Dental pulp fibroblast: A star Cell

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## Abstract

**Introduction**: Dental pulp fibroblasts (DPF) are the most abundant cell type in the dental pulp. They play pivotal roles; however, they are often mistaken to be involved only in the repair and maintenance of this connective tissue.

**Methods:** We used the search terms “pulp fibroblast,” “complement system proteins,” “pulp inflammation,” “angiogenesis,” and “dentin pulp regeneration” to identify articles from the PubMed and Scopus databases.

**Result:** These sentinel cells produce all complement system proteins participating in defense processes, control of inflammation, and dentin-pulp regeneration; produce several proinflammatory cytokines and chemokines and express pattern-recognition receptors, demonstrating their involvement in immunoregulatory mechanisms; express neuropeptides and their receptors, playing an important role in neurogenic inflammation and dental pulp wound healing; secrete angiogenic growth factors as well as neurotrophic proteins, essential for dentin-pulp regeneration; regulate neuronal plasticity processes; and can sense the external environment.

Conclusion: This review highlights that DPFs are more than mere passive cells in pulp biology and presents an integrative analysis of their roles and functions.

**Keywords:** dental pulp, fibroblasts, regenerative endodontics, dental pulp disease, complement system proteins

## Introduction

The dental pulp is a unique connective tissue containing fibers, cells, extracellular matrix, and a wide nerve and vascular plexus (1). The most studied cell populations of this tissue are the odontoblasts, representing the first line of defense against injury (2); dental pulp stem cells (DPSC), which have a high proliferative potential for self-renewal and the ability to differentiate into classical mesodermal lineages (3); and dental pulp fibroblasts (DPF), which are the most abundant cells in the dental pulp (1,4). DPFs exhibit some singularities with respect to fibroblasts present in other connective tissues, such as the expression of tenascin, osteonectin, and tissue-related extracellular matrix (ECM) proteins (5).

The role of DPFs in the synthesis and replacement of collagen and other components of the ECM is well known (1); however, these cells reportedly have an important role in pulp function. Investigations show that DPFs produce all complement system proteins (6), indicating their participation in defense processes, control of inflammation, and dentin-pulp regeneration (4,7,8). These cells also produce several proinflammatory cytokines and chemokines (9–11) and express pattern-recognition receptors (PRRs) (12), which are involved in many immunoregulatory mechanisms and express some neuropeptides and their receptors (13,14), thus playing an important role in neurogenic inflammation. Another study has cited the role of pulp myofibroblasts (specialized fibroblasts) in dental pulp wound

healing (15), and others have analyzed the role of DPFs in neuronal plasticity processes (16–18), in sensing the external environment (19), and in the synthesis of angiogenic growth factors (20–24) and neurotrophic proteins (16,17). Figure 1 summarizes their pivotal aspects.

Notwithstanding the abovementioned investigations that describe many physiological and pathological functions of DPFs, to our knowledge, the present review is the first study to integrate all the roles of DPF that have been ascribed, until now, in a single review, including their potential clinical application in pulp therapy. This review presents an integrative analysis of the roles and functions of DPFs, highlighting that DPFs are not mere passive cells, as is often mistakenly assumed, but play a leading role in various functional and pathological situations. For this purpose, we searched the available literature in PubMed and Scopus databases to identify relevant articles published until June 30, 2021, using the search terms "pulp fibroblast," "complement system proteins," "pulp inflammation," "angiogenesis," and "dentin pulp regeneration." In this study, we included only articles published in English. A manual search of the reference lists of the initially selected articles was performed to complement the electronic search. We also examined endodontic journals for papers in press or with an early view.



## 1. Fibroblasts: basic aspects and physiological roles

Fibroblasts are ubiquitous mesenchymal cells and one of the most abundant cell types present in the stroma of many tissues. They exhibit a spindle or stellate shape and regulate tissue homeostasis, scaffolding support, repair, and maintenance of connective tissue (25)

through the synthesis of collagen and fibronectin, and degradation of the ECM by matrix metalloproteinases (MMP) (26). Moreover, fibroblasts also play significant physiological roles in innate immunity (27) and dentin-pulp complex regeneration through the secretion of growth factors (16,23,28,29).

The exact cell that gives rise to fibroblasts is unknown due to the lack of definitive cell surface markers (30,31), their cellular heterogeneity (32–34), and similarities with mesenchymal stem cells (MSCs) (35–37). It has been suggested that fibroblasts should be named MSCs based on the current criteria provided by the International Society for Cell Therapy (35,38). Both cells are described as plastic-adherent cells, with an almost identical proliferation potential (36,37,39,40) and are phenotypically indistinguishable in culture (36). Furthermore, both fibroblast and MSCs have similar immunomodulatory properties *in vitro* (36,41) and have the potential to differentiate into many cell types *in vivo* (42,43) and *in vitro* (39,41,42,44–51). Conversely, other studies report that fibroblasts lack this latter capacity (37,52,53). These contradictory results may be due to the presence of external factors such as the age of the donor (35,54), *in vitro* life of the cells (55), and the topographical differentiation of the fibroblast (56). It is speculated that MSCs are immature fibroblasts, and an aging-associated process distinguishes the two cells more than the differentiation process (35). Therefore, the distinction capacity between these two cells remain unclear (35,36,41).

Although it has been stated that fibroblasts and MSCs express the same surface markers (36,37), some markers and genes have been found to allow their correct differentiation. CD

106 (39,57–59), CD 146 (57,59–63), ITGA11 (57,64), SSEA-4 (57,65), GD-2 (57), Stro-1 (66), CD 271 (66), CD 166 (57), and IGF-2 (57) are specific to MSCs, and CD 9 (57,58), CD 10 (52,57,67), and CD 26 (52,57) to fibroblasts. Therefore, although some surface markers have been used to identify fibroblasts in distinct tissues, when used alone or in combination, they do not reliably identify fibroblast subpopulations in all tissues (68).

Specifically, concerning DPF, fibroblast surface protein (FSP) staining analysis by immunofluorescence microscopy and flow cytometry has been used in *in vitro* experiments to properly characterize these cells by the explant outgrowth method (6,16,69–72). As for DPSCs, immunostaining with six stem-cell markers (STRO-1, CD 44, CD 90, CD 105, CD 146, and CD 166) has been used in cells obtained by STRO-1 magnetic cell sorting. The co-expression of these stem-cell markers and STRO-1 by sorted cells was clearly visible under fluorescence microscopy and flow cytometry, thus ensuring a high quality and purity of DPSCs obtained by this method (6).

In general, fibroblasts are a heterogeneous population, depending on the stage of development, anatomical sites, or the tissue microenvironment (36); different subtypes can be present within the same tissue (32,73). Differences in cell behaviors likely result from a combination of intrinsic and extrinsic factors, such as mechanical forces originating from gravity, blood flow, and body movement, which differ between different regions of the body (32,56). Unicellular technologies have studied the heterogeneity in fibroblasts, which accurately determine the differences in genes, gene expression profiles, and protein production within individual cells (74–77).

A progressive decrease in fibroblasts is part of the pulp aging process (78). DPFs show great variation in proliferative activity, which cannot be explained by the age of the donor, the source, or the number of passages (73). Additionally, after injury, mechanical stress, or inflammation (15), fibroblasts can undergo a phenotypic transformation into cells that possess the features of fibroblasts with cytoskeletal characteristics of contractile smooth muscle cells. These specialized fibroblasts are known as myofibroblasts (79), which are regularly present in a few tissues (25). However, in injured tissues, such as the dental pulp, they synthesize abundant collagen to restore the damaged tissues (80).

## 2. DPFs in innate immunity and inflammation

Fibroblasts act as sentinel cells in the connective tissues, producing inflammatory mediators in response to several microorganisms (81). These cells recognize pathogens, induce the recruitment of inflammatory cells, and express antimicrobial peptides, proinflammatory cytokines, chemokines, and growth factors, thus displaying immunological attributes that regulate the innate immune response (82,83). DPFs also exert these functions to maintain homeostasis in the pulp and support tissue repair and regeneration (83,84), as described in the following sections. However, no reports regarding the production of antimicrobial peptides by DPFs have yet been published, unlike fibroblasts in other tissues (85–87) and odontoblasts (88,89).

### 2.1 DPFs and complement system

The dentin-pulp complex defends itself against injury by generating inflammatory reactions and eliminating bacteria, which are considered the initial steps of tissue regeneration

(84,90). Complements are a powerful innate immune response involved in initiating inflammation and its subsequent resolution (7,17,91). The liver is the primary origin of these proteins (92); however, poorly vascularized tissues, such as the dental pulp, are a possible extrahepatic source when there is tissue damage (6). Within the dental pulp, mechanical trauma (93), carious lesions (6,94), and restorative procedures activate complements (95) and initiate dentin-pulp regeneration. In this sense, DPFs constitutively express C1q and C7; however, after stimulation with lipoteichoic acid (LTA), which is used to simulate the presence of gram-positive bacteria in the pulp, these cells express all complement molecules, C1 to C9 (6), including the membrane attack complex (MAC) (90). The function and fixation of MAC are assumed to be similar in gram-negative bacteria (96).

Complement proteins secreted by DPFs allow direct lysis of the pathogens through the formation of MAC, which is clearly visible after 30 min of coculture with cariogenic bacteria (70). Furthermore, DPFs allow the release of proinflammatory mediators, recruitment of leukocytes to the site of inflammation, and modulation of their phagocytic activity by producing C5a and C3a (7,97). The opsonization of cariogenic bacteria stimulates phagocytosis through the expression of C3b (72). These steps inhibit bacterial progression through the dentin-pulp complex (98), and thus, DPFs provide powerful control of inflammation through local activation of the complement system (6).

### 2.2 DPFs as producers and target cells of proinflammatory cytokines

DPFs play an important role in local immune regulation by expressing various receptors for cytokines, such as interleukin-10 receptor (99), interleukin-17 receptor, and various pro-

inflammatory cytokines (11), which regulate the intensity and duration of pulp and periapical inflammatory processes (99–101), such as CCL3, CXCL12 (10,11), interleukin (IL)-6 (9,83,102), IL-1β (103), IL-8 (83,104,105), CXCL10 (106), and CCL2 (78,107), in response to bacterial stimulation (11,107). However, other cytokines can also stimulate these cells (83,106,108–110), sensory neuropeptides (111), leptin (112), and dental materials (113), and thus produce cytokines/chemokines (111), MMPs (114–116), tissue inhibitor of metalloproteinase-1 (116), colony-stimulating factor (110), and cyclooxygenase 2 (COX-2) (100,117), which amplify the immune response (106,109).

IL-1β is one of the most potent proinflammatory cytokines among various cytokines, and a multi-protein complex called NLRP3/caspase-1 inflammasome controls its release (87). This molecular platform boosts the innate immune response and regulates the adaptive immune response (118,119). In the dental pulp, DPFs express inflammasomes in response to bacteria and bacterial products (120–122) and get activated by lipopolysaccharides through a process involving the ATP-activated P2X7 receptor and reactive oxygen species (121,122). Therefore, inflammasome regulates the secretion and bioactivity of IL-1β, which is crucial for the immunological defense of the dentin-pulp complex (123,124).

**2.3 PRRs in DPFs**

DPFs express innate immune receptors such as PRRs (12) to recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (124,125). PRRs are toll-like receptors (TLRs) bound to the cell membrane (125,126) and nucleotide-binding oligomerization domain (NOD), which are implicated in the intracellular recognition of

bacterial components (125,127). DPFs constitutively express TLR2 (12,27), TLR3 (27), TLR4 (12,27), and TLR 5 (128) in response to LTA, viral double-stranded RNA, lipopolysaccharides, and flagellin, respectively, and NOD1 and NOD2 (12). These receptors recognize invading microorganisms (109) and regulate the expression of various proinflammatory mediators (12,27,128,129). TLR2 acts synergistically with NOD2 and the histamine-1 receptor to induce an inflammatory response during microbial infection (12,128). All these functions actively show that DPFs recognize invading microorganisms and initiate immune/inflammatory events in the pulp (128).

### 2.4 DPFs and neurogenic inflammation

DPFs are known to express neuropeptides such as substance P (SP) and its neurokinin-1 receptor (NK-1) (14). Similarly, these cells respond to neuropeptide Y (NPY) since they express its receptor NPY Y1; however, unlike SP, these cells do not synthesize NPY (13). The expression of SP, NK-1, and NPYY1 increases in response to inflammatory mediators (13,14); therefore, their presence is marked in inflamed pulps compared to healthy pulps (130,131). SP is a pro-inflammatory neuropeptide that induces the release of inflammatory mediators that cause local vasodilatation, increase vascular permeability and local blood flow, and increase tissue pressure (132,133). In contrast, NPY plays an inhibitory role in neurogenic inflammation, antagonizing the effects of SP to avoid excessive inflammation in tissues (13). In addition, SP, neurokinin A, and calcitonin gene-related peptide (CGRP) induce pulp fibroblast growth *in vitro* (134). All these data confirm the role of DPFs as producers and

target cells of neuropeptides, which play an important role in inflammation and wound healing after pulpal injury (131).

### 2.5. DPFs and inflammation resolution

Recently, a notable investigation using an elegant model (69) proposed that, during the carious process, DPFs subjected to cariogenic bacteria are located in the central inflammatory zone and stimulate M1 macrophage differentiation (with high phagocytic capacity), whereas DPFs located in the peripheral inflammatory zone, which is not in direct contact with bacteria, induce M2 macrophage differentiation, which has anti-inflammatory activity and thus limits pulp tissue damage. These results contradict the long-believed notion that macrophages are strictly of the M1 phenotype during the inflammatory process and strictly of the M2 phenotype during the healing process. Nevertheless, a mixed M1/M2 population was present in both inflammatory zones, indicating that phenotypic plasticity is essential to regulate the balance of pulp inflammation and repair to avoid cell damage and chronic inflammation (69). This study is the only one that shows the indirect anti-inflammatory role of DPFs in the context of dental pulp inflammation, which is significant because this cell is known to trigger the production of pro-inflammatory mediators.

The interaction between fibroblasts and macrophages during inflammation and resolution is well recognized (135). Fibroblasts and macrophages are present in all tissues, and recent evidence supports that these cells maintain direct communication to influence the tissue microenvironment and thus affect disease onset, progression, disease outcomes, and resolution (136). Molecular programs linking these cell types could help understand their

interactions and regulatory networks, for example, in pulp disease. One of these programs is The Atlas of Inflammation Resolution (137), a platform that covers over 30 highly interconnected submaps associated with acute inflammation onset, transition, resolution, and homeostasis at the molecular level, providing the user with an interactive interface to map time-series omics data.

## 3. DPFs in dentin-pulp regeneration

Dental pulp has great regenerative capacity due to the release of growth factors after the acid dissolution of carious dentin (21,51,138). Nonetheless, in germ-free animals, it has been observed that after surgical pulp amputation, the formation of reparative dentin can occur regardless of the growth factors released from dentin (139), indicating that dental pulp represents another source of signals that induce regeneration of the dentin-pulp complex following traumatic injury (20,140,141). DPFs secrete various growth factors involved in the recruitment and differentiation of stem cells into odontoblast-like cells for reparative dentin synthesis (28,29), neoangiogenesis (23), and nerve regeneration (17). In addition, DPFs can form crystals with an X-ray diffractometry pattern similar to that of hydroxyapatite, which demonstrates that fibroblasts themselves can produce mineralized nodules as a defense mechanism (142). Further, DPFs can play important roles in pulp-dentin complex regeneration (90), as emphasized in the following subsections.

### 3.1 Stem cell recruitment and differentiation

Odontoblasts are considered postmitotic cells (143) with no potential for self-renewal. Severe carious lesions, traumatic injuries, or aggressive restorative procedures can cause

irreversible damage, such as apoptosis (144). For repair of the pulp-dentin complex, it is necessary to trigger signals that induce migration and differentiation of DPSCs into odontoblasts-like cells, which can synthesize reparative dentin (145). In this regard, DPFs secrete transforming growth factor beta-1 (TGF-ß1) (29), basic fibroblast growth factor (bFGF or FGF-2) (20), vascular endothelial growth factor (VEGF) (146), and complement proteins such as C3a and C5a, when LTA stimulates fibroblasts (6). These factors are important promoters of DPSC migration (147,148) and, TGF-ß1 (138), VEGF (149), FGF-2 (28), and neural growth factor (NGF) (150) participate in the differentiation of DPSCs into odontoblast-like cells, which generate reparative dentin (149).

These factors act in the repair process through their actions. For instance, TGF-ß1 increases collagen secretion by DPFs (151), plays a role in the differentiation and activation of myofibroblasts (80), and induces odontoblast-like cell differentiation and mineralization when capping materials such as calcium hydroxide, biodentine, or mineral trioxide aggregate (MTA) are applied directly to the dental pulp (152–154). VEGF promotes stem cells from exfoliated deciduous teeth to differentiate into functional odontoblasts, which generate tubular dentin, and endothelial cells with angiogenic capacity (149), whereas FGF-2 induces neovascularization (23), stimulates proliferation of fibroblasts in the proximity of the wound, and promotes hyaluronan secretion by pulp cells, which influences tissue repair by promoting anti-inflammatory effects (155).

Inflammatory reactions are a prerequisite for the migration of progenitor cells involved in pulp repair (3). An *in vitro* model of inflammation induced by nemosis verified this

(156,157). In nemosis, normal fibroblasts are induced to form groups, called spheroids, which do not grow but undergo cellular activation and simultaneous programmed cell death (158,159); it occurs in DPFs *in vitro* and could occur *in vivo* during pulp inflammation (156,160). Nemotic fibroblasts release significant amounts of proinflammatory cytokines, chemokines (156), COX-2 (156,157), VEGF (156), and hepatocyte growth factor (160), triggered through direct cell-to-cell adhesion rather than external stimuli (158,160). Thus, under experimental conditions, nemotic DPFs could be a source of chemokines and cytokines that induce stem cell migration (160) and proangiogenic factors that induce angiogenic responses from endothelial cells during tissue repair (161).

**3.2 Role of DPFs in angiogenesis**

Angiogenesis is an extremely complex process that is essential to provide nutrients and oxygen during the healing process and for the migration of progenitor cells to the injury site (162). This process occurs in physiological conditions, such as development and wound healing (163), and pathological conditions, such as irreversible pulpitis (146). DPFs play a pivotal role in dental pulp angiogenesis because they produce and release growth factors, such as VEGF (20,146), FGF-2 (20,24), TGF-ß1 (164), platelet-derived growth factor (PDGF) (22,23), angiogenin, angiopoietin-2 (ANG-2), epidermal growth factor (EGF), leptin, heparin-binding EGF (HB-EGF), hepatocyte growth factor, and placental growth factor (22).

The most potent and abundant factors in the revascularization and wound healing process are VEFG and FGF-2 (20). However, an *in vitro* study showed that ANG-2, PDGF, and HB-EGF were higher than the VEFG and FGF-2 levels, suggesting potential physiological roles in

dental pulp (22). It is important to note that neuropeptides such as NPY, vasoactive intestinal peptide, CGRP, and SP can regulate the release of all angiogenic factors from DPF (22).

### 3.3 Complement system and regeneration

The complement system is widely known for its role in immune surveillance and inflammation (90); however, it also participates in pulp regeneration (6,148). As mentioned earlier, despite being non-immune and non-hepatic cells, DPFs can efficiently produce and activate their own complement components (6). C5a, secreted by DPFs, binds to progenitor pulp cells, which express C5aR(6). This interaction induces the migration of these cells to the site of injury in a gradient-dependent manner (91), thus allowing the synthesis of reparative dentin, which is an essential step in the regeneration of the dentin-pulp complex (91,94). In contrast, DPFs express the C3aR receptor; the interaction between C3a and C3aR is involved in the proliferation and recruitment of DPFs following the C3a gradient (71). Furthermore, similar to that with C5a, DPSCs are mobilized and proliferate when subjected to a C3a gradient (148). Thus, it is evident that the complement molecules secreted by DPFs orchestrate the processes necessary for pulp regeneration.

### 3.4 DPFs in nerve sprouting, regeneration, and nociception

DPFs express the C5aR receptor for C5a, both *in vivo* and *in vitro* (16), being the major cell type to do so in the inflamed pulp of carious teeth (165). The interaction between C5a and its receptor results in the upregulation of brain-derived neurotrophic factor (BDNF) in DPFs (17), which acts as a negative regulator of NGF, also expressed by this cell (16). The secretion

of these neurotrophins by LTA-stimulated pulp fibroblasts promotes prominent neurite outgrowth toward the site of carious injury (16,17), which is required for pulp integrity and sensory function in dentin-pulp regeneration (18). In contrast, C5L2, another C5aR, is considered a non-functional receptor (166) that is co-expressed with C5aR under LTA stimulation (17,165); however, it represses BDNF secretion by human DPFs (165). The silencing of C5L2 dramatically increases the number of neurites at the injured site (167).

Transient receptor potential channels (TRP) are sensors for environmental stimuli and transduce various external stimuli into electrical signals that are ultimately perceived as pain (19). In dental pulp, odontoblasts express certain TRP channels that act as mechanoreceptors (168). Similar to what occurs in odontoblasts, DPFs express TRPs such as TRPA1 (19), TRPV1 (169), transient receptor potential melastatin 8 (TRPM8) (19), and TRPM2 (170). TRPA1 is activated by cold (19) and chemical irritants (171–173), TRPV1 by capsaicin, noxious heat, and acid conditions (169), TRPM8 by cold temperatures (174), and TRPM2 by oxidative stress (175). All these receptors demonstrate the ability of DPFs as sensing cells for noxious stimuli in the dental pulp (19). In contrast, the communication between odontoblasts and neurons could be an ephaptic transmission, that is, ion fields generated by odontoblasts may alter the excitability of nearby neurons (176). DPFs could also perform this transmission if we take into account that TRP channels respond to stimuli by the activation of calcium fluxes (177). In non-neural tissues, these channels act in concert with neurons to mediate pain and inflammation (178).

Further, TRP channels potentially participate in pulp inflammation. The binding of capsaicin to TRPV1 induces the production of IL-6 in DPFs, which contributes to pulp inflammation (169). Similarly, TRPA1 and TRPM8 mediate neurogenic inflammation by releasing neuropeptides and inflammatory cytokines in the airway cells (172,179). In the case of the dental pulp, given that DPFs can recognize and synthesize neuropeptides (13,14) and express TRP, it is reasonable to speculate that the activation of TRPA1 in these cells could modulate neurogenic pulp inflammation. However, further studies are required to elucidate this process (19).

**3.5 Myofibroblast in pulp regeneration**

As mentioned previously, myofibroblasts are specialized contractile fibroblasts. They originate from various precursors, including resident fibroblasts, pericytes, vascular smooth muscle cells, epithelial to mesenchymal transition cells, endothelial to mesenchymal transition cells (180), and fibrocytes (181). In the dental pulp, myofibroblasts can originate from MSCs (182) or perivascular mesenchymal stem cells expressing Gli1 (15). Myofibroblasts are of two types: (i) proto-myofibroblasts, which are cellular intermediates between fibroblasts and myofibroblasts and contain actin microfilament bundles (stress fibers) but do not possess α-SMA-positive microfilament bundles; and (ii) myofibroblasts, which contain both bundles (183). The presence of α-SMA confers this cell with a high contractile capacity (184) and, therefore, is a defining property that helps distinguish proto-myofibroblasts from myofibroblasts and "normal" fibroblasts (183).

Myofibroblast activation is based on a positive feedback control, in which stress levels or mechanical tension are key stimuli for differentiation into proto-myofibroblasts (185). In addition, the degradation of fibrillin-1 is necessary for myofibroblast differentiation in the dental pulp wound healing (186). Fibrillin-1 is a structural component of extracellular microfibrils that contributes to the maintenance of connective tissue architecture (187) but disappears during the healing of dental pulp wounds (186), which allows the release and activation of TGF-ß1 (188), the most important cytokine in the transdifferentiation of fibroblasts into α-SMA-positive myofibroblasts (80,189).

Myofibroblasts are key players in physiological and pathological tissue remodeling. They generate tension during normal wound healing due to intracytoplasmic stress fibers, deposit and remodel the ECM (183), and secrete proangiogenic factors (190). In the dental pulp, myofibroblasts have been temporarily characterized in rat molars after pulpotomy with MTA, migrating to sites of injury in response to released chemokines (15). Pulp myofibroblasts were detected at the wound site on day 5 and disappeared on day 14 after the dentin bridge formation was complete. Therefore, myofibroblasts may facilitate the reorganization of the ECM in injured pulp (15).

In addition, some myofibroblasts could be the source of newly differentiated odontoblast-like cells with the capacity to synthesize reparative dentin (15,191). Therefore, these cells participate in dental pulp wound healing. After its regenerative action, myofibroblasts disappear mainly through apoptosis (192) or may even undergo dedifferentiation or senescence (193). However, when these mechanisms are delayed, myofibroblast activity

becomes excessive and persistent, causing fibrosis (194). The latter is not precise in dental pulp, rather it involves pulp aging with a decrease in cell density and accumulation of fibrous tissue from the connective tissue sheaths of blood vessels and nerves (78,195). In these scenarios, fibroblasts do not show signs of high metabolic activity (196).

## 4. DPFs as therapeutic agents

Cell cultures have been extensively used to evaluate dental materials (197–199). Pulp cells, especially human (200–202) and animal (203,204) fibroblasts, are the models of choice for biocompatibility testing of dental materials, the cytotoxic effects of which directly affect the dental pulp (205). Furthermore, DPFs are highly sensitive to toxic substances and are therefore ideal to elucidate the possible adverse effects of restorative (206–208), endodontic (209–211), and novel therapeutic materials (212–215). It must be borne in mind that for cell cultures to be considered an acceptable model, it is necessary to demonstrate that the response of cells to the tested materials can be reproduced, that pulp cell cultures can be easily established, and that cell lines can be standardized (205).

Fibroblasts are difficult to cultivate (205) and show great variation in proliferative activity, which the source, age of the donor, or the number of passages cannot explain (73). They also have a low long-term survival rate (216), which may be related to the age of the patient (73). These drawbacks can influence the reproducibility of the results among researchers, despite the use of identical culture techniques (73,217). Therefore, the data obtained from *in vitro* studies must be interpreted with caution. However, these cells remain

representative of the dental pulp cell cultures (202,218), and therefore, the need for greater uniformity in the establishment of these cells and their use in experiments is evident (73).

Further, DPFs can recognize warning signs and initiate inflammatory responses (122). Inflammation can be controlled at the point of initiation and resolution by regulating fibroblasts (160). Therefore, these cells are potentially important targets for future anti-inflammatory therapies in pulp inflammation (219) and regeneration of the dentin-pulp complex (4).

**Conclusions and future perspectives**

Overall, considering the pivotal role of DPFs in health and disease, as well as their potential therapeutic application in regenerative endodontics, it is clear that this cell type is not a mere bystander in the pulp-dentin complex. In the near future, molecular programs (137,220), proteomic profiling (221), and artificial intelligence (222), owing to their unique characteristics and performance, could help confirm known findings and unveil novel functions of DPFs, further establishing their status as star cells of the pulp tissue. These approaches could also be an important milestone in developing fibroblast-based therapies.

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The authors declare no conflicts of interest.

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**Author Contribution**

José Luis Álvarez-Vásquez: Conceptualization, Methodology, Literature search, Writing - original draft preparation, Writing-review and editing, Figure editing, Supervision, Project administration.

Cristina Paola Castañeda-Alvarado: Literature search, Writing–original draft preparation, Writing-review and editing, Figure design.

Both authors have approved the submitted manuscript.

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FIGURE LEGEND

**A SCHEMATIC REPRESENTATION OF THE LEADING ROLES OF DENTAL PULP FIBROBLASTS (DPF) IN PULP PATHOPHYSIOLOGY.**

**Figure 1**. DPF participate in extracellular matrix (ECM) remodeling through the synthesis of collagen and fibronectin and its degradation by matrix metalloproteinases (MMP). In response to bacterial stimulation, DPFs express all complement proteins, including the membrane attack complex (MAC), allowing the lysis of cariogenic bacteria. Furthermore, these cells have the ability to secrete and respond to various cytokines. DPFs also express the NLRP3/caspase-1 inflammasome, generating the release of IL-β, the most potent proinflammatory cytokine. They express toll-like (TLR) and nucleotide-binding oligomerization domain (NOD) receptors, and thus recognize pathogen-associated molecular patterns (PAMPs) and regulates the expression of various proinflammatory mediators, through the recognition of PAMPs like lipoteichoic acid (LTA) and lipopolysaccharides (LPS). In response to inflammatory mediators, DPFs secrete Substance P (SP) and receptors for neuropeptides such as neurokinin-1 receptor (NK-1) and NPY Y1 receptor (NPY Y1), thus participating in neurogenic inflammation and dental pulp wound healing and amplifying the pulp immune response. DPFs contribute to dental nociception, by secretion of neural growth factor (NGF) that sensitizes afferent nerve fibers and expresses transient receptor potential channels (TRP) that are sensors of external environment. TRP channels could allow DPFs to perform ephaptic transmission, although this is yet to be elucidated. DPFs interact with macrophages and modulate their

differentiation into M1 (proinflammatory) macrophages to control infection and M2 (anti-inflammatory) to start pulp healing, demonstrating the active participation of DPFs in local immune response and inflammation. DPFs also actively participate in dentin-pulp regeneration through the secretion of growth factors such as transforming growth factor beta-1 (TGF-ß1), basic fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF), complement proteins (C3a and C5a) and NGF, allowing dental pulp stem migration and odontoblast-like differentiation. They also secrete angiogenic growth factors for pulp angiogenesis. DPFs secrete the brain-derived neurotrophic factor (BDNF) when C5a binds to their C5aR receptor, decreasing the expression of NGF, which increases in inflamed pulp tissue; BDNF decreases when C5a binds to the C5L2 receptor. Both, NGF and BDNF are essential for neuronal plasticity. Finally, DPFs participate in dental pulp wound healing and dentin-pulp regeneration by transdifferentiating into myofibroblasts; these latter cells facilitate the reorganization of the ECM in injured pulp and can differentiate into odontoblast-like cells, with the capacity to synthesize reparative dentin. The figure was created with BioRender.