

UCUENCA

Facultad de Odontología

Especialización en Endodoncia

“Dental Pulp Fibroblast: A star cell.”

Trabajo de titulación previo a la
obtención del título de **Especialista
en Endodoncia**

Modalidad: Artículo Científico

Autora:

Cristina Paola Castañeda Alvarado

CI: 0106512056

Correo electrónico: cris.castaneda@hotmail.com

Director:

Dr. José Luis Álvarez Vásquez

CI: 0103374120

Cuenca, Ecuador

19-septiembre-2022

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

Results

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.

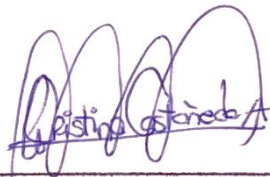
Keywords: Dental pulp. Fibroblasts. Regenerative endodontics. Dental pulp disease. Complement system proteins

Cláusula de licencia y autorización para publicación en el Repositorio Institucional

Yo Cristina Paola Castañeda Alvarado en calidad de autor/a y titular de los derechos morales y patrimoniales del trabajo de titulación "Dental Pulp Fibroblast: A star cell", de conformidad con el Art. 114 del CÓDIGO ORGÁNICO DE LA ECONOMÍA SOCIAL DE LOS CONOCIMIENTOS, CREATIVIDAD E INNOVACIÓN reconozco a favor de la Universidad de Cuenca una licencia gratuita, intransferible y no exclusiva para el uso no comercial de la obra, con fines estrictamente académicos.

Asimismo, autorizo a la Universidad de Cuenca para que realice la publicación de este trabajo de titulación en el repositorio institucional, de conformidad a lo dispuesto en el Art. 144 de la Ley Orgánica de Educación Superior.

Cuenca, 19 de septiembre del 2022.



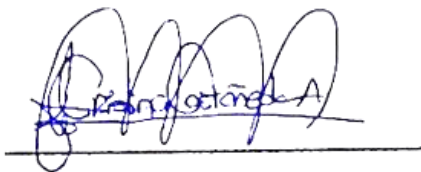
Nombres completos: Cristina Paola Castañeda Alvarado

C.I: 0106512056

Cláusula de Propiedad Intelectual

Cristina Paola Castañeda Alvarado, autora del artículo académico (trabajo de titulación) "Dental Pulp Fibroblast: A star cell", certifico que todas las ideas, opiniones y contenidos expuestos en la presente investigación son de exclusiva responsabilidad de su autor/a.

Cuenca, 19 de septiembre del 2022.



Cristina Paola Castañeda Alvarado

C.I: 0106512056

1. 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 3 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.

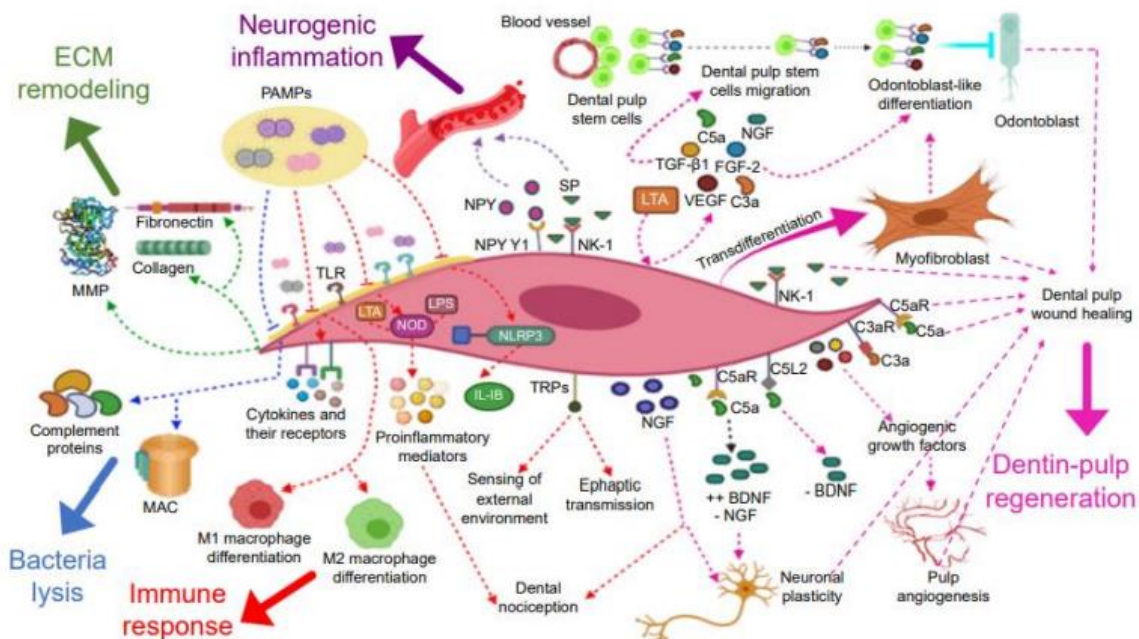


Figure 1 A schematic representation of the leading roles of dental pulp fibroblasts (DPF) in pulp pathophysiology. Dental pulp fibroblasts (DPFs) participate in

extracellular matrix (ECM) remodeling through the synthesis of collagen and fibronectin and its degradation by matrix metalloproteinases (MMPs). 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 interleukin (IL)- β , the most potent proinflammatory cytokine. They express tolllike (TLR) and nucleotide-binding oligomerization domain (NOD) receptors, and thus recognize pathogen-associated molecular patterns (PAMPs) and regulate the expression of various proinflammatory mediators, through the recognition of PAMPs like lipoteichoic acid 4 (LTA) and lipopolysaccharides (LPS). In response to inflammatory mediators, DPFs secrete Substance P (SP) and express 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 express 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 (antiinflammatory) 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- β 1), 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 odontoblastlike cells, with the capacity to synthesize reparative dentin. The figure was created with BioRender.

Notwithstanding the abovementioned investigations that describe many physiological and pathological functions of DPFs, to our knowledge, the present 5 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.

2. 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 remains 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).

3. 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).

3.1. 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).

3.2. 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, 10 CXCL12 (10,11), interleukin (IL)-6 (9,83,102), IL-1 β (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 metalloproteinase1 (116), colony-stimulating factor (110), and cyclooxygenase 2 (COX-2) (100,117), which amplify the immune response (106,109).

IL-1 β 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-1 β , which is crucial for the immunological defense of the dentin-pulp complex (123,124).

3.3. 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 11 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).

3.4. 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).

3.5. 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 antiinflammatory 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.

4. 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.

4.1. Stem cell recruitment and differentiation

Odontoblasts are considered postmitotic cells (143) with no potential for selfrenewal. 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 β 1) (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- β 1 (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- β 1 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 antiinflammatory 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 15 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).

4.2. 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- β 1 (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 VEGF and FGF-2 (20). However, an in vitro study showed that ANG-2, PDGF, and HB-EGF were higher than the VEGF and FGF-2 levels, suggesting potential physiological roles in dental pulp (22). It is important to note that 16 neuropeptides such as NPY, vasoactive intestinal peptide, CGRP, and SP can regulate the release of all angiogenic factors from DPF (22).

4.3. 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.

4.4. 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-17 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 nonfunctional 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).

4.5. 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 protomyofibroblasts (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- β 1 (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 20 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).

5. 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).

The chemical adhesion of epoxy resins to the tooth structure is produced by covalent bonds between the open epoxy groups and the exposed amino groups in the collagen network of the dentin. This is one of the reasons for the good dislodgment 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 21 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).

6. 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.

Acknowledgements: Figures were created with BioRender.

Declaration of Interest: The authors declare no conflicts of interest. Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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

References

1. Goldberg M, Smith AJ. Cells and extracellular matrices of dentin and pulp: A biological basis for repair and tissue engineering. *Crit Rev Oral Biol Med.* 2004;15(1):13-27. doi:10.1177/154411130401500103
2. Yu C, Abbott P. An overview of the dental pulp: its functions and responses to injury. *Aust Dent J.* 2007;52(1 Suppl):S4-S6. doi:10.1111/j.1834-7819.2007.tb00525.x
3. Sloan A, Smith A. Stem cells and the dental pulp: potential roles in dentine

- regeneration and repair. *Oral Dis.* 2007;13(2):151-157. doi:10.1111/j.1601-0825.2006.01346.x
4. Jeanneau C, Lundy FT, El Karim IA, About I. Potential Therapeutic Strategy of Targeting Pulp Fibroblasts in Dentin-Pulp Regeneration. *J Endod.* 2017;43(9):S17-S24. doi:10.1016/j.joen.2017.06.007
 5. Martinez EF, Araujo VC. In vitro immunoexpression of extracellular matrix proteins in dental pulpal and gingival human fibroblasts. *Int Endod J.* 2004;37(11):749-755. doi:10.1111/j.1365-2591.2004.00864.x
 6. Chmilewsky F, Jeanneau C, Laurent P, et al. Pulp fibroblasts synthesize functional complement proteins involved in initiating dentin-pulp regeneration. *Am J Pathol.* 2014;184(7):1991-2000. doi:10.1016/j.ajpath.2014.04.003
 7. Bergmann M, Jeanneau C, Giraud T, et al. Complement activation links inflammation to dental tissue regeneration. *Clin Oral Investig.* 2020;24(12):4185-4196. doi:10.1007/S00784-020-03621-W
 8. Le Fournis C, Jeanneau C, Roumani S, et al. Pulp Fibroblast Contribution to the Local Control of Pulp Inflammation via Complement Activation. *J Endod.* 2020;46(9):S26-S32. doi:10.1016/j.joen.2020.06.029
 9. Matsushima K, Ohbayashi E, Takeuchi H, et al. Stimulation of interleukin-6 production in human dental pulp cells by peptidoglycans from *Lactobacillus casei*. *J Endod.* 1998;24(4):252-255. doi:10.1016/S0099-2399(98)80107-6
 10. Sipert CR, Morandini AC, Dionísio TJ, et al. In Vitro Regulation of CCL3 and CXCL12 by Bacterial By-products Is Dependent on Site of Origin of Human Oral Fibroblasts. *J Endod.* 2014;40(1):95-100. doi:10.1016/j.joen.2013.09.031
 11. Sipert CR, Morandini AC de F, Modena KC da S, et al. CCL3 and CXCL12 production in vitro by dental pulp fibroblasts from permanent and deciduous teeth stimulated by *Porphyromonas gingivalis* LPS. *J Appl Oral Sci.* 2013;21(2):99-105. doi:10.1590/1678-7757201300004
 12. Hirao K, Yumoto H, Takahashi K, et al. Roles of TLR2, TLR4, NOD2, and

- NOD1 in Pulp Fibroblasts. *J Dent Res.* 2009;88(8):762-767. doi:10.1177/0022034509341779
13. Killough SA, Lundy FT, Irwin CR. Dental pulp fibroblasts express neuropeptide Y Y1 receptor but not neuropeptide Y. *Int Endod J.* 2010;43(10):835-842. doi:10.1111/j.1365-2591.2010.01741.x
 14. Killough SA, Lundy FT, Irwin CR. Substance P Expression by Human Dental Pulp Fibroblasts: A Potential Role in Neurogenic Inflammation. *J Endod.* 2009;35(1):73-77. doi:10.1016/j.joen.2008.10.010
 15. Edanami N, Yoshida N, Ohkura N, et al. Characterization of Dental Pulp Myofibroblasts in Rat Molars after Pulpotomy. *J Endod.* 2017;43(7):1116-1121. doi:10.1016/j.joen.2017.02.018
 16. Chmilewsky F, Ayaz W, Appiah J, et al. Nerve Growth Factor Secretion From Pulp Fibroblasts is Modulated by Complement C5a Receptor and Implied in Neurite Outgrowth. *Sci Rep.* 2016;6:31799. doi:10.1038/srep31799
 17. Chmilewsky F, About I, Chung SH. Pulp Fibroblasts Control Nerve Regeneration through Complement Activation. *J Dent Res.* 2016;95(8):913-922. doi:10.1177/0022034516643065
 18. Byers MR, Suzuki H, Maeda T. Dental neuroplasticity, neuro-pulpal interactions, and nerve regeneration. *Microsc Res Tech.* 2003;60(5):503-515. doi:10.1002/jemt.10291
 19. El Karim IA, Linden GJ, Curtis TM, et al. Human dental pulp fibroblasts express the "cold-sensing" transient receptor potential channels TRPA1 and TRPM8. *J Endod.* 2011;37(4):473-478. doi:10.1016/j.joen.2010.12.017
 20. Tran-Hung L, Mathieu S, About I. Role of Human Pulp Fibroblasts in Angiogenesis. *J Dent Res.* 2006;85(9):819-823. doi:10.1177/154405910608500908
 21. Roberts-Clark D., Smith A. Angiogenic growth factors in human dentine matrix. *Arch Oral Biol.* 2000;45(11):1013-1016. doi:10.1016/S0003-9969(00)00075-3

22. El Karim IA, Linden GJ, Irwin CR, et al. Neuropeptides regulate expression of angiogenic growth factors in human dental pulp fibroblasts. *J Endod.* 2009;35(6):829-833. doi:10.1016/j.joen.2009.03.005
23. Tran-Hung L, Laurent P, Camps J, et al. Quantification of angiogenic growth factors released by human dental cells after injury. *Arch Oral Biol.* 2008;53(1):9-13. doi:10.1016/j.archoralbio.2007.07.001
24. Asahara T, Bauters C, Zheng LP, et al. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. *Circulation.* 1995;92(9):365-371. doi:10.1161/01.CIR.92.9.365
25. Phan SH. Biology of Fibroblasts and Myofibroblasts. *Proc Am Thorac Soc.* 2008;5(3):334-337. doi:10.1513/pats.200708-146DR
26. Wisithphrom K, Murray PE, Windsor LJ. Interleukin-1 alpha alters the expression of matrix metalloproteinases and collagen degradation by pulp fibroblasts. *J Endod.* 2006;32(3):186-192. doi:10.1016/j.joen.2005.10.055
27. Staquet MJ, Durand SH, Colomb E, et al. Different Roles of Odontoblasts and Fibroblasts in Immunity. *J Dent Res.* 2008;87(3):256-261. doi:10.1177/154405910808700304
28. Kikuchi N, Kitamura C, Morotomi T, et al. Formation of dentin-like particles in dentin defects above exposed pulp by controlled release of fibroblast growth factor 2 from gelatin hydrogels. *J Endod.* 2007;33(10):1198-1202. doi:10.1016/j.joen.2007.07.025
29. Piattelli A, Rubini C, Fioroni M, et al. Transforming Growth Factor-beta 1 (TGF-beta 1) expression in normal healthy pulps and in those with irreversible pulpitis. *Int Endod J.* 2004;37(2):114-119. doi:10.1111/j.0143-2885.2004.00758.x
30. Shamis Y, Hewitt KJ, Carlson MW, et al. Fibroblasts derived from human embryonic stem cells direct development and repair of 3D human skin equivalents. *Stem Cell Res Ther.* 2011;2(1):10. doi:10.1186/scrt51

31. LeBleu VS, Neilson EG. Origin and functional heterogeneity of fibroblasts. *FASEB J.* 2020;34(3):3519-3536. doi:10.1096/fj.201903188R
32. Lynch MD, Watt FM. Fibroblast heterogeneity: implications for human disease. *J Clin Invest.* 2018;128(1):26-35. doi:10.1172/JCI93555
33. Kis K, Liu X, Hagood JS. Myofibroblast differentiation and survival in fibrotic disease. *Expert Rev Mol Med.* 2011;13:e27. doi:10.1017/S1462399411001967
34. Sorrell JM, Caplan AI. Fibroblasts-a diverse population at the center of it all. *Int Rev Cell Mol Biol.* 2009;276:161-214. doi:10.1016/S1937-6448(09)76004-6
35. Soundararajan M, Kannan S. Fibroblasts and mesenchymal stem cells: Two sides of the same coin?. *J Cell Physiol.* 2018;233(12):9099-9109. doi:10.1002/jcp.26860
36. Denu RA, Nemcek S, Bloom DD, et al. Fibroblasts and Mesenchymal Stromal/Stem Cells Are Phenotypically Indistinguishable. *Acta Haematol.* 2016;136(2):85-97. doi:10.1159/000445096
37. Alt E, Yan Y, Gehmert S, et al. Fibroblasts share mesenchymal phenotypes with stem cells, but lack their differentiation and colony-forming potential. *Biol Cell.* 2011;103(4):197-208. doi:10.1042/BC20100117
38. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8(4):315-317. doi:10.1080/14653240600855905
39. Blasi A, Martino C, Balducci L, et al. Dermal fibroblasts display similar phenotypic and differentiation capacity to fat-derived mesenchymal stem cells, but differ in anti-inflammatory and angiogenic potential. *Vasc Cell.* 2011;3(1):5. doi:10.1186/2045-824X-3-5
40. Flavell SJ, Hou TZ, Lax S, et al. Fibroblasts as novel therapeutic targets in

- chronic inflammation. *Br J Pharmacol.* 2008;153(S1):S241-S246. doi:10.1038/sj.bjp.0707487
41. Haniffa MA, Wang XN, Holtick U, et al. Adult Human Fibroblasts Are Potent Immunoregulatory Cells and Functionally Equivalent to Mesenchymal Stem Cells. *J Immunol.* 2007;179(3):1595-1604. doi:10.4049/jimmunol.179.3.1595
 42. Lysy PA, Smets F, Sibille C, et al. Human skin fibroblasts: From mesodermal to hepatocyte-like differentiation. *Hepatology.* 2007;46(5):1574-1585. doi:10.1002/hep.21839
 43. Kuznetsov SA, Krebsbach PH, Satomura K, et al. Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. *J Bone Miner Res.* 1997;12(9):1335-1347. doi:10.1359/jbmr.1997.12.9.1335
 44. Hanson SE, Kim J, Johnson BHQ, et al. Characterization of mesenchymal stem cells from human vocal fold fibroblasts. *Laryngoscope.* 2010;120(3):546-551. doi:10.1002/lary.20797
 45. Kim SW, Cho JH, Hong MW, et al. Induction of chondrogenic differentiation in cultured fibroblasts isolated from the inferior turbinate. *Otolaryngol Neck Surg.* 2008;139(1):143-148. doi:10.1016/j.otohns.2008.04.015
 46. Chen FGFF, Zhang WJ, Bi D, et al. Clonal analysis of nestin- vimentin+ multipotent fibroblasts isolated from human dermis. *J Cell Sci.* 2007;120(16):2875-2883. doi:10.1242/jcs.03478
 47. Strakova Z, Livak M, Krezalek M, et al. Multipotent properties of myofibroblast cells derived from human placenta. *Cell Tissue Res.* 2008;332(3):479-488. doi:10.1007/s00441-008-0604-x
 48. Sudo K, Kanno M, Miharada K, et al. Mesenchymal Progenitors Able to Differentiate into Osteogenic, Chondrogenic, and/or Adipogenic Cells In Vitro Are Present in Most Primary Fibroblast-Like Cell Populations. *Stem Cells.* 2007;25(7):1610-1617. doi:10.1634/stemcells.2006-0504

49. Brohem CA, De Carvalho CM, Radoski CL, et al. Comparison between fibroblasts and mesenchymal stem cells derived from dermal and adipose tissue. *Int J Cosmet Sci.* 2013;35(5):448-457. doi:10.1111/ics.12064
50. Bi D, Chen FG, Zhang WJ, et al. Differentiation of human multipotent dermal fibroblasts into islet-like cell clusters. *BMC Cell Biol.* 2010;11(1):46. doi:10.1186/1471-2121-11-46
51. Sabatini F, Petecchia L, Taviani M, et al. Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities. *Lab Invest.* 2005;85(8):962-971. doi:10.1038/labinvest.3700300
52. Cappellesso-Fleury S, Puissant-Lubrano B, Apoil PA, et al. Human Fibroblasts Share Immunosuppressive Properties with Bone Marrow Mesenchymal Stem Cells. *J Clin Immunol.* 2010;30(4):607-619. doi:10.1007/s10875-010-9415-4
53. Brendel C, Kuklick L, Hartmann O, et al. Distinct gene expression profile of human mesenchymal stem cells in comparison to skin fibroblasts employing cDNA microarray analysis of 9600 genes. *Gene Expr.* 2004;12(4-6):245-257. doi:10.3727/000000005783992043
54. Choudhery MS, Badowski M, Muise A, et al. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. *J Transl Med.* 2014;12(1):8. doi:10.1186/1479-5876-12-8
55. Laschober GT, Brunauer R, Jamnig A, et al. Age-Specific Changes of Mesenchymal Stem Cells Are Paralleled by Upregulation of CD106 Expression As a Response to an Inflammatory Environment. *Rejuvenation Res.* 2011;14(2):119-131. doi:10.1089/rej.2010.1077
56. Chang HY, Chi JT, Dudoit S, et al. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc Natl Acad Sci.* 2002;99(20):12877-12882. doi:10.1073/pnas.162488599
57. Halfon S, Abramov N, Grinblat B, et al. Markers distinguishing mesenchymal

stem cells from fibroblasts are downregulated with passaging. *Stem Cells Dev.* 2011;20(1):53-66. doi:10.1089/scd.2010.0040

58. Lindroos B, Mäenpää K, Ylikomi T, et al. Characterisation of human dental stem cells and buccal mucosa fibroblasts. *Biochem Biophys Res Commun.* 2008;368(2):329-335. doi:10.1016/j.bbrc.2008.01.081
59. Mafi P. Adult Mesenchymal Stem Cells and Cell Surface Characterization - A Systematic Review of the Literature. *Open Orthop J.* 2011;5(1):253-260. doi:10.2174/1874325001105010253
60. Shafiei F, Tavangar M, Razmkhah M, et al. Cytotoxic effect of silorane and methacrylate based composites on the human dental pulp stem cells and fibroblasts. *Med Oral Patol Oral y Cir Bucal.* 2014;19(4):e350-e358. doi:10.4317/medoral.19340
61. Tavangar MS, Attar A, Razmkhah M, et al. Differential expression of drug resistance genes in CD146 positive dental pulp derived stem cells and CD146 negative fibroblasts. *Clin Exp Dent Res.* 2020;6(4):448-456. doi:10.1002/cre2.297
62. Diar-Bakirly S, El-Bialy T. Human gingival fibroblasts: Isolation, characterization, and evaluation of CD146 expression. *Saudi J Biol Sci.* 2021;28(4):2518-2526. doi:10.1016/j.sjbs.2021.01.053
63. Al Bahrawy M. Comparison of the Migration Potential through Microperforated Membranes of CD146+ GMSC Population versus Heterogeneous GMSC Population. *Stem Cells Int.* 2021;2021:1-14. doi:10.1155/2021/5583421
64. Kundrotas G. Surface markers distinguishing mesenchymal stem cells from fibroblasts. *Acta medica Litu.* 2012;19(2):75-79. doi:10.6001/actamedica.v19i2.2313
65. Gang EJ, Bosnakovski D, Figueiredo CA, et al. SSEA-4 identifies mesenchymal stem cells from bone marrow. *Blood.* 2007;109(4):1743-1751. doi:10.1182/blood-2005-11-010504

66. Jones E, Yang X. Mesenchymal stem cells and bone regeneration: current status. *Injury*. 2011;42(6):562-568. doi:10.1016/j.injury.2011.03.030
67. Xie L, Moroi Y, Takahara M, et al. CD10 expressed by fibroblasts and melanoma cells degrades endothelin-1 secreted by human keratinocytes. *Eur J Dermatology*. 2011;21(4):505-509. doi:10.1684/ejd.2011.1371
68. Worthen CA, Cui Y, Orringer JS, et al. CD26 Identifies a Subpopulation of Fibroblasts that Produce the Majority of Collagen during Wound Healing in Human Skin. *J Invest Dermatol*. 2020;140(12):2515-2524.e3. doi:10.1016/j.jid.2020.04.010
69. Le Fournis C, Jeanneau C, Giraud T, et al. Fibroblasts Control Macrophage Differentiation during Pulp Inflammation. *J Endod*. 2021;47(9):1427-1434. doi:10.1016/j.joen.2021.06.015
70. Jeanneau C, Rufas P, Rombouts C, et al. Can Pulp Fibroblasts Kill Cariogenic Bacteria? Role of Complement Activation. *J Dent Res*. 2015;94(12):1765-1772. doi:10.1177/0022034515611074
71. Rufas P, Jeanneau C, Rombouts C, et al. Complement C3a Mobilizes Dental Pulp Stem Cells and Specifically Guides Pulp Fibroblast Recruitment. *J Endod*. 2016;42(9):1377-1384. doi:10.1016/j.joen.2016.06.011
72. Le Fournis C, Hadjichristou C, Jeanneau C, et al. Human Pulp Fibroblast Implication in Phagocytosis via Complement Activation. *J Endod*. 2019;45(5):584-590. doi:10.1016/j.joen.2018.10.023
73. Moule AJ, Li H, Bartold PM. Donor variability in the proliferation of human dental pulp fibroblasts. *Aust Dent J*. 1995;40(2):110-114. doi:10.1111/j.1834-7819.1995.tb03125.x
74. Rinkevich Y, Walmsley GG, Hu MS, et al. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. *Science*. 2015;348(6232):aaa2151. doi:10.1126/science.aaa2151
75. Driskell RR, Lichtenberger BM, Hoste E, et al. Distinct fibroblast lineages

determine dermal architecture in skin development and repair. *Nature*. 2013;504(7479):277-281. doi:10.1038/nature12783

76. Bacher R, Kendzierski C. Design and computational analysis of single-cell RNA-sequencing experiments. *Genome Biol*. 2016;17:63. doi:10.1186/s13059-016-0927-y
77. Wen L, Tang F. Single-cell sequencing in stem cell biology. *Genome Biol*. 2016;17:71. doi:10.1186/s13059-016-0941-0
78. Murray PE, Stanley HR, Matthews JB, et al. Age-related odontometric changes of human teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;93(4):474-482. doi:10.1067/moe.2002.120974
79. Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia*. 1971;27(5):549-550. doi:10.1007/BF02147594
80. Martinez EF, Araújo VC, Sousa SO, et al. TGF- β 1 Enhances the Expression of α -Smooth Muscle Actin in Cultured Human Pulpal Fibroblasts: Immunochemical and Ultrastructural Analyses. *J Endod*. 2007;33(11):1313-1318. doi:10.1016/j.joen.2007.07.040
81. Bautista-Hernández LA, Gómez-Olivares JL, Buentello-Volante B, et al. Fibroblasts: the unknown sentinels eliciting immune responses against microorganisms. *Eur J Microbiol Immunol*. 2017;7(3):151-157. doi:10.1556/1886.2017.00009
82. Smith RS, Smith TJ, Blieden TM, et al. Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. *Am J Pathol*. 1997;151(2):317-322.
83. Tsai CL, Hung SL, Lee YY, et al. The role of fibroblasts in the modulation of dental pulp inflammation. *J Formos Med Assoc*. 2022;121(1 Pt 2):342-349. doi:10.1016/j.jfma.2021.05.007
84. Goldberg M, Farges J, Lacerda-Pinheiro S, et al. Inflammatory and

- immunological aspects of dental pulp repair. *Pharmacol Res.* 2008;58(2):137-147. doi:10.1016/j.phrs.2008.05.013
85. Castañeda-Sánchez J, García-Pérez B, Muñoz-Duarte A, et al. Defensin Production by Human Limbo-Corneal Fibroblasts Infected with Mycobacteria. *Pathogens.* 2013;2(1):13-32. doi:10.3390/pathogens2010013
86. Rizzo A, Paolillo R, Buommino E, et al. Modulation of cytokine and β -defensin 2 expressions in human gingival fibroblasts infected with *Chlamydia pneumoniae*. *Int Immunopharmacol.* 2008;8(9):1239-1247. doi:10.1016/j.intimp.2008.04.015
87. Rodriguez-Martinez S, Cancino-Diaz ME, Cancino-Diaz JC. Expression of CRAMP via PGN-TLR-2 and of β -defensin-3 via CpG-ODN-TLR-9 in corneal fibroblasts. *Br J Ophthalmol.* 2006;90(3):378-382. doi:10.1136/bjo.2005.082289
88. Dommisch H, Winter J, Willebrand C, et al. Immune regulatory functions of human beta-defensin-2 in odontoblast-like cells. *Int Endod J.* 2007;40(4):300-307. doi:10.1111/J.0143-2885.2007.01228.X
89. Lundy FT, Irwin CR, McLean DF, et al. Natural Antimicrobials in the Dental Pulp. *J Endod.* 2020;46(9):S2-S9. doi:10.1016/j.joen.2020.06.021
90. Chmilewsky F, Jeanneau C, Dejou J, et al. Sources of dentin-pulp regeneration signals and their modulation by the local microenvironment. *J Endod.* 2014;40(4):S19-S25. doi:10.1016/j.joen.2014.01.012
91. Chmilewsky F, Jeanneau C, Laurent P, et al. Pulp progenitor cell recruitment is selectively guided by a C5a gradient. *J Dent Res.* 2013;92(6):532-539. doi:10.1177/0022034513487377
92. Morgan BP, Gasque P. Extrahepatic complement biosynthesis: where, when and why?. *Clin Exp Immunol.* 2003;107(1):1-7. doi:10.1046/j.1365-2249.1997.d01-890.x
93. Chakraborty S, Karasu E, Huber-Lang M. Complement After Trauma: Suturing

- Innate and Adaptive Immunity. *Front Immunol.* 2018;9(SEP):2050. doi:10.3389/fimmu.2018.02050
94. Chmilewsky F, Jeanneau C, Laurent P, et al. LPS induces pulp progenitor cell recruitment via complement activation. *J Dent Res.* 2015;94(1):166-174. doi:10.1177/0022034514555524
95. Andersson J, Ekdahl KN, Larsson R, et al. C3 adsorbed to a polymer surface can form an initiating alternative pathway convertase. *J Immunol.* 2002;168(11):5786-5791. doi:10.4049/jimmunol.168.11.5786
96. Berends ETM, Dekkers JF, Nijland R, et al. Distinct localization of the complement C5b-9 complex on Gram-positive bacteria. *Cell Microbiol.* 2013;15(12):1955-1968. doi:10.1111/cmi.12170
97. Haas PJ, Van Strijp J. Anaphylatoxins: Their role in bacterial infection and inflammation. *Immunol Res.* 2007;37(3):161-175. doi:10.1007/BF02697367
98. Berends ET, Mohan S, Mielliet WR, et al. Contribution of the complement Membrane Attack Complex to the bactericidal activity of human serum. *Mol Immunol.* 2015;65(2):328-335. doi:10.1016/j.molimm.2015.01.020
99. Tokuda M, Nagaoka S, Torii M. Interleukin-10 receptor expression in human dental pulp cells in response to lipopolysaccharide from *Prevotella intermedia*. *J Endod.* 2003;29(1):48-50. doi:10.1097/00004770-200301000-00013
100. Chang YC, Yang SF, Huang FM, et al. Proinflammatory cytokines induce cyclooxygenase-2 mRNA and protein expression in human pulp cell cultures. *J Endod.* 2003;29(3):201-204. doi:10.1097/00004770-200303000-00009
101. Chang YC, Yang SF, Huang FM, et al. Induction of tissue plasminogen activator gene expression by proinflammatory cytokines in human pulp and gingival fibroblasts. *J Endod.* 2003;29(2):114-117. doi:10.1097/00004770-200302000-00007
102. Coil J, Tam E, Waterfield J. Proinflammatory cytokine profiles in pulp fibroblasts stimulated with lipopolysaccharide and methyl mercaptan. *J Endod.*

2004;30(2):88-91. doi:10.1097/00004770-200402000-00006

103. Barkhordar R, Ghani Q, Russell T, et al. Interleukin-1 β Activity and Collagen Synthesis in Human Dental Pulp Fibroblasts. *J Endod.* 2002;28(3):157-159. doi:10.1097/00004770-200203000-00003
104. Nagaoka S, Tokuda M, Sakuta T, et al. Interleukin-8 gene expression by human dental pulp fibroblast in cultures stimulated with *Prevotella intermedia* lipopolysaccharide. *J Endod.* 1996;22(1):9-12. doi:10.1016/S0099-2399(96)80228-7
105. Silva AC, Faria MR, Fontes A, et al. Interleukin-1 beta and interleukin-8 in healthy and inflamed dental pulps. *J Appl Oral Sci.* 2009;17(5):527-532. doi:10.1590/S1678-77572009000500031
106. Adachi T, Nakanishi T, Yumoto H, et al. Caries-related bacteria and cytokines induce CXCL10 in dental pulp. *J Dent Res.* 2007;86(12):1217-1222. doi:10.1177/154405910708601215
107. Colombini-Ishikiriama BL, Dionisio TJ, Garbieri TF, et al. What is the response profile of deciduous pulp fibroblasts stimulated with *E. coli* LPS and *E. faecalis* LTA?. *BMC Immunol.* 2020;21(1):38. doi:10.1186/s12865-020-00367-8
108. Xiong H, Wei L, Peng B. IL-17 stimulates the production of the inflammatory chemokines IL-6 and IL-8 in human dental pulp fibroblasts. *Int Endod J.* 2015;48(6):505-511. doi:10.1111/iej.12339
109. Wei L, Liu M, Xiong H, et al. Up-regulation of IL-23 expression in human dental pulp fibroblasts by IL-17 via activation of the NF- κ B and MAPK pathways. *Int Endod J.* 2018;51(6):622-631. doi:10.1111/iej.12871
110. Sawa Y, Horie Y, Yamaoka Y, et al. Production of colony-stimulating factor in human dental pulp fibroblasts. *J Dent Res.* 2003;82(2):96-100. doi:10.1177/154405910308200204
111. Yamaguchi M, Kojima T, Kanekawa M, et al. Neuropeptides stimulate production of interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha

- in human dental pulp cells. *Inflamm Res.* 2004;53(5):199-204. doi:10.1007/s00011-003-1243-z
112. Wei L, Chen Y, Zhang C, et al. Leptin induces IL-6 and IL-8 expression through leptin receptor Ob-Rb in human dental pulp fibroblasts. *Acta Odontol Scand.* 2019;77(3):205-212. doi:10.1080/00016357.2018.1536280
113. Ferreira DC, Brito DG, Cavalcanti BN. Cytokine production from human primary teeth pulp fibroblasts stimulated by different pulpotomy agents. *J Dent Child (Chic).* 2009;76(3):194-198.
114. Tamura M, Nagaoka S, Kawagoe M. Interleukin-1 alpha stimulates interstitial collagenase gene expression in human dental pulp fibroblast. *J Endod.* 1996;22(5):240-243. doi:10.1016/S0099-2399(06)80140-8
115. Wisithphrom K, Windsor LJ. The effects of tumor necrosis factor-alpha, interleukin-1beta, interleukin-6, and transforming growth factor-beta1 on pulp fibroblast mediated collagen degradation. *J Endod.* 2006;32(9):853-861. doi:10.1016/j.joen.2006.03.017
116. Lin S, Wang C, Huang S, et al. Induction of dental pulp fibroblast matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 gene expression by interleukin-1alpha and tumor necrosis factor-alpha through a prostaglandin-dependent pathway. *J Endod.* 2001;27(3):185-189. doi:10.1097/00004770-200103000-00012
117. Nakanishi T, Shimizu H, Hosokawa Y, et al. An immunohistological study on cyclooxygenase-2 in human dental pulp. *J Endod.* 2001;27(6):385-388. doi:10.1097/00004770-200106000-00003
118. Chen M, Wang H, Chen W, et al. Regulation of adaptive immunity by the NLRP3 inflammasome. *Int Immunopharmacol.* 2011;11(5):549-554. doi:10.1016/j.intimp.2010.11.025
119. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol.* 2009;27(1):229-265.

doi:10.1146/annurev.immunol.021908.132715

120. Song Z, Lin Z, He F, et al. NLRP3 Is Expressed in Human Dental Pulp Cells and Tissues. *J Endod.* 2012;38(12):1592-1597. doi:10.1016/j.joen.2012.09.023
121. Zhang A, Wang P, Ma X, et al. Mechanisms that lead to the regulation of NLRP3 inflammasome expression and activation in human dental pulp fibroblasts. *Mol Immunol.* 2015;66(2):253-262. doi:10.1016/j.molimm.2015.03.009
122. Jiang W, Lv H, Wang H, et al. Activation of the NLRP3/caspase-1 inflammasome in human dental pulp tissue and human dental pulp fibroblasts. *Cell Tissue Res.* 2015;361(2):541-555. doi:10.1007/s00441-015-2118-7
123. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1 β secretion. *Cytokine Growth Factor Rev.* 2011;22(4):189-195. doi:10.1016/j.cytogfr.2011.10.001
124. Wang D, Sun S, Xue Y, et al. MicroRNA-223 negatively regulates LPS-induced inflammatory responses by targeting NLRP3 in human dental pulp fibroblasts. *Int Endod J.* 2021;54(2):241-254. doi:10.1111/iej.13413
125. Staquet MJ, Carrouel F, Keller JF, et al. Pattern-recognition receptors in pulp defense. *Adv Dent Res.* 2011;23(3):296-301. doi:10.1177/0022034511405390
126. Takeda K. Toll-like receptors in innate immunity. *Int Immunol.* 2004;17(1):1-14. doi:10.1093/intimm/dxh186
127. Lin ZM, Song Z, Qin W, et al. Expression of nucleotide-binding oligomerization domain 2 in normal human dental pulp cells and dental pulp tissues. *J Endod.* 2009;35(6):838-842. doi:10.1016/j.joen.2009.03.047
128. Park C, Lee SY, Kim HJ, et al. Synergy of TLR2 and H1R on Cox-2 Activation in Pulpal Cells. *J Dent Res.* 2010;89(2):180-185. doi:10.1177/0022034509354720

129. Keller JF, Carrouel F, Colomb E, et al. Toll-like receptor 2 activation by lipoteichoic acid induces differential production of pro-inflammatory cytokines in human odontoblasts, dental pulp fibroblasts and immature dendritic cells. *Immunobiology*. 2010;215(1):53-59. doi:10.1016/j.imbio.2009.01.009
130. Rodd HD, Boissonade FM. Substance P expression in human tooth pulp in relation to caries and pain experience. *Eur J Oral Sci*. 2000;108(6):467-474. doi:10.1034/j.1600-0722.2000.00924.x
131. El Karim IA, Lamey PJ, Linden GJ, et al. Neuropeptide Y Y1 receptor in human dental pulp cells of noncarious and carious teeth. *Int Endod J*. 2008;41(10):850-855. doi:10.1111/j.1365-2591.2008.01436.x
132. Caviedes-Bucheli J, Gutierrez-Guerra JE, Salazar F, et al. Substance P receptor expression in healthy and inflamed human pulp tissue. *Int Endod J*. 2007;40(2):106-111. doi:10.1111/j.1365-2591.2006.01189.x
133. O'Connor TM, O'Connell J, O'Brien DI, et al. The role of substance P in inflammatory disease. *J Cell Physiol*. 2004;201(2):167-180. doi:10.1002/jcp.20061
134. Bongenhielm U, Hægerstrand A, Theodorsson E, et al. Effects of neuropeptides on growth of cultivated rat molar pulp fibroblasts. *Regul Pept*. 1995;60(2-3):91-98. doi:10.1016/0167-0115(95)00115-8
135. Watanabe S, Alexander M, Misharin A, et al. The role of macrophages in the resolution of inflammation. *J Clin Invest*. 2019;129(7):2619-2628. doi:10.1172/JCI124615
136. Buechler MB, Fu W, Turley SJ. Fibroblast-macrophage reciprocal interactions in health, fibrosis, and cancer. *Immunity*. 2021;54(5):903-915. doi:10.1016/j.immuni.2021.04.021
137. Serhan CN, Gupta SK, Perretti M, et al. The Atlas of Inflammation Resolution (AIR). *Mol Aspects Med*. 2020;74:100894. doi:10.1016/j.mam.2020.100894
138. Smith AJ, Tobias RS, Murray PE. Transdental stimulation of reactionary

- dentinogenesis in ferrets by dentine matrix components. *J Dent.* 2001;29(5):341-346. doi:10.1016/S0300-5712(01)00020-3
139. Inoue T, Shimono M. Repair dentinogenesis following transplantation into normal and germ-free animals. *Proc Finn Dent Soc.* 1992;88 Suppl 1:183-194.
140. About I. Dentin regeneration in vitro: the pivotal role of supportive cells. *Adv Dent Res.* 2011;23(3):320-324. doi:10.1177/0022034511405324
141. Botero TM, Shelburne CE, Holland GR, et al. TLR4 Mediates LPS-Induced VEGF Expression in Odontoblasts. *J Endod.* 2006;32(10):951-955. doi:10.1016/j.joen.2006.03.018
142. Tsukamoto Y, Fukutani S, Shin-ike T, et al. Mineralized nodule formation by cultures of human dental pulp-derived fibroblasts. *Arch Oral Biol.* 1992;37(12):1045-1055. doi:10.1016/0003-9969(92)90037-9
143. Rajan S, Ljunggren A, Manton DJ, et al. Post-mitotic odontoblasts in health, disease, and regeneration. *Arch Oral Biol.* 2020;109:104591. doi:10.1016/j.archoralbio.2019.104591
144. Mitsiadis TA, De Bari C, About I. Apoptosis in developmental and repair-related human tooth remodeling: A view from the inside. *Exp Cell Res.* 2008;314(4):869-877. doi:10.1016/j.yexcr.2007.11.001
145. Qvist V. Pulp reactions in human teeth to tooth colored filling materials. *Eur J Oral Sci.* 1975;83(2):54-66. doi:10.1111/j.1600-0722.1975.tb00420.x
146. Artese L, Rubini C, Ferrero G, et al. Vascular endothelial growth factor (VEGF) expression in healthy and inflamed human dental pulps. *J Endod.* 2002;28(1):20-23. doi:10.1097/00004770-200201000-00005
147. Howard C, Murray PE, Namerow KN. Dental Pulp Stem Cell Migration. *J Endod.* 2010;36(12):1963-1966. doi:10.1016/j.joen.2010.08.046
148. Schraufstatter IU, DiScipio RG, Zhao M, et al. C3a and C5a are chemotactic factors for human mesenchymal stem cells, which cause prolonged ERK1/2

- phosphorylation. J Immunol. 2009;182(6):3827-3836. doi:10.4049/jimmunol.0803055
149. Sakai VT, Zhang Z, Dong Z, et al. SHED differentiate into functional odontoblasts and endothelium. J Dent Res. 2010;89(8):791-796. doi:10.1177/0022034510368647
150. Mitsiadis TA, Magloire H, Pagella P. Nerve growth factor signalling in pathology and regeneration of human teeth. Sci Rep. 2017;7(1):1327. doi:10.1038/s41598-017-01455-3
151. Chan CP, Lan WH, Chang MC, et al. Effects of TGF- β s on the growth, collagen synthesis and collagen lattice contraction of human dental pulp fibroblasts in vitro. Arch Oral Biol. 2005;50(5):469-479. doi:10.1016/j.archoralbio.2004.10.005
152. Graham L, Cooper PR, Cassidy N, et al. The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. Biomaterials. 2006;27(14):2865-2873. doi:10.1016/j.biomaterials.2005.12.020
153. Laurent P, Camps J, About I. BiodentineTM induces TGF- β 1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012;45(5):439-448. doi:10.1111/j.1365-2591.2011.01995.x
154. Tomson PL, Grover LM, Lumley PJ, et al. Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. J Dent. 2007;35(8):636-642. doi:10.1016/j.jdent.2007.04.008
155. Shimabukuro Y, Ueda M, Ichikawa T, et al. Fibroblast growth factor-2 stimulates hyaluronan production by human dental pulp cells. J Endod. 2005;31(11):805-808. doi:10.1097/01.don.0000158242.44155.49
156. Le Clerc J, Pérard M, Pellen-Mussi P, et al. Characterization of a programmed necrosis process in 3-dimensional cultures of dental pulp fibroblasts. Int Endod J. 2013;46(4):308-316. doi:10.1111/j.1365-2591.2012.02114.x
157. Le Clerc J, Tricot-Doleux S, Pellen-Mussi P, et al. Expression of factors

- involved in dental pulp physiopathological processes by nemotic human pulpal fibroblasts. *Int Endod J.* 2018;51:e94-e106. doi:10.1111/iej.12762
158. Vaheiri A, Enzerink A, Räsänen K, et al. Nemosin, a novel way of fibroblast activation, in inflammation and cancer. *Exp Cell Res.* 2009;315(10):1633-1638. doi:10.1016/j.yexcr.2009.03.005
159. Kankuri E, Babusikova O, Hlubinova K, et al. Fibroblast nemosin arrests growth and induces differentiation of human leukemia cells. *Int J Cancer.* 2008;122(6):1243-1252. doi:10.1002/ijc.23179
160. Zhai S, Wang Y, Jiang W, et al. Nemotic human dental pulp fibroblasts promote human dental pulp stem cells migration. *Exp Cell Res.* 2013;319(10):1544-1552. doi:10.1016/j.yexcr.2013.03.018
161. Enzerink A, Rantanen V, Vaheiri A. Fibroblast nemosin induces angiogenic responses of endothelial cells. *Exp Cell Res.* 2010;316(5):826-835. doi:10.1016/j.yexcr.2009.11.012
162. Saghiri MA, Asatourian A, Sorenson CM, et al. Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. *J Endod.* 2015;41(6):797-803. doi:10.1016/j.joen.2014.12.019
163. Flamme I, Frölich T, Risau W. Molecular mechanisms of vasculogenesis and embryonic angiogenesis. *J Cell Physiol.* 1997;173(2):206-210. doi:10.1002/(SICI)1097-4652(199711)173:2<206::AID-JCP22>3.0.CO;2-C
164. Sloan AJ, Perry H, Matthews JB, et al. Transforming growth factor-beta isoform expression in mature human healthy and carious molar teeth. *Histochem J.* 2000;32(4):247-252. doi:10.1023/A:1004007202404
165. Chmielewski F, About I, Chung SH. C5L2 Receptor Represses Brain-Derived Neurotrophic Factor Secretion in Lipoteichoic Acid-Stimulated Pulp Fibroblasts. *J Dent Res.* 2017;96(1):92-99. doi:10.1177/0022034516673832
166. Li R, Coulthard LG, Wu MCL, et al. C5L2: a controversial receptor of

- complement anaphylatoxin, C5a. *FASEB J.* 2013;27(3):855-864. doi:10.1096/fj.12-220509
167. Chmilewsky F, About I, Cooper LF, et al. C5L2 Silencing in Human Pulp Fibroblasts Enhances Nerve Outgrowth Under Lipoteichoic Acid Stimulation. *J Endod.* 2018;44(9):1396-1401. doi:10.1016/j.joen.2018.05.004
168. Son AR, Yang YM, Hong JH, et al. Odontoblast TRP channels and thermo/mechanical transmission. *J Dent Res.* 2009;88(11):1014-1019. doi:10.1177/0022034509343413
169. Miyamoto R, Tokuda M, Sakuta T, et al. Expression and characterization of vanilloid receptor subtype 1 in human dental pulp cell cultures. *J Endod.* 2005;31(9):652-658. doi:10.1097/01.don.0000155259.22746.ae
170. Hossain M, Bakri M, Yahya F, et al. The Role of Transient Receptor Potential (TRP) Channels in the Transduction of Dental Pain. *Int J Mol Sci.* 2019;20(3):526. doi:10.3390/ijms20030526
171. Bandell M, Story GM, Hwang SW, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron.* 2004;41(6):849-857. doi:10.1016/S0896-6273(04)00150-3
172. André E, Campi B, Materazzi S, et al. Cigarette smoke–induced neurogenic inflammation is mediated by α,β -unsaturated aldehydes and the TRPA1 receptor in rodents. *J Clin Invest.* 2008;118(7):2574-2582. doi:10.1172/JCI34886
173. Sawada Y, Hosokawa H, Matsumura K, et al. Activation of transient receptor potential ankyrin 1 by hydrogen peroxide. *Eur J Neurosci.* 2008;27(5):1131-1142. doi:10.1111/j.1460-9568.2008.06093.x
174. Peier AM, Moqrich A, Hergarden AC, et al. A TRP channel that senses cold stimuli and menthol. *Cell.* 2002;108(5):705-715. doi:10.1016/S0092-8674(02)00652-9
175. Rowland KC, Kanive CB, Wells JE, et al. TRPM2 immunoreactivity is

- increased in fibroblasts, but not nerves, of symptomatic human dental pulp. *J Endod.* 2007;33(3):245-248. doi:10.1016/j.joen.2006.11.020
176. Allard B, Magloire H, Couble ML, et al. Voltage-gated Sodium Channels Confer Excitability to Human Odontoblasts. *J Biol Chem.* 2006;281(39):29002-29010. doi:10.1074/jbc.M601020200
177. Shibukawa Y, Sato M, Kimura M, et al. Odontoblasts as sensory receptors: transient receptor potential channels, pannexin-1, and ionotropic ATP receptors mediate intercellular odontoblast-neuron signal transduction. *Pflügers Arch.* 2015;467(4):843-863. doi:10.1007/s00424-014-1551-x
178. El Karim IA, Linden GJ, Curtis TM, et al. Human odontoblasts express functional thermo-sensitive TRP channels: implications for dentin sensitivity. *Pain.* 2011;152(10):2211-2223. doi:10.1016/j.pain.2010.10.016
179. Sabnis AS, Reilly CA, Veranth JM, et al. Increased transcription of cytokine genes in human lung epithelial cells through activation of a TRPM8 variant by cold temperatures. *Am J Physiol Cell Mol Physiol.* 2008;295(1):L194-L200. doi:10.1152/ajplung.00072.2008
180. Yang X, Chen B, Liu T, et al. Reversal of myofibroblast differentiation: A review. *Eur J Pharmacol.* 2014;734(1):83-90. doi:10.1016/j.ejphar.2014.04.007
181. Schmidt M, Sun G, Stacey MA, et al. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J Immunol.* 2003;171(1):380-389. doi:10.4049/jimmunol.171.1.380
182. Dimitrova-Nakov S, Baudry A, Harichane Y, et al. Pulp stem cells: implication in reparative dentin formation. *J Endod.* 2014;40(4):S13-S18. doi:10.1016/j.joen.2014.01.011
183. Tomasek JJ, Gabbiani G, Hinz B, et al. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol.* 2002;3(5):349-363. doi:10.1038/nrm809

184. Matthijs Blankesteyn W. Has the search for a marker of activated fibroblasts finally come to an end?. *J Mol Cell Cardiol.* 2015;88:120-123. doi:10.1016/j.yjmcc.2015.10.005
185. Hinz B, Mastrangelo D, Iselin CE, et al. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. *Am J Pathol.* 2001;159(3):1009-1020. doi:10.1016/S0002-9440(10)61776-2
186. Yoshida N, Yoshida K, Ohkura N, et al. Correlation between Fibrillin-1 Degradation and mRNA Downregulation and Myofibroblast Differentiation in Cultured Human Dental Pulp Tissue. *J Histochem Cytochem.* 2015;63(6):438-448. doi:10.1369/0022155415580622
187. Ramirez F, Sakai LY, Dietz HC, et al. Fibrillin microfibrils: multipurpose extracellular networks in organismal physiology. *Physiol Genomics.* 2004;19(2):151-154. doi:10.1152/physiolgenomics.00092.2004
188. Ramirez F, Sakai LY. Biogenesis and function of fibrillin assemblies. *Cell Tissue Res.* 2010;339(1):71-82. doi:10.1007/s00441-009-0822-x
189. Vaughan MB, Howard EW, Tomasek JJ. Transforming growth factor-beta1 promotes the morphological and functional differentiation of the myofibroblast. *Exp Cell Res.* 2000;257(1):180-189. doi:10.1006/excr.2000.4869
190. Vong S, Kalluri R. The role of stromal myofibroblast and extracellular matrix in tumor angiogenesis. *Genes Cancer.* 2011;2(12):1139-1145. doi:10.1177/1947601911423940
191. Yoshida N, Yoshida K, Ohkura N, et al. Immunohistochemical analysis of two stem cell markers of α -smooth muscle actin and STRO-1 during wound healing of human dental pulp. *Histochem Cell Biol.* 2012;138(4):583-592. doi:10.1007/s00418-012-0978-4
192. Desmoulière A, Redard M, Darby I, et al. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am J Pathol.* 1995;146(1):56-66.

193. Glasser SW, Hagood JS, Wong S, et al. Mechanisms of Lung Fibrosis Resolution. *Am J Pathol.* 2016;186(5):1066-1077. doi:10.1016/j.ajpath.2016.01.018
194. Hinz B, Phan SH, Thannickal VJ, et al. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am J Pathol.* 2012;180(4):1340-1355. doi:10.1016/j.ajpath.2012.02.004
195. Goga R, Chandler NP, Oginni AO. Pulp stones: a review. *Int Endod J.* 2008;41(6):457-468. doi:10.1111/j.1365-2591.2008.01374.x
196. Quigley MB. Functional and geriatric changes of the human pulp. *Oral Surgery, Oral Med Oral Pathol.* 1971;32(5):795-806. doi:10.1016/0030-4220(71)90306-9
197. Leirskar J, Helgeland K. A methodologic study of the effect of dental materials on growth and adhesion of animal cells in vitro. *Scand J Dent Res.* 1972;80(2):120-133. doi:10.1111/j.1600-0722.1972.tb00272.x
198. Meryon SD, Riches DWH. A comparison of their vitro cytotoxicity of four restorative materials assessed by changes in enzyme levels in two cell types. *J Biomed Mater Res.* 1982;16(4):519-528. doi:10.1002/jbm.820160417
199. Spångberg LSW. Correlation of in vivo and in vitro screening tests. *J Endod.* 1978;4(10):296-299. doi:10.1016/S0099-2399(78)80275-1
200. Shi BQ, Yuan XJ, Zhao YM. Effects of mineral trioxide aggregate and ethanolic extracts of Shandong propolis on the biological properties of human dental pulp fibroblasts. *Beijing Da Xue Xue Bao.* 2019;51(6):1108-1114. doi:10.19723/J.ISSN.1671-167X.2019.06.023
201. Escobar-García M, Rodríguez-Contreras K, Ruiz-Rodríguez S, et al. Eugenol Toxicity in Human Dental Pulp Fibroblasts of Primary Teeth. *J Clin Pediatr Dent.* 2016;40(4):312-318. doi:10.17796/1053-4628-40.4.312
202. Aguilar-Perez D, Vargas-Coronado R, Cervantes-UC JM, et al. Antibacterial activity of a glass ionomer cement doped with copper nanoparticles. *Dent*

- Mater J. 2020;39(3):389-396. doi:10.4012/dmj.2019-046
203. Mahasti S, Sattari M, Romoozi E, et al. Cytotoxicity Comparison of Harvard Zinc Phosphate Cement Versus Panavia F2 and Rely X Plus Resin Cements on Rat L929-fibroblasts. *Cell J.* 2011;13(3):163-168.
204. Jontell M, Hanks CT, Bratel J, et al. Effects of unpolymerized resin components on the function of accessory cells derived from the rat incisor pulp. *J Dent Res.* 1995;74(5):1162-1167. doi:10.1177/00220345950740050401
205. van Wyk CW, Olivier A, Maritz JS. Cultured pulp fibroblasts: are they suitable for in vitro cytotoxicity testing?. *J Oral Pathol Med.* 2001;30(3):168-177. doi:10.1034/j.1600-0714.2001.300307.x
206. Ersahan S, Oktay EA, Sabuncuoglu FA, et al. Evaluation of the cytotoxicity of contemporary glass-ionomer cements on mouse fibroblasts and human dental pulp cells. *Eur Arch Paediatr Dent.* 2020;21(3):321-328. doi:10.1007/s40368-019-00481-1
207. Modena KCDS, Calvo AM, Sipert CR, et al. Dental Pulp Fibroblasts Response after Stimulation with HEMA and Adhesive System. *Braz Dent J.* 2018;29(5):419-426. doi:10.1590/0103-6440201802558
208. Kurata S, Morishita K, Kawase T, et al. Cytotoxic effects of acrylic acid, methacrylic acid, their corresponding saturated carboxylic acids, HEMA, and hydroquinone on fibroblasts derived from human pulp. *Dent Mater J.* 2012;31(2):219-225. doi:10.4012/dmj.2011-085
209. Karapınar-Kazandağ M, Bayrak ÖF, Yalvaç ME, et al. Cytotoxicity of 5 endodontic sealers on L929 cell line and human dental pulp cells. *Int Endod J.* 2011;44(7):626-634. doi:10.1111/j.1365-2591.2011.01863.x
210. Alliot-Licht B, Jean A, Gregoire M. Comparative effect of calcium hydroxide and hydroxyapatite on the cellular activity of human pulp fibroblasts in vitro. *Arch Oral Biol.* 1994;39(6):481-489. doi:10.1016/0003-9969(94)90144-9

211. Mondelli JASS, Hoshino RA, Weckwerth PH, et al. Biocompatibility of mineral trioxide aggregate flow and biodentine. *Int Endod J.* 2019;52(2):193-200. doi:10.1111/iej.12989
212. Bulbule A, Mandroli P, Bhat K, et al. In vitro evaluation of cytotoxicity of *Emblica officinalis* (amla) on cultured human primary dental pulp fibroblasts. *J Indian Soc Pedod Prev Dent.* 2019;37(3):251. doi:10.4103/JISPPD.JISPPD_85_18
213. Zare Jahromi M, Ranjbarian P, Shiravi S. Cytotoxicity evaluation of Iranian propolis and calcium hydroxide on dental pulp fibroblasts. *J Dent Res Dent Clin Dent Prospects.* 2014;8(3):130-133. doi:10.5681/joddd.2014.024
214. Al-Shaher A, Wallace J, Agarwal S, et al. Effect of propolis on human fibroblasts from the pulp and periodontal ligament. *J Endod.* 2004;30(5):359-361. doi:10.1097/00004770-200405000-00012
215. Mandrol PS, Bhat K, Prabhakar AR. An in vitro evaluation of cytotoxicity of curcumin against human dental pulp fibroblasts. *J Indian Soc Pedod Prev Dent.* 2016;34(3):269. doi:10.4103/0970-4388.186757
216. Das S. Effect of certain dental materials on human pulp in tissue culture. *Oral Surgery, Oral Med Oral Pathol.* 1981;52(1):76-84. doi:10.1016/0030-4220(81)90177-8
217. Cortés O, García C, Pérez L, et al. Pulp cell cultures obtained with two different methods for in vitro cytotoxicity tests. *Eur Arch Paediatr Dent.* 2006;1(2):96-99. doi:10.1007/BF03320822
218. Adıgüzel M, Ahmetoğlu F, Ünverdi Eldeniz A, et al. Comparison of cytotoxic effects of calcium silicate-based materials on human pulp fibroblasts Mehmet. *J Dent Res Dent Clin Dent Prospects.* 2019;13(4):241-246. doi:10.15171/joddd.2019.037
219. Song J, Wu Q, Jiang J, et al. Berberine reduces inflammation of human dental pulp fibroblast via miR-21/KBTBD7 axis. *Arch Oral Biol.* 2020;110:104630.

doi:10.1016/j.archoralbio.2019.104630

220. Zeigler AC, Nelson AR, Chandrabhatla AS, et al. Computational model predicts paracrine and intracellular drivers of fibroblast phenotype after myocardial infarction. *Matrix Biol.* 2020;91-92:136-151. doi:10.1016/J.MATBIO.2020.03.007
221. Berberich B, Thriene K, Gretzmeier C, et al. Proteomic Profiling of Fibroblasts Isolated from Chronic Wounds Identifies Disease-Relevant Signaling Pathways. *J Invest Dermatol.* 2020;140(11):2280-2290.e4. doi:10.1016/j.jid.2020.02.040
222. Mäkelä K, Mäyränpää MI, Sihvo HK, et al. Artificial intelligence identifies inflammation and confirms fibroblast foci as prognostic tissue biomarkers in idiopathic pulmonary fibrosis. *Hum Pathol.* 2021;107:58-68. doi:10.1016/j.humpath.2020.10.008