[17]

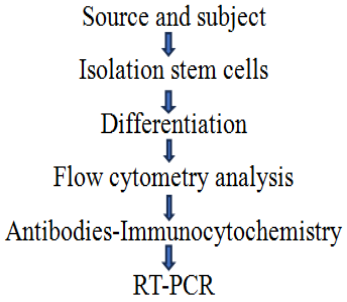
Gioventù et al. 2012 The researchers investigated four human deciduous whole teeth that were cryopreserved using a method involving the creation of micro-channels within the tooth using a laser beam. These teeth were then stored at -80°C. The results indicated that this approach streamlines the isolation of DPSCs before cryopreservation, thereby reducing initial costs and workload associated with tooth banking. DPSCs isolated using this method exhibited normal morphology, cell viability, proliferation rate, and maintained a normal mesenchymal phenotype, akin to cells isolated from fresh non-cryopreserved teeth. Additionally, they found that DPSCs isolated without laser piercing showed a significant loss of cell viability and proliferation rate compared to teeth cryopreserved using laser piercing.[18]

DIFFERENTIATION OF DPSC

DPSCs differentiate into different kinds of cells and tissues and their multi-potency has been compared to those of Bone Marrow Stem Cells (BMSCs). It has been demonstrated that proliferation, availability, and cell number of DPSCs are greater than BMSC.[3]

DPSCs are characterized by their rapid proliferation and impressive capacity to differentiate into various cell lineages, including osteoblasts, neural cells, muscle cells, and hepatocytes. This multi-lineage capacity of these DPSC suggests that they may have a more broad therapeutic application than lineage-restricted adult stem cell populations.[19]

BIOMARKERS IDENTIFICATION PROCESS



SOURCE AND SUBJECT ISOLATION OF STEM CELLS

Dental pulp stem cells (DPSCs) were isolated from permanent teeth as previously described. A collagenase blend (2 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added to the isolated pulp tissues, which were then minced. The tissues were incubated in a Thermo Electron Corporation incubator (Milford, MA, USA) at 37°C with 5% CO2 for 1 hour. After incubation, the samples were centrifuged at 400g for 5 minutes using a Thermo Electron Corporation centrifuge. The resulting tissue pellets were plated and kept in the incubator. The cells were cultured in knockout Dulbecco's modified Eagle's medium (KO-DMEM) basal media (Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Hyclone, Victoria, Australia), 5 mmol/L L-glutamine (Invitrogen), and 50 U/mL penicillin-streptomycin (Invitrogen).[20]

DIFFERENTIATION

Differentiation of DPSC was initiated by seeding cultures at a density of 1000 cells/cm2.The cells were grown until confluence and then subjected to differentiation into adipogenic, chondrogenic, and osteogenic lineages. For adipogenic differentiation, cells were induced with a combination of 10% FBS, 200µM indomethacin, 0.5mM 3-isobutyl-1-methylxanthine (IBMX), 10 µg/mL insulin, and 1µM dexamethasone (all

from Sigma Aldrich). Lipid droplets were visualized using oil red staining (Sigma-Aldrich).

Chondrogenic differentiation was achieved by culturing cells in medium supplemented with ITS+1 (Sigma Aldrich), 50µM L-ascorbic acid 2-phosphate, 55µM sodium pyruvate (Invitrogen), 25µM L-proline (Sigma-Aldrich), and 10 ng/mL transforming growth factor-β (TGF-β; Sigma Aldrich). Proteoglycan accumulation was assessed and visualized by Alcian Blue staining (Sigma-Aldrich).

To induce osteogenic differentiation, a three-week culture period was conducted in a medium supplemented with specific components, including 10% fetal bovine serum (FBS), 10–7M dexamethasone, 10mM glycerol phosphate (Fluka, Buchs, Switzerland), and 100µM L-ascorbic acid 2-phosphate. The resulting calcium accumulation was evaluated and visualized using von Kossa staining (Sigma-Aldrich).[21]

Neurogenic differentiation studies— demonstrated that the expression of βIII-tubulin and NFM were reduced in the presence of cytokines. DPSCs underwent morphologic transformation with elongated cellular processes after differentiation induction.[14]

FLOW CYTOMETRY ANALYSIS

Flow cytometry-based immunophenotyping was performed on cultured dental pulp stem cells (DPSCs) to characterize these cells using a candidate set of mesenchymal stromal cell-specific surface markers. The cells were harvested and counted, and 10 µL of tagged antibody was added to the appropriate number of cells. The samples were stained for 1 hour at 4°C, then washed with fluorescence-activated cell sorting buffer for 5 minutes. The cells were fixed in 4% paraformaldehyde, which was added to the pellet and resuspended. Various types of markers were used, and data analysis was optimized against control cells incubated with specific isotypes on a flow cytometer. Cells were identified by light scatter for 10,000 gated events and analysed using flow cytometry software. Overall flow cytometry analysis plays a crucial role in characterizing DPSCs and understanding their potential for regenerative medicine applications in dentistry.[20]

IMMUNOCYTOCHEMISTRY ANALYSIS (ANTIBODIES)

In brief, undifferentiated DPSCs, along with islet-like cell clusters (ICCs), were fixed for 20 minutes in 4% paraformaldehyde and treated with 0.1% Triton X-100 as a cell membrane penetration agent. Following fixation and permeabilization, cells were blocked at room temperature in a 0.5% BSA solution for 30 minutes and then incubated with primary antibodies overnight at 4°C. Subsequently, cells were washed with PBS and incubated with FITC-conjugated secondary antibodies targeting Neurogenin(Ngn3), Insulin gene enhance(Isl-1), C-peptide, Glucose transporter2(Glut-2), and PDX1 (Abcam, Cambridge, UK) at room temperature for 30 minutes. Finally, slides were counterstained with 4',6-diamidino-2-phenylindole, dihydrochloride for 5 minutes, and fluorescent images were captured.[21]

PROLIFERATION ASSAYS

DPSC seeded onto a Falcon Optilux 96-well cell culture plate (BD Biosciences) in basal medium containing a Alpha modified minimum essential medium (a-MEM; Gibco) supplemented with 10% FCS. The cell culture plates were incubated at 37°C with 5% CO2 in air. A proliferation assay was performed for a minimum of 12 days after seeding. The cells were washed with PBS−, and 0.1 mg/ml of calcein AM solution diluted with PBS− was added to each well. The plates were incubated for 30 min at room temperature. The fluorescence intensity (FI) of cellular calcein-AM (lex = 490 nm, lem=515 nm) was measured using the Wallac 1420 ARVO-SX Fluorescence Multilabel Counter (Perkin-Elmer, Waltham, MA, USA). The background FI of the PBS− was subtracted from the FI of each well, and these FIs were then compared with the intensity of the calcein-AM solution free from cells. For the fibroblastic colony formation (CFU-F) assay, 50 sorted DPSCs and BM-MSCs were seeded onto a 10 cm in diameter cell culture dish (BD Biosciences) in basal medium containing a-MEM supplemented with 10% FCS. The cell culture dishes were incubated at 37 °C with 5% CO2 in air. After 2 weeks, the cells were stained with 0.1 mg/ml of calcein-AM solution, and the fluorescence of cellular calcein-AM from fibroblastic colonies was visualized using an image analyzer.[22]

REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION

Total RNAs were isolated using the RNeasy Plus Mini kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Subsequently, cDNAs were synthesized using the iScript DNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). Amplification of cDNAs was carried out utilizing AmpliTaq Gold DNA polymerase (Applied Biosystems, Tokyo, Japan). The resulting RT-PCR products were visualized using an ultraviolet transilluminator. For quantitative real-time RT-PCR, Power SYBR Green PCR Master Mix (Applied Biosystems) was employed in a Rotor-Gene Q (Qiagen), and data analysis was performed utilizing the comparative CT method.[22]

PROPERTIES OF HUMAN DENTAL PULP DERIVED STEM CELLS

The HDPSCs share the common properties of mesenchymal stem cells. They have undifferentiated lineage with long-term self-renewal capacity. Additionally, DPSCs possess the capacity to differentiate into progenitor cells. They can differentiate into mesodermal, ectodermal, endodermal, osteogenic, chondrogenic, and adipogenic lineages.[23]

THE PROPERTIES OF HDPSCS INCLUDE

Multipotency: Stem cell technology allows for the induction of HDPSCs into various lineages, including ectodermal lineage cells such as neural cells; mesenchymal lineage cells such as odontoblasts, osteoblasts, chondrocytes, adipocytes, and myocytes; and endodermal lineage cells such as vascular endothelial cells, hepatocytes, and pancreatic islet-insulin-producing β cells.[4]

High proliferation activity: The population doubling assay indicates that HDPSCs exhibit a significantly higher proliferative capacity, approximately 3–4 times greater than that of human bone marrow-deriv1ed mesenchymal stem cells (BMMSCs). Moreover, HDPSCs also demonstrate elevated telomerase activity compared to BMMSCs.[4]

Self-renewal capacity: Following in vivo transplantation, human DPSCs exhibit the capacity for self-renewal. Theoretically, DPSCs possess the ability to respond to distinct specific environmental signals and either to generate new stem cells or to select a particular differentiation program, allowing them to either generate new stem cells or choose a specific differentiation pathway.[24]

In vivo tissue regeneration capacity: Upon subcutaneous transplantation with hydroxyapatite/tricalcium phosphate (HA/TCP) powders as a carrier into the dorsal surface of immune-compromised mice, individual HDPSCs exhibit distinct and remarkable regenerative capabilities. DPSCs and SCAP not only facilitate dentin regeneration but also induce the formation of dental-pulp-like tissues characterized by blood capillary vessels and dense collagen fibres enveloped by newly formed dentin.[4]

Paracrine action: DPSCs have the capability to secrete a diverse array of cytokines in a paracrine fashion, including the chemokine stromal cell-derived factor-1, brain-derived neurotrophic factor, ciliary neurotrophic factor, nerve growth factor, vascular endothelial growth factor, granulocyte-colony-stimulating factor, and stem cell factor, which are all growth factors. These cytokines possess the potential to stimulate angiogenesis, inhibit apoptosis, and maintain the integrity of regenerated tissue.[25]

APPLICATIONS OF HUMAN DENTAL PULP DERIVED STEM CELLS

Recent studies have demonstrated the diverse applications of HDPSCs, leveraging their tissue regenerative capacity, multipotency, and immunomodulatory properties. Human DPSC-based therapies, utilizing either cells or secretome /conditioned media, have shown promising results in various disease conditions, including diabetes, neuropathy, hepatic diseases, oculopathies, spinal cord injury, peripheral nerve injury, cerebral ischemia, muscular dystrophy, myocardial infarction, Parkinson's disease, lung injury, and stroke.[26] Moreover, DPSCs derived from permanent teeth are utilized in bone repair and regeneration, periodontal intra bony defects, and dental defects. They have been effectively employed for larger bone defects such as impacted lower third molar (ITM) post-extraction sockets.[8]

TISSUE ENGINEERING REGENERATION OF DENTIN/PULP COMPLEX

The regeneration of the dentin-pulp complex relies on vascularization, which can be facilitated by the administration of vascular endothelial growth factor (VEGF). However, VEGF's short half-life can be prolonged by binding to heparin. In experiments, DPSCs were combined with a carrier and filled into root canal-treated extracted teeth.[4]

ODONTOGENESIS

Recent studies have revealed significant findings regarding the utilization of various scaffold materials for DPSC-related applications. For instance, fibronectin-coated chitosan scaffolds have demonstrated superior cellular attachment compared to arginine-glycine-aspartic acid coating, making them promising substrates for three-dimensional (3D) DPSC attachment and proliferation.

Furthermore, DPSCs loaded onto collagen-chitosan scaffolds have shown the capability to undergo odontogenic differentiation without the need for exogenous growth and differentiation factors. Incorporation of nanoHA (nHA) into electrospun poly(epsilon-caprolactone)/gelatin scaffolds or HA/TCP alone has been shown to upregulate the expression of odontogenic genes and enhance DPSC differentiation toward odontoblasts.[9]

BLOOD VESSEL CONSTRUCTION

Dental pulp stem cells (DPSCs) offer a novel approach to address diseases associated with small blood vessels. VEGF stands out as a crucial regulator of vasculogenesis and angiogenesis in both physiological and pathological conditions. VEGF plays a pivotal role in inducing the differentiation of DPSCs, thereby contributing to their potential therapeutic application.[1]

NEUROGENESIS

Dental pulp stem cells have also been proposed as a treatment for peripheral nerve injury. The dental pulp cells formed blood vessels and myelinating tissue and contributed to the promotion of normal nerve regeneration.[3]

Furthermore, DPSCs possess a neuroprotective function, preventing the death of retinal ganglion cells and protecting their axons in a rat model of glaucoma induced by elevated intraocular pressure. These findings underscore the neuroprotective potential of DPSCs,

suggesting their therapeutic relevance in in vitro models of Alzheimer's and Parkinson's diseases.[9]

CARTILAGE FORMATION

Certain populations of DPSCs extracted from the pulp exhibit the expression of bone-specific factors, such as type II collagen and chitosan. Approximately 30% of these cells can undergo transformation into chondrocytes. Furthermore, dental pulp stem cells in the early stages of culture demonstrate the ability to differentiate into dentin, bone, and cartilage structures.[25]

ISLET PRODUCTION

The differentiation of DPSCs into insulin-producing islet cells has emerged as a promising strategy to address the limitations associated with conventional pharmaceutical and insulin-based therapies. Several researchers have investigated the differentiation of DPSCs into insulin-producing islet-like aggregates from both monoclonal and polyclonal DPSCs. This approach provides a non-pancreatic and minimally invasive source of cells for islet regeneration.[8]

TOOTH REGENERATION

In experimental studies, researchers successfully generated tooth structures from single-cell suspensions of cultured rat tooth bud cells. They demonstrated the development of bioengineered rat teeth within a span of 12 weeks using a PGA and PLGA scaffold. Additionally, Honda et al. engineered tissue teeth, which were implanted into the omentum of rats using porcine tooth bud cells and a PGA fibermesh scaffold, mimicking odontogenesis. Histological analysis revealed a pattern of tissue-engineered odontogenesis similar to that of natural tooth development, with notable regeneration of enamel, dentin, and cementum.[4]

OTHER APPLICATIONS

The distal carboxy terminus of a voltage-gated L-type Ca2+ channel has been recognized for its role in guiding DPSCs towards a neuronal phenotype. Additionally, in certain studies, tissue-engineered DPSC sheets were transplanted onto the corneal bed and covered with de-epithelialized human amniotic membrane, resulting in a reduction in corneal transparency in rabbit eyes and the reconstruction of corneal epithelium.[8] The potential of DPSCs can also be used in the treatment of infertility. Leake and Templeton conducted a study where they isolated human dental pulp stem cells (HDPSCs) and introduced them via injection into the testes of live male mice. The mice were killed at various intervals after the injection, and their testes were examined to see whether the stem cells survived. It was found that stem cells settled in the testes and also differentiated into cells that were producing viable sperm.[4]

Furthermore, neurotransmitters and stem cells contribute to regeneration. Notably, 5-hydroxytryptophan, a necessary precursor of 5-HT found in the cell's cytoplasm, plays a role in dentin repair by modulating endogenous pulpal stem cells.[27]

DPSCs, with their anti-inflammatory property and regenerative capabilities, are under investigation for potential therapeutic use in COVID-19 patients. In COVID-19 patients which causes breakdown of alveolar structures and the buildup of proinflammatory M1 macrophages. This cascade event triggers the release of proinflammatory cytokines, exacerbating tissue fibrosis. In a mouse model study of acute lung injury, Wakayama et al. 2015 found that intravenous infusion of DPSCs, as well as conditioned media from these cells, enhanced anti-inflammatory effects by activating M2 macrophages. This intervention effectively enhanced the pathophysiology of the disease.[26]

DPSC isolates were studied in relation to growth factors such as vascular endothelial growth factor (VEGF) and bone morphogenetic protein (BMP-2), as well as functional biomaterials like mineralized trioxide aggregates (MTA).[28]

CONCLUSION

Stem cell-based therapies hold great promise for addressing systemic and oral diseases, with tissue regeneration hinging on identifying optimal stem cell sources. Among these, human Dental Pulp Stem Cells (DPSCs) have gained attention for their accessibility, minimally invasive harvesting, and potential as mesenchymal stem cells.

DPSCs are widely applied in dentistry, particularly for pulp regeneration, essential for preserving infected pulp and maintaining tooth vitality. Since their discovery in 2000, research on DPSCs has expanded rapidly, though questions about their variable biological capacity remain unresolved.

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