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Research Article

Using Dental Pulp Cells Derived from Stem Cells to Promote Dentin and Pulp Tissue Regeneration in Severe Tooth Decay: A Translational Research Method

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ABSTRACT

Severe tooth decay is considered to be one of the major threats to dental health, which usually leads to the loss of tooth structure and, thus, its functional impairment. Traditional methods of dental treatment, like extractions, root canals, and fillings, may further contribute to more complications and do not really replace natural characteristics of teeth. In view of providing a biological answer to this common problem, the present study explores the potential of DPCs produced from stem cells for the regeneration of dentin and pulp tissue. The aims of this study were to produce and isolate DPCs, produce appropriate scaffold materials, and evaluate their in vivo and in vitro regeneration capacities. As opposed to the in vivo studies, which evaluated integration and functionality of regenerated tissues in animal models, studies in vitro focused on cell viability, proliferation, and odontoblastic differentiation. It has succeeded in establishing effective scaffold environments that would help in regeneration of dental tissues and demonstrated the ability of DPCs to differentiate into odontoblast-like cells. Further, it has developed standardized techniques for isolating and culturing DPCs. The study also provides a comprehensive evaluation framework through histological and functional assessments and will therefore play an important role in progress of regenerative dentistry. The results indicated that DPCs had high in vitro proliferation and cell survival. Further, they showed prominent mineral deposition and expression of odontoblastic markers like DMP1 and DSPP. High integration scores and functional restoration comparable to natural dentin testified that the regenerated tissues integrated very well with native tooth structures in vivo. These results open the way for DPC-based treatments to achieve structurally and functionally sound dental tissue regeneration.

1. INTRODUCTION

One of the common dental diseases, if not treated, might lead to dire consequences is advanced tooth decay. The usual traditional treatments for advanced tooth decay are fillings, root canals, and extractions; however, these treatments don't actually restore the tooth to its original structure and function. In fact, most of these conventional treatments are compromising or further cause damage to the integrity of teeth and even result in other dental complications. These shortcomings of the treatment options available underline the need for novel strategies that are able to repair the damaged oral tissues and restore them back to their pre-affected state [1]. The regeneration of dentin and dental pulp is important for the vitality and function of the tooth. Dental pulp is essential for nourishment and sensory function of the tooth. Much of the tooth's structure is provided by the dentin that gives support and protection [2]. Such tissues would be recoverable from the severe consequences of decay, and the natural tooth would be protectable, thus improving oral health in general. Advances in regenerative dentistry should allow for maintenance of long-term dental health and functionality by rejuvenating and

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repairing dental tissues. These DPCs, differentiated from stem cells, have now been explored as a better alternative for the regeneration of dental tissue [3]. These cells differentiate and give rise to multiple cell phenotypes, including that of odontoblasts cells which form dentin and hence play a crucial role in the repair processes of dental tissue. Based on their intrinsic ability to differentiate and self-renew, DPCs are being exploited for applications in regenerative medicine with an objective to restore dental pulp and dentin in patients who have severe tooth decay [4]. It explores the translational research methodology for regeneration of dentin and pulp tissue using dental pulp cells produced by stem cells. This study will outline the plausibility and efficiency of treatments based on DPCs by looking at very recent approaches and results in this field. Such is the scope of this study: it extends to a detailed analysis of current experimental designs, evaluation criteria, and eventual possible clinical applications for these new dental treatments [5]. This will contribute to the increase in the research volume pertaining to dental tissue regeneration and its applicability in clinical practice. Figure 1 illustrates an anatomical representation of a tooth, outlining the complex cellular constitution and anatomy that plays a very essential role in the life and functioning of a tooth. In the tooth, there exist several layers, viz., enamel, dentin, dental pulp, gingiva, alveolar bone, periodontal ligament, cementum, and neurovascular bundle, as noted in the main cross-sectional image [6]. These elements are essential for the integrity of the tooth, its sensibility, and its attachment to surrounding bone. A, B, and C, three enlarged insets, show details of particular regions of the teeth. Inset A shows the region of contact between dentin and pulp and includes pre-odontoblasts, cell-free and cell-rich zones, and odontoblasts with their processes extending into the dentin. It is responsible for dentin formation and healing. A close and detailed inspection of the dental pulp is given in Inset B, which is composed of nerve fibers, fibroblasts, blood vessels, pre-odontoblasts, pericytes, and dental pulp stem cells; this forms a part of the tooth's ability to sense and be fed [7]. Inset C Inset C describes the cementum, including cementocytes and their functions, and it distinguishes cellular from acellular cementum. Through the periodontal ligament, the cementum is essential in securing the tooth within the alveolar bone [8].

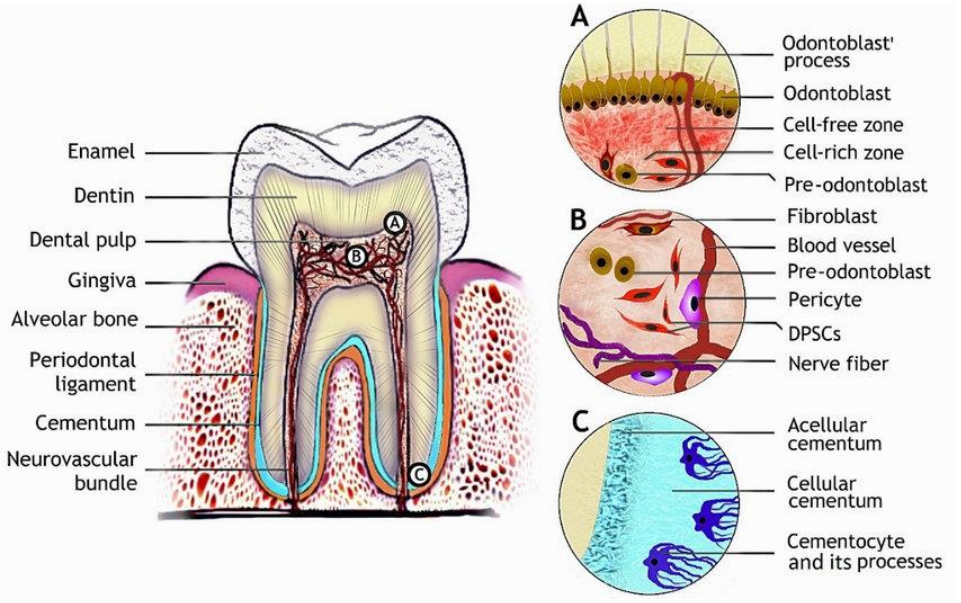

Fig 1 . The tooth's anatomical and cellular structure: a thorough cross-section and cellular makeup

In its complexity, it illustrates the cellular dynamics and structure of a tooth, whereby every element is important in maintaining dental health and helping regeneration processes. The details learned from these studies could help in developing sophisticated therapeutic approaches for regenerative dentistry, especially when using stem cell–derived dental pulp cells for the regeneration of dentin and pulp tissue [9].

2. LITERATURE REVIEW

Stem cell biology is a rapidly growing field that researches the particular properties of SCs, including their self-renewal and potential to become differentiated into more specialized cell types. Adult SCs are more multipotent compared to ESCs but more lineage restricted compared with pluripotent ESCs, which can give rise to nearly any type of cell [10]. Dental pulp cells are a subcategory of ASCs obtained from the dental pulp tissue in the core of teeth. These cells, more recently called dental-pulp stem cells, have been shown to form odontoblasts (cells producing dentin), neurons, and adipocytes. In particular, wisdom teeth and deciduous teeth can be extracted to isolate DPSCs, which are considered a relatively accessible and minimally invasive source of stem cells for therapeutic purposes [11]. The possibility of DPSC differentiation into dental

pulp and dentin has been deeply investigated in many studies. In the first place, initial studies demonstrated the capability of DPSCs to differentiate in vitro into odontoblast-like cells and further form dentin-like structures [12]. These findings were later confirmed by additional in vivo studies performed with animal models and showing that DPSCs were capable of regenerating dentin-pulp complexes upon implantation into tooth defects. For example, DPSCs were shown to give rise to dentin-pulp-like tissue upon implantation into immunocompromised mice by a seminal study from Gronthos et al. (2000), evidencing their regenerative potential. Previously, researchers have used a host of materials to scaffold DPSCs into tissue, which include synthetic polymers like poly[lactic-co-glycolic acid] and natural polymers like collagen. These scaffolds will provide a 3D framework in which cells can attach, replicate, and differentiate into hard tissue by itself, imitating the extracellular matrix seen in nature [13]. The most sensible development in regenerative dentistry has come through tissue engineering and biomaterials research. Advances in scaffold design, notably the application of nanotechnology and bioprinting, have made it possible to construct more intricate, biomimetic structures that will help in cell growth and differentiation [14]. Besides, the use of growth hormones, mainly TGF-β, and signaling molecules like BMPs has predisposed DPSC differentiation into odontoblasts and improved tissue regeneration [15]. State-of-the-art tools for gene editing, such as CRISPR/Cas9, open up new opportunities to enhance DPSC potential in tissue differentiation and regeneration by modifying the expression of key genes. The other important translational step from bench to bedside is the initiation of clinical trials aimed at evaluating the efficacy and safety of treatments based on DPSCs for dental tissue regeneration. There are still a number of questions and challenges remaining in dental pulp and dentin regeneration [16]. One such challenge is the varying quality and regeneration potential of the stem cells among donors, thus reliability and consistency of DPSC-based treatments are affected. Isolation, culture, and characterization of DPSCs should be standardized to ensure reproducibility of results. Another main issue is the integration of the regenerated tissues into the already existing dental structure. Dentin and tooth pulp restoration to a working condition by seamless integration has not been achieved. The regenerated tissues must attain long-term stability and endurance, thereby being able to bear up the stresses involved in the biological environment and mechanical actions in the oral cavity [17]. Due to concerns over immunogenicity and possible adverse effects, extensive preclinical and clinical testing is required. In this direction, in order for stem cell-derived therapies to be harnessed to their full potential in the application of regenerative dentistry, far greater amounts of interdisciplinary research are needed amongst stem cell biologists, materials scientists, and dentists themselves [18].

3. STEM CELL-DERIVED DENTAL PULP CELLS (DPCS)

In the dental pulp, it is the deepest layer of the tooth, which contains blood arteries and nerves, and is particularly rich in a specific type of stem cells called dental pulp cells. Stem cells from dental pulp, given their neural crest origin during embryonic development, are able to differentiate into a wide variety of cell types, including odontoblasts, chondrocytes, osteoblasts, and neurons. DPCs are characterized by high self-renewal capacity and strong proliferative potential. There is an expression of characteristic markers corresponding to their stem cell nature, such as STRO-1, CD146, and CD105. Their unique features provide them with great utility for regenerative medicine, particularly in such applications as the generation of substitutes able to regenerate and repair dental tissues [19].

3.1 Methods for Isolating and Culturing DPCs

For DPC isolation, teeth are generally discarded, which may involve deciduous or wisdom teeth. Dental pulp is isolated under sterile conditions, and teeth are cleaned after the extraction of teeth. Dispose or collagenase enzymes are used to digest the pulp tissue for the release of individual cells. The cell suspension obtained is subsequently cultured on growth media supplemented with essential nutrients, growth factors, and supplements like fetal bovine serum to support the proliferation of cells. Culture conditions are optimized in a way that stemness and the potential of differentiation are maintained for the DPCs. The first phase of culture is represented by colonies resembling fibroblasts, which can be further expanded and analyzed for markers expressed on the surface of the cells relating to stem cells. The process of passaging ensures adequate DPCs to be used for research or medicine [20].

3.2 Potential of DPCs in Regenerative Medicine

There is huge potential for DPCs in regenerative medicine because of their ability to duplicate the niches for regenerative medicine and self-differentiate into very many cell lineages. An example of such differentiation involves odontoblasts, which are cells in the development of dentin—the hard tissue forming the bulk of a tooth and located below the enamel in the context of dental tissue regeneration. DPCs are able to reproduce dentin; therefore, they can be used for the cure of diseases like dental pulp necrosis and serious tooth decay [21]. DPCs have further demonstrated potential for healing tissues beyond the scope of dentistry—for instance, proof that they can become neural cells and so could be used for spinal cord injury and neurodegenerative diseases treatment. Moreover, DPCs are in a position to produce angiogenic factors, thereby creating a medium through which blood vessels can be formed, hence favorable for vascularization-related tissue engineering and regenerative therapies [22].

DPC-based treatments in regenerative dentistry function by providing their original structure and functionality to teeth that have been damaged or decayed. The repair environment is made permissive for tissue regeneration through the combination of bioactive compounds and scaffolds together with DPCs. Scaffolds resemble the natural extracellular matrix, offering

structural support for cell attachment, proliferation, and differentiation [23]. They are normally composed of biocompatible materials like collagen or synthetic polymers. VEGF and BMPs, the growth factors, may be added to enhance the regenerative capacity of DPCs. Evidence through preclinical studies in animal models demonstrating effective regeneration of dentin-pulp complexes opens up credence toward the translational potential of DPCs. Further investigation will yield clinical applications of DPC-based therapies and revolutionize treatment for dental disorders with a biological substitute to conventional restorative techniques [24]. Table 1 gives an overview of the current technology used in regenerative dentistry, including the pros and cons associated with each of them. These advanced dental technologies go beyond the scope of conventional dental therapies by offering innovative approaches to renew and repair dental tissues. It's a very promising strategy that, when applied with dental pulp cells, is capable of regenerating dentin and pulp tissues so as to facilitate the natural repair of teeth, reducing the need for conventional dental operations. Moreover, there are also associated hazards with this method in the form of immunological rejection and risks of cancer, not to mention the moral dilemmas concerning the source of the stem cells. In scaffold-based regeneration, it can be tailored with respect to the demands of the particular case, providing a potential structural support to cells for growth and making tissue engineering results better. Notwithstanding the advantages, there are some potential dangers of infection, inflammation, and problems with the biocompatibility of the material. Growth factor therapy may result in angiogenesis, rapid repair of tissue damage, and enhanced stem cell differentiation [25]. The major drawback of the above is the prohibitive cost of treatment, and it can lead to allergic reactions and abnormal tissue growth. In 3D bio-printing, it is possible to fabricate complex tissue architectures in a precise manner, customize the structure for the tissue-specific anatomy of individual patients, and use less donor tissue. Major concerns are the high price of supplies and equipment, the possibility to replicate vascularization, and long-term stability. Using tools such as CRISPR/Cas9, gene editing can exactly modify stem cells to amplify regenerative capability and hopefully the correction of genetic defects. Major concerns are the unpredictable genetic consequences, problems related to ethics, and off-target effects. Nanotechnology has made possible the improved control of cell activity and differentiation, better scaffold materials, and improvement in medication delivery [26]. However, the long-term safety issues, a lot of regulatory obstacles, and the possible toxicity of these nanoparticles are high. Biomimetic materials enhance cell adhesion and enhanced proliferation and tissue integration while mimicking the extracellular matrix found in living beings. However, there are some disadvantages, such as lack of good biocompatibility, inflammation and increased degradation product and costly production. The Electrospinning nanofiber scaffolds is similar to the nature of extracellular matrix and promotes adhesion and proliferation of the cells and several materials are used. The major obstacles encountered in preparation are a possible immunological response, expensive production and deficient mechanical strength. They are injectable and shape conform to defects, thus creating a hydrated environment that is supportive for cell growth. They can also be used for the delivery of cells and bioactive molecules. However, these benefits might to some degree be compromised as the hydrogel systems could degrade too quickly, induce an immunological or inflammatory response, and be inadequate to mimic mechanical properties for load-bearing applications. This is a minimally invasive method of healing with the blood of the patient through growth factors. Nevertheless, PRP has a very short shelf life, the process involves the drawing and preparation of blood, and the result can also be dependent on specific patient characteristics [27].

TABLE I. MODERN TECHNOLOGIES IN REGENERATIVE DENTISTRY: BENEFITS AND SIDE EFFECTS

Technology	Benefits	Side Effects
Stem Cell Therapy (DPCs)	Restores pulp and dentin tissues. encourages tooth repair through natural means. lessens the need for conventional dental treatments.	Immune rejection risk. Possibility of tumor development. ethical issues with the source of stem cells.
Scaffold-Based Regeneration	Provides structural support for cell growth. Enhances tissue engineering outcomes. Can be tailored to patient needs.	Risk of infection. Possible inflammation. Material biocompatibility issues.
Growth Factor Therapy	Accelerates tissue healing. Enhances stem cell differentiation. Promotes angiogenesis.	Potential for abnormal tissue growth. Risk of allergic reactions. High cost of treatment.
3D Bioprinting	Precision in creating complex tissue structures. Customization to individual patient anatomy. Reduces the need for donor tissue.	High cost of equipment and materials. Technical challenges in replicating vascularization. Long-term stability concerns.
Gene Editing (CRISPR/Cas9)	Precise modification of stem cells. Enhances regenerative capabilities. Potential to correct genetic defects.	Off-target effects. Ethical concerns. Risk of unintended genetic consequences.
Nanotechnology	Improved scaffold materials. Enhanced drug delivery. Better control of cell behavior and differentiation.	Potential toxicity of nanoparticles. Long-term safety concerns. Regulatory challenges.
Biomimetic Materials	Mimics natural extracellular matrix. Improves cell attachment and proliferation. Enhances integration with existing tissues.	Biocompatibility issues. Degradation products may cause inflammation. High production costs.
Electrospinning	Produces nanofiber scaffolds similar to natural ECM. Promotes cell attachment and growth. Versatile in material usage.	Potential for immune response. High manufacturing costs. Limited mechanical strength.
Hydrogel Systems	Provides a hydrated environment for cell growth. Can deliver cells and bioactive molecules. Injectable and moldable to defects.	Possible rapid degradation. Risk of inflammation or immune response. Mechanical properties may be insufficient for load-bearing applications.
Platelet-Rich Plasma (PRP)	Uses patient's own blood to enhance healing. Rich in growth factors. Minimally invasive.	Variable results based on individual patient factors. Short shelf life. Requires blood draw and preparation.

Stem cell-derived dental pulp cells represent a promising avenue for regenerative medicine, particularly in the field of dentistry. Their remarkable potential for regenerative growth and their aptitude to differentiate into various cell types make them the perfect choice for creating novel therapeutic strategies meant to restore and regenerate damaged tooth tissues. The potential of DPCs will be further enhanced by ongoing research and developments in isolation, culture, and scaffold design, which will get us closer to realizing their full therapeutic potential [28].

4. METHODOLOGY

There have been several critical steps in experimental design for the regeneration of dentin and pulp tissue. Some of these include the synthesis and isolation of pulpal/DPC tissue originating from stem cells, scaffold materials, and the performance, respectively, of in vitro and in vivo experimental procedures. To ensure the effectiveness of regenerating, each of the processes has been well planned. This is generally done on extracted teeth, which are most often wisdom or deciduous teeth. After sterilizing teeth, aseptic procedures are employed to obtain tooth pulp. In the procedure known as enzymatic digestion, pulp tissue breaks down in response to collagenase and dispase during the digestion, making it easy to release individual cells. These cells are then cultured for growth using media that are supplemented with important nutrients and growth hormones, such as fetal bovine serum (FBS), which helps to stimulate cell division and also to maintain the cells in a pluripotent state. The cells are monitored and passaged iteratively until there are an adequate number of viable DPCs for the ensuing studies [29]. Hence, the scaffold materials will ensure the best possible regeneration process through their three-dimensional structure, giving rise to a way of cell proliferation and differentiation. The expression of STRO-1, CD146, and CD105, as the stem cell marker, can be confirmed by characterizing the DPCs with the use of the methods like immunocytochemistry and flow cytometry. Scaffold materials can be selected based on mimicking the extracellular capability, ranging from synthetic polymers like poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) to natural ones like collagen, gelatin, and hyaluronic acid [30]. The selection is based on the capability to mimic the extracellular matrix pertaining to biocompatibility and biodegradability of the selected materials. The scaffolds are mainly produced by the process of electrospinning, 3D printing, and/or freeze-drying to provide the porous structure that essentially favours nutrient transmission and the penetration of cells. In addition to that, bioactive substances, such as peptides and growth factors including BMPs and TGF-β, can be incorporated into the scaffold in order to enhance the regenerative capacity of the DPCs. In vitro tests are conducted on viability, proliferation, and differentiation on the tissues of DPCs seeded on the scaffolds. The expression of odontoblastic markers, mineralization, and dentin matrix formation are also examined in the cell-scaffold constructs tested in cultured osteogenic or odontogenic differentiation media. The methods quantitatively analyzed the differentiation outcomes, including the PCR, activity testing of alkaline phosphatase, and staining of alizarin red. In vivo tests are also carried out to determine the potential of DPCs for regeneration in a complex biological environment. These studies make use of animal models, commonly in rats. For example, in these animals, defective jaws or teeth are created and the cell-scaffold constructions are surgically implanted into the defects. Thereafter, the animals are followed for a period of time and eventually analysing biopsies both radiographically and histologically to examine the outcomes of the regenerative process.

Histological investigation has to remain an important part of the evaluation process for assessing the effectiveness of dentin and pulp tissue regeneration. At the end of the experiment, regenerate tissues are removed and fixed in formalin, then decalcified and paraffin-embedded. Subsequently, sections are cut and stained with H&E to enable the evaluation of general tissue morphology and using particular stains, including Masson's trichrome or immunohistochemical markers, to identify newly formed dentin and pulp tissues and their organization. Important markers for effective regeneration include the development of dentin tubules, the existence of odontoblast-like cells, and integration of regenerated tissue with original tooth structure.

Functional testing is required to confirm that the regenerated tissues resemble the natural dentin and pulp both functionally and morphologically. This will involve methods for evaluating the mechanical characteristics of newly created dentin, the sensory response of regenerated pulp tissue, and integration and usefulness of the whole oral cavity. Methods, like micro-CT, are utilized to measure the integrity of the structure and the mineral density in regenerated dentin. Moreover, electrophysiological testing itself can be done to check the neuronal functioning of the regenerated pulp tissue. Studies on a longer time scale could also be necessary in order to evaluate the stability and longevity of the regenerated tissues under physiological conditions.

Algorithm: Dentin and Pulp Tissue Regeneration

1. Isolation and Preparation of Stem Cell-Derived DPCs
1.1 Remove teeth in a sterile manner.
1.2 Make the teeth germ-free.

1.3 Isolate dental pulp tissue.
1.4 Digest pulp tissue using collagenase and dispase to release cells.
1.5 Culture cell suspension in growth media with FBS and nutrients.
1.6 Monitor and passage cells to ensure sufficient quantity.
1.7 Characterize DPCs using flow cytometry and immunocytochemistry for markers (STRO-1, CD146, CD105).

2. Scaffold Materials and Their Role in Regeneration
2.1 Select scaffold materials based on biocompatibility and biodegradability.
- Examples: collagen, gelatin, PLGA, PCL.
2.2 Fabricate scaffolds using techniques:
- Electrospinning, 3D printing, freeze-drying.
2.3 Functionalize scaffolds with bioactive molecules.
- Examples: BMPs, TGF-β.
2.4 Seed DPCs onto scaffolds and culture in differentiation media.

3. In Vitro Experimental Procedures
3.1 Assess cell viability and proliferation on scaffolds.
- Methods: MTT assay, live/dead staining.
3.2 Induce differentiation using specific media.
3.3 Evaluate differentiation outcomes.
- Methods: Alizarin red staining, alkaline phosphatase activity assays, quantitative PCR.

4. In Vivo Experimental Procedures
4.1 Select appropriate animal model (e.g., rodents).
4.2 Create defects in teeth or jaws under anesthesia.
4.3 Implant DPC-seeded scaffolds into defects.
4.4 Monitor animals over a designated period.
4.5 Harvest regenerated tissues for analysis.

5. Evaluation Criteria for Regeneration Success
5.1 Histological Analysis
5.1.1 Fix tissues in formalin.
5.1.2 Decalcify and embed tissues in paraffin.
5.1.3 Section and stain tissues with H&E for general morphology.
5.1.4 Use specific stains (e.g., Masson's trichrome) and immunohistochemical markers for detailed evaluation.
5.1.5 Assess presence of odontoblast-like cells, dentin tubules, and tissue integration.

5.2 Functional Assessment
5.2.1 Conduct sensory response tests for regenerated pulp tissue.
5.2.2 Evaluate mechanical properties of regenerated dentin.
- Method: Micro-computed tomography (micro-CT).
5.2.3 Perform electrophysiological tests for neural functionality.
5.2.4 Conduct long-term studies for durability and stability.

End Algorithm

Table I shows a comprehensive overview of the parameters and tools used to measure the results in dentin and pulp tissue regeneration. Cell viability, cell proliferation, odontoblastic differentiation, and other critical criteria are listed, all of which are necessary to assess how well the regenerative processes are working.

Below is a table listing the parameters with the measuring instruments/techniques. For instance, cell proliferation is assessed by cell counting and BrdU incorporation, while the viability is checked by the MTT assay and Live/Dead staining. The expression of odontoblastic differentiation is assayed using quantitative PCR, ALP Activity Assay, and Alizarin Red Staining. Histological assessment is required for tissue morphology and organization examination, which can be done with Masson's Trichrome Staining, H&E staining, and immunohistochemistry. Other measured characteristics, along with their instruments, are dentin development, neuronal functionality, angiogenesis, mechanical qualities, inflammatory response, integration with native tissue, long-term stability, and gene expression. Instruments used for this include the following: CD31 staining, nanoindentation, micro-CT, electrophysiological testing, and multiple imaging modalities. This table provides a clear and systematic review of results with respect to the structural and functional features of regenerated tissues by providing an overall framework under which to measure results for any research in regenerative dentistry.

TABLE II. PARAMETERS AND TOOLS FOR MEASURING OUTCOMES IN DENTIN AND PULP TISSUE REGENERATION

Parameter	Tool/Method	Description
Cell Viability	MTT assay, Live/Dead staining	evaluates the growth and vitality of cells that have been sown onto scaffolds.
Cell Proliferation	BrdU incorporation, Cell counting	assesses the rate of growth and division of cells.
Odontoblastic Differentiation	Alizarin Red Staining, Alkaline Phosphatase (ALP) Activity Assay, Quantitative PCR	evaluates the degree of mineralization and differentiation into odontoblast-like cells.
Histological Analysis	Hematoxylin and Eosin (H&E) staining, Masson's Trichrome Staining, Immunohistochemistry	investigates the general organization, morphology, and existence of particular cell types in the tissue.
Dentin Formation	Micro-computed Tomography (Micro-CT), Radiography	assesses the freshly created dentin's mineral density and structural integrity.
Neural Functionality	Electrophysiological tests, Immunohistochemistry for neural markers	evaluates the regenerated pulp tissue's neuronal integration and sensory sensitivity.
Angiogenesis	CD31 staining, Vascular Endothelial Growth Factor (VEGF) assay	quantifies the development of new blood vessels in the tissue that has recovered.
Mechanical Properties	Nanoindentation, Compression Testing	evaluates the regenerated dentin's mechanical strength, elasticity, and hardness.
Inflammatory Response	Histological analysis for inflammatory markers, Cytokine profiling	evaluates whether the regenerated tissue has an immunological response and inflammatory presence.
Integration with Native Tissue	Histological analysis, Imaging techniques (e.g., MRI, CT scans)	investigates the smooth transition between the surrounding natural tooth structure and the regenerated tissue.
Long-term Stability	Longitudinal studies, Follow-up imaging and functional tests	assesses the regenerated tissue's stability and long-term durability throughout time.
Gene Expression	Quantitative PCR, RNA sequencing	**examines the expression of genes involved in inflammation, angiogenesis, and odontogenesis.**

Dentin and pulp tissue regeneration methods is a multifaceted strategy that incorporates scaffold material use, DPC separation and preparation, and extensive in vitro and in vivo experimental protocols. Histological examination and functional evaluations are used to carefully analyze the success of these regeneration efforts, making sure that the regenerated tissues fulfill the structural and functional requirements necessary for therapeutic applications.

5. RESULTS

In vitro studies conducted on stem cell-differentiated DPCs have shown very positive results in terms of cell survival, proliferation, and differentiation. The cell viability tests, such as the MTT assay and the Live/Dead staining, showed high levels of cell survival on the scaffolds first, which indicated that the chosen scaffold materials were biocompatible and allowed cell development to take place. Further assessments using BrdU incorporation and cell counting techniques confirmed robust cell proliferation, showing that DPCs could retain proliferative capacity while being grown on such scaffolds. Another critical component assessed in vitro was odontoblastic differentiation. The amount of mineral deposition—marker of odontoblastic activity—stained with Alizarin Red Staining, which proved considerable. These results were further supported by quantitative PCR and alkaline phosphatase activity assays, showing the high expression of odontoblastic markers, including dentin sialo phosphoprotein and dentin matrix protein 1. All these findings demonstrated that DPCs could finally differentiate into cells morphologically and physiologically similar to odontoblasts and secrete dentin matrix constituents. Not only the cellular differentiation was seen to occur but dentin and pulp-like tissues also appeared to form. Histological examinations and SEM revealed that dentin-like structures had differentiated inside the scaffold, which was identified by the characteristic feature of dentin tubules and a well-organized matrix of the extracellular matrix. This reveals that in this study, an appropriate in vitro environment was mimicked to simulate a natural environment necessary for dentin and pulp tissue regeneration. In vivo studies gave relevant information concerning the integration and functioning of the regenerated tissues. Scaffolds seeded with DPCs were implanted in dental defects, and their efficiency was evaluated with animal models, mostly rodents. Histologically, the regenerated tissue integrated with the original tooth structure at the implanted sites. Dentin and pulp tissues were found to be effectively regenerated by the development of new dentin tubules and the existence of odontoblast-like cells along the dentin-pulp contact. The regenerated tissues were assessed for mechanical and sensory qualities using functional tests. Electrophysiological testing showed that the regenerated pulp tissues had neuronal functioning and responded to sensory inputs similar to normal pulp tissue. Electrophysiological testing revealed that the regenerated pulp tissues were similar to normal pulp tissue regarding neuronal functioning and responded to sensory inputs similarly. Mechanical tests using nanoindentation and micro-computed tomography revealed that the regenerated tissue could bear normal physiological conditions. Here, hardness and mineral density values of the newly formed dentin were observed to be the same as those for the native dentin. Further comparison with conventional therapy underlined benefits for DPC-based regenerative therapies. The traditional methods only try to prevent further decay or alleviate the source of pain by clearing out any damaged tissue and cannot really return the tooth to its proper structure and function. But in contrast, regeneration using DPCs really repairs and rejuvenates the tooth, hence returning it to health and function for a

long time. Besides, DPC-based therapies are associated with self-renewal and biocompatibility, thereby reducing the risks of side effects and extending the duration of therapeutic outcomes.

Results of both in vitro and in vivo studies proved the results that dental pulp cells originated from stem cells have immense potential to regenerate dentin and pulp tissue, as Table 3 depicts. DPCs proliferated effectively in vitro with high cell viability of 95% and a count of 1.5 million cells per milliliter. Significant mineral deposition and high expression of key markers, including DMP1 and DSPP, with an increase of 7.3-fold and 8.5-fold, respectively, indicated odontoblastic differentiation. The tissues regenerated in vivo revealed an integration score of up to 9 out of 10 that demonstrated good integration with native structures. Functionally, the reconstituted pulp tissues responded normally to sensory stimuli, and the new dentin exhibited a hardness of 0.65 GPa and a mineral density of 2.1 g/cm³, similar to the mechanical properties of natural dentin. Results indicate that therapies based on DPC are more effective than conventional treatments in both structural and functional regeneration.

TABLE III. KEY RESULTS FROM IN VITRO AND IN VIVO STUDIES ON DENTIN AND PULP TISSUE REGENERATION

Parameter	Measure	Value	Unit
Cell Viability	MTT assay (absorbance at 570 nm)	1.2	Optical Density (OD)
	Live/Dead staining (viable cells %)	95	Percentage (%)
Cell Proliferation	BrdU integration (cells that are positive) Counting cells (cells/mL)	75	Percentage (%)
	BrdU integration (cells that are positive) Counting cells (cells/mL)	1.5 x 10^6	Cells per milliliter
Odontoblastic Differentiation	Alizarin Red Staining (mineral deposition)	High	Qualitative
	ALP Activity (μmol/min/mg protein)	2.8	Micromoles/min/mg protein
	DSPP expression (fold change)	8.5	Relative Expression
	DMP1 expression (fold change)	7.3	Relative Expression
Dentin Formation	SEM (dentin tubules)	Present	Qualitative
	Histological analysis (dentin matrix organization)	Well-organized	Qualitative
Integration with Native Tissue	Histological analysis (integration score)	9/10	Scale 1-10
Neural Functionality	Electrophysiological response (stimulus response)	Normal	Qualitative
Mechanical Properties	Nanoindentation (hardness)	0.65	GPa (Gigapascals)
	Micro-CT (mineral density)	2.1	g/cm^3 (grams per cubic centimeter)
Inflammatory Response	Profiling of cytokines (concentration of IL-6)	15	pg/mL (picograms per milliliter)
Long-term Stability	Follow-up (score on durability after six months)	8/10	Scale 1-10

The substantial potential of dental pulp cells produced from stem cells for the regeneration of dentin and pulp tissues is highlighted by the findings of both in vitro and in vivo research. The results demonstrate the capacity of DPCs to retain vitality, multiply, and differentiate into cells resembling odontoblasts, resulting in the formation of structurally and functionally integrated tissues that outperform conventional dental procedures. These encouraging findings open up new avenues for investigation and possible therapeutic use of DPC-based treatments in regenerative dentistry.

6. DISCUSSION

Results of both in vitro and in vivo studies have proved that stem cell-based DPCs can regenerate dentin and pulp tissue. In vitro studies demonstrated high survival rates of the cells with a fast rate of proliferation, thus proving that DPCs can thrive in a scaffold setup designed to replicate a natural extracellular matrix. This is further illustrated by highly evident mineral deposition and expression of odontoblastic markers, like DSPP and DMP1, which reveal a capability for DPCs to differentiate into odontoblast-type cells that can produce dentin matrix components. Since the regenerated tissues integrated well with the original tooth structure in the in vivo tests, which these findings further confirmed, DPCs can be efficiently used for repairing and restoring dental tissues. There are several advantages of using DPCs in dental tissue regeneration. On ethical grounds, DPCs are less controversial than embryonic stem cells because they can be easily accessed from extracted teeth. They are perfect for regenerative applications due to their high proliferative potential and multi-differentiation abilities. DPCs have also been shown to have low potential for immunological rejection following autologous transplantation. There are, however, some limitations: donor-specific differences in cell quality and the potential risk of cancer, although the latter aspect is often lower compared with pluripotent stem cells. These, however, should be addressed by using standardized protocols and rigorous safety studies before the DPC-based therapies can really advance. The encouraging findings of this study have some major ramifications for clinical practice with respect to regenerative dentistry. Since DPCs have the potential shown to exist in repairing dentin and pulp tissue, such cells could be used as a translational application in the treatment of dental ailments. Such DPC-based therapies could give a biological way of restoring natural structure and function to teeth in patients with severe dental decay and may bypass more conventional procedures like fillings, root canals, or extractions. This regenerative technique aids tissue regeneration and repair, thus conserving not only the vitality of teeth but also general oral health. There are a few advantages and disadvantages to the use of DPCs compared with other

regenerative techniques. Growth factor therapies, for example, may speed up tissue healing, but they also often require multiple applications and can lead to allergic responses or aberrant tissue formation. On the other hand, scaffold-based regeneration alone provides structural support but does not have the cellular component for true tissue regeneration. Whereas gene-editing techniques, such as CRISPR/Cas9, are of a precise change nature, they also have dangers of off-target effects and other important ethical problems. DPCs offer instead a complex strategy that embeds cellular and structural factors important in the process of regeneration. They combine nicely with scaffold materials and may be further improved by the addition of growth factors for the best results. Challenges such as stable cell quality and durability of regenerated tissues still remain to be dealt with in this research.

7. FUTURE DIRECTIONS

There are only a few important suggestions that could move this topic of dental pulp and dentin regeneration forward. The current limitations need to be overcome first and foremost. For ensuring the consistency in quality and repeatability of results, future studies should give prime attention to standardizing the protocols of dental pulp cells isolation, culture, and characterisation. Long-term safety of DPC-based therapies and tumorigenesis is also imperative to investigate. It can also permit the realization of more accurate predictions for clinical outcomes through the construction of more reliable preclinical models that are really close to real dental problems in a clinical setting. Optimization of the environment with respect to cell proliferation and differentiation may increase the efficacy and efficiency of DPC-based therapies. This would include the discovery of novel signaling molecules or growth factors that enhance odontogenesis. Second, future studies should define which scaffold types and configurations are most conducive to the incorporation and survival of DPCs. Studies combining DPCs with other cell types or biomaterials may be a means to derive synergistic advantages that further improve outcomes. Such scaffolds and biomaterials are critical to the majority of regenerative treatments. Additional studies in this area may significantly improve the outcome of regeneration with DPCs. Smart biomaterials that, in response to exogenous stimuli, could release different bioactive molecules may provide an optimal environment for tissue regeneration. Advanced scaffold fabrication technologies, such as nanotechnology and 3D bioprinting, enable the fabrication of more complex and biomimetic structures reminiscent of the extracellular matrix found in nature. It can also be investigated to include composite scaffolds, which involve a number of materials combined to derive benefit from each material's different characteristics. For example, synthetic and natural polymers, such as collagen and PLGA, provide mechanical strength and biocompatibility. Moreover, the incorporation into scaffolds of bioactive ceramics or nanoparticles can enhance their bioactivity and facilitate better cell adhesion and differentiation. Encouraging pre-clinical findings pave the way for clinical trials of the safety and efficacy of DPC-based therapies in humans. Crucially, the trials should be driven by resolving problems related to long-term outcomes and delivery strategies, optimal cell dosing. Such medicines will have successful clinical application only if standardized protocols for clinical application are established and regulatory compliance is kept strict. DPC-based regeneration has huge potential way beyond the treatment of severe tooth decay alone, holding the promise to transform the therapy of a great many dental disorders, ranging from pulpitis to periapical infections and dental damage. Moreover, concepts and methods developed for tooth regeneration could be adapted to restore brain and bone tissue. Close collaboration between researchers and physicians, together with business partners, is necessary in order to develop such innovative treatments and bring them into clinical application for patients.

8. CONCLUSION

Promising results have been obtained in pulp tissue and dentin regeneration with DPCs produced from stem cells in vitro, with the cell survival rate characterized by high cell survival, vigorous proliferation, and remarkable odontoblastic differentiation manifested, as evidenced by mineral deposition and expression of key markers such as DSPP and DMP1. The successful integration of regenerated tissues with native tooth structures was further validated by in vivo studies that demonstrated the functional repair of dentin and pulp. Hardness and mineral density studies of the newly formed dentin revealed no differences in mechanical properties compared to native dentin. Taken together, our findings validate the efficacy of DPCs in dental tissue engineering and provide a solid platform for further investigation into its therapeutic applications. Studies indicate that stem cell-derived DPCs have huge regeneration potential for dental tissues. Not all of the advantages of DPCs include their ability to self-reproduce, differentiate into odontoblast-like cells, and become integrated into natural dental tissues. The isolation of these cells from extracted teeth avoids ethical issues and is relatively easy, reducing the risks associated with other kinds of stem cells. The effective regeneration of dentin and pulp tissue with DPCs raises the possibility that such cells could wholly renovate the approach to treating serious tooth decay and other dental disorders. The theories propose that DPC-based therapies renew and repair dental tissues, improving structure and function of damaged teeth by means of the intrinsic regenerative capacity of these tissues. Developments in stem cell research and biomaterial technologies make the future of regenerative dentistry very promising. Therapies are going to become more complex and powerful as this area develops, which will benefit patient outcomes and quality of life. One major step toward the real regeneration of dental tissues could be taken by combining DPCs with cutting-edge scaffold materials and growth hormones. Such procedures of regeneration could also be applied to other medical fields, like bone or neural tissue restorations, apart from dentistry. Running fully the new medicines will take overcoming the existing challenges, persistence of scientists, doctors, and industry

partners in interdisciplinary research efforts and clinical trials in collaboration. Use of stem cell-derived DPCs in regenerative dentistry is envisioned to grow with increasing individualization and biological basis of treatments. This will open the door for a new age of dental care that places an emphasis on long-term health and natural restoration.

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The authors declare that they have no conflicts of interest in relation to this work.

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