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Potential Therapeutic Strategy of Targeting Pulp Fibroblasts in Dentin-Pulp Regeneration

Charlotte Jeanneau, PhD,* Fionnuala T. Lundy, PhD,[†] Ikbls A. El Karim, PhD,[†] and Imad About, PhD*

Abstract

Fibroblasts represent the most abundant population within the dental pulp. Although other cell types such as odontoblasts and stem cells have been extensively investigated, very little attention was given to the fibroblasts, which have major roles in regulating the pulp biology and function under normal and pathologic conditions. Indeed, although pulp fibroblasts control the pulp vascularization and innervation under physiological conditions, these cells synthesize growth factors that enhance dentin-pulp regeneration, vascularization, and innervation. Pulp fibroblasts also represent a unique cell population because they are the only non-hepatic and non-immune cell type capable of synthesizing all complement proteins leading to production of biologically active fragments such as C3a, C5a, and membrane attack complex, which play major roles in the pulp regeneration processes. C3a fragment is involved in inducing the proliferation of both stem cells and pulp fibroblasts. It is also involved in stem cell mobilization and pulp fibroblast recruitment. C5a guides nerve sprouting and stem cell recruitment. The membrane attack complex fixes on cariogenic bacteria walls, leading to their direct destruction. These data demonstrate the central role played by pulp fibroblasts in regulating the dentin-pulp tissue by directly destroying cariogenic bacteria and by releasing bioactive fragments involved in nerve sprouting and stem cell recruitment and pulp regeneration. Taken together, this shows that targeting pulp fibroblasts represents a realistic strategy to induce complete dentin-pulp regeneration. (*J Endod* 2017;■:1–8)

Key Words

Complement, dentin-pulp, growth factors, pulp fibroblast, regeneration

Unlike any other tissue of the human body, the dental pulp is located within inextensible and rigid dentin walls. Although the inflammatory reaction and subsequent increased vascularization and blood flow may have no serious consequences in all body tissues, this inflammation may be deleterious in case of severe pulp inflammation leading to its necrosis. However, several lines of evidence suggest that there is a local regulation of the pulp response to external insults. This is particularly true in the context of dentin-pulp regeneration studies. Indeed, since the demonstration of the presence of the dental pulp stem cells (DPSCs), a huge number of studies were devoted to investigate the potential of these cells in regenerating the dentin pulp by differentiating into odontoblast-like cells secreting dentin (1, 2). Also, a significant number of studies were carried out to understand the signals involved in their activation and recruitment (3, 4). In addition, more and more studies have investigated the differentiation potential of these cells into other cell types *in vitro* and their promising potential in the regeneration of other tissues *in vivo* such as bone, cartilage, and vascularization (5, 6).

The presence of these cells has been reported within the dental pulp, which is mainly composed of fibroblasts. Although the latter represent a great majority of pulp cell populations, very few studies were devoted to understand the interest of having such a high number of fibroblasts within the pulp. The major part of studies of fibroblasts focused on the role of these cells in the pulp simply as in all connective tissues: their capacity to synthesize and to secrete different types of collagen. Although collagen synthesis is essential in extracellular matrix (ECM) synthesis for cell adhesion and function in the dental pulp, it is also essential in providing support and stabilization of blood vessels mainly by contributing to basement membrane formation. However, recent data reported that these cells do much more than synthesizing collagen. Indeed, in case of pulp infection/injury, fibroblasts synthesize growth factors that are involved in reestablishing blood vascularization, nerve sprouting, and dentin-pulp regeneration by recruiting stem cells and nerve endings and directing their migration/sprouting to the injury site (7–9). Fibroblasts also synthesize all complement proteins and lead to production of

Significance

Pulp fibroblasts play a major role in bacterial progression control and in regulating dentin-pulp regeneration through the secretion of complement active fragments and growth factors. After pulp injury, they induce pulp cell proliferation, DPSC recruitment/differentiation, pulp vascularization, and innervation. Thus, in addition to other pulp cell types such as odontoblasts and pulp stem cells, the pulp fibroblast should be considered as a real target in dentin-pulp regeneration strategies.

complement bioactive fragments (10). These bioactive fragments are able to initiate the pulp and nervous regeneration processes and, at the same time, are efficient in cariogenic bacteria destruction (9, 11, 12). This review will shed light on pulp fibroblasts as essential cells in defending the pulp against cariogenic bacteria invasion. It will also put the fibroblast under the light as a source of the major part of signals required to initiate the regeneration process by providing the activation, guidance, and pulp regeneration signals. At the same time, this review will highlight the anti-inflammatory role of fibroblasts through their capacity in destroying cariogenic bacteria directly.

Fibroblast: Definition and Physiological Roles

The fibroblast is often defined as an irregular shaped cell involved in the synthesis of the ECM that provides support to all animal tissues. Indeed, fibroblasts are mesenchymal cells that form fibers of the connective tissue and contribute to their structural integrity. They originate from a multipotent mesenchymal stem cell (MSC) that also gives rise to adipoblasts, chondroblasts, osteoblasts, and myoblasts (13). Fibroblasts are fusiform or stellate cells with long cytoplasmic processes (14). They play a vital role in ECM production and remodeling because they secrete both its major components (fibrous collagen, elastin, laminin, fibronectin, glycosaminoglycans such as hyaluronan, and glycoproteins) and also many matrix metalloproteinases. Their role in ECM synthesis and mineralization was illustrated in *Mia3*-null embryos, where the inhibition of collagens' secretion by fibroblasts leads to severe defects in chondrocyte maturation and bone mineralization (15). Beyond ECM production, fibroblasts also play significant physiological roles. Fibroblasts have a low proliferation index and low metabolic activities under physiological conditions. However, during the healing process, they have a high proliferation rate and a high metabolic rate. They secrete more matrix components and acquire contractile properties (16). These fibroblasts, called "activated," will then secrete a large number of chemokines, leading to the recruitment of inflammatory cells at the wound site (17).

Fibroblasts also play pivotal roles in angiogenesis. They facilitate angiogenesis into injured tissues beyond the reach of existing blood vessels (18). This response requires the migration of endothelial cells to construct tubes through the ground substance of connective tissue. A major mechanism for this phenomenon is the fibroblast-mediated production and release of vascular endothelial growth factor (VEGF), which acts on VEGF receptors expressed on endothelial cells to promote neoangiogenesis (19).

Human Pulp Fibroblasts Secrete Growth Factors and Induce Pulp Regeneration

The dental pulp is rather complex and contains a heterogeneous population of fibroblasts (20). They all express fibroblast surface protein (FSP-1). They also express type IA and type II receptors of bone morphogenetic proteins (21) and transforming growth factor beta (TGF- β) receptors (22). Carious/traumatic tooth injuries may alter the dentin-pulp complex and lead to an inflammatory reaction, which is the initial step of tissue regeneration. This process aims at restoring the integrity of the dentin-pulp complex and also at maintaining tooth vitality and function. Depending on the severity of the tissue alteration, dentin-pulp regeneration can vary from an upregulation of the odontoblast synthetic activity, which leads to regenerating a protective reactionary dentin (23), to complete pulp-dentin regeneration. This complete regeneration requires reparative dentin synthesis, neoangiogenesis, and innervation. All these processes are orchestrated by growth factors mainly secreted by pulp fibroblasts.

Pulp Fibroblasts as a Source of Growth Factors

Dentin was the first identified source of molecules capable of inducing dentin-pulp regeneration (24–26). However, after surgical pulp amputation, healing can occur, with hard tissue formation in germ-free animals independent of growth factor release from the acid dissolution of dentin because of bacteria metabolism (27, 28). This suggests that the pulp could represent another source of signals inducing dentin-pulp regeneration after traumatic injuries. Indeed, it has been demonstrated that human pulp fibroblasts secrete basic fibroblast growth factor (FGF-2), VEGF, and platelet-derived growth factor (PDGF) *in vitro*, and that this secretion was significantly increased 6 hours after traumatic injury (8). Therefore, this information suggests that the lesion itself induces a change in the local microenvironment by inducing the secretion of growth factors. Moreover, pulp cells from both rats and humans express messenger RNAs and release the corresponding neurotrophic proteins (29–31). This also indicates a potential of these cells in nerve growth and pulp innervation.

Role of Human Pulp Fibroblast in Angiogenesis

An interesting aspect of fibroblast involvement in pulp physiology and function has been illustrated through a co-culture system between pulp fibroblast and endothelial cells. This allowed demonstration of the direct influence of fibroblasts on neoangiogenesis *in vitro* (32). Indeed, direct culture of fibroblasts with endothelial cells induced the organization of endothelial cells into tubular structure *in vitro*, reflecting their angiogenic capacity. This organization of endothelial cells started after 24 hours of co-culture with fibroblasts, and completely closed structures were obtained after 6 days. When both cell types were cultured separately and physical injuries were performed on pulp fibroblasts, the culture medium containing the soluble factors was collected and was applied onto endothelial cell cultures. Surprisingly, endothelial cells started to organize into closed tubular structures corresponding to neoangiogenesis *in vitro* (32). Quantification of growth factors released in the culture medium of pulp fibroblasts revealed the presence of angiogenic factors such as FGF-2, VEGF, and PDGF. When this quantification was performed on injured fibroblasts, there was a significant increase of these factors a few hours after the cells' injury (8).

Role of Human Pulp Fibroblasts in Nerve Regeneration

In addition to their implication in neoangiogenesis, pulp cells from both rats and humans express mRNAs and release the corresponding neurotrophic proteins. Although the production of neurotrophic factors by dental pulp cells plays an important role in tooth innervation during development, continued production by mature pulp cells seems to be involved in other functions such as the control of neuronal survival, guidance of nerve processes, and regulation of innervation density (31). For example, it has been shown that explants of young rat trigeminal ganglia extend neurites when co-cultivated with pulpal tissue explants, suggesting that pulp cells stimulate growth of trigeminal ganglia axons by secreting soluble molecules (33).

Role of Pulp Fibroblast in DPSC Recruitment and Differentiation

Severe carious lesions or deep cavity preparation during restorative procedures may lead to odontoblast apoptosis/destruction (34). In this case, dentin-pulp regeneration requires the activation and proliferation of progenitor cells as well as their migration and differentiation at the injury site. The damaged dentin is then replaced by a reparative dentin secreted by newly differentiated odontoblast-like cells (35). Several studies reported the involvement of growth factors such as

TGF- β 1 in the odontoblastic differentiation and reparative dentin secretion (26). It has been shown that components of the ECM (collagen I, collagen IV, laminin, and fibronectin) and growth factors including sphingosine 1-phosphate, FGF-2, epidermal growth factor, and TGF- β 1 induce the migration of stem cells (36). In a recent study, FGF-2 and TGF- β 1 were encapsulated in poly(lactic polyglycolic acid) beads to control their release. This study clearly demonstrated that TGF- β 1 induced migration of progenitor pulp STRO-1+ cells, whereas FGF-2 induced pulp fibroblast proliferation, indicating the involvement of these factors in pulp-dentin regeneration (7). Moreover, FGF-2 and VEGF secreted from pulp fibroblasts have been shown to directly affect progenitor cell differentiation. They are involved in the differentiation of the side population CD34⁺, VEGFR2/FLK⁺, CD31⁻, and CD146⁻ into both odontoblasts and endothelial cells (37). The role of pulp fibroblasts in growth factor secretion and pulp regeneration is summarized in Figure 1.

Human Pulp Fibroblasts Express and Secrete Complement Proteins

Complement is one of the most powerful and efficient plasma immune surveillance systems. It consists of more than 40 plasma and membrane proteins that act as cascades of finely regulated enzymatic reactions (38–40). The local activation of complement proteins is rapidly amplified by successive enzymatic reactions, rendering it extremely effective in inflammatory reactions (41, 42).

The complement system can be activated by 3 principal enzymatic cascades (Fig. 2): the classical, alternative, and lectin pathways, which converge at the cleavage of the main element, the C3.

Its activation leads to the formation of the cytolytic membrane attack complex (MAC) on the pathogen surface (43), the recruitment of immune cells by the release of anaphylatoxins such as C3a and C5a (44–46), and clearance of immune complexes and damaged cells.

Activation of the complement system leads to very important signal amplification, for example, one activated C3 convertase leads to the formation of more than 1000 MACs that form on pathogen membranes. This is why the complement system is subject to a fine and powerful control of its activation.

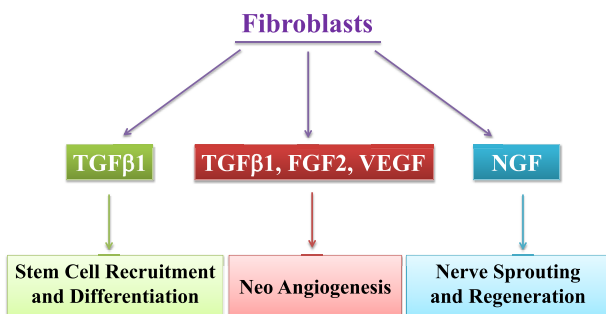


Figure 1. Human pulp fibroblasts secrete growth factors involved in dentin-pulp regeneration. Complete dentin-pulp regeneration requires reparative dentin synthesis, neoangiogenesis, and pulp innervation. All these processes are orchestrated by growth factors secreted by pulp fibroblasts. Some of these are well-characterized. They include TGF- β 1, FGF-2, and VEGF, which play a major role in neoangiogenesis; TGF- β 1 for stem cell recruitment and differentiation; and nerve growth factor (NGF) is required for nerve sprouting and regeneration. The above factors are only examples. However, there are many more factors and redundancies involved in the system because some factors may be involved in more than 1 process.

The liver is the primary site for circulating complement proteins synthesis (47, 48). However, during the past 25 years, it has been shown that many organs/tissues/cells were able to produce some complement molecules (including C1q, factor D, properdin, and C7) either constitutively or in response to stimulation. This extrahepatic production is especially true in poorly vascularized areas of the body where the plasma supply of complement molecules is insufficient for effective activation. This is also true in areas prone to infections that need rapid complement activation (49).

The tooth is prone to both trauma and carious injuries. Also, all events that alter the dentin-pulp complex and initiate its regeneration also activate the complement system. These include the following:

1. Carious lesions activate the complement system via the classical, lectin, and alternative pathways (50, 51).
2. Mechanical trauma leads to the formation not only of necrotic cells but also of apoptotic cells. Membrane modifications undergone by these 2 types of cells activate the classical pathway of the complement system (52–56).
3. The therapeutic procedures imply the application of biomaterials containing free OH, NH₃, or COOH groups, which are known to activate the complement classical pathway (57–59).

Although the dental pulp is a highly vascularized tissue, local synthesis and regulation of complement may be of benefit to dental pulp hemostasis. Indeed, recent studies (10) have shown local expression of complement molecules by fibroblasts obtained from pulp explants. These cells were identified as fibroblasts after their characterization by immunofluorescence and flow cytometry (10). After analysis by reverse transcriptase polymerase chain reaction (RT-PCR) (Fig. 3A), these cells were found to constitutively express a large portion of the complement system molecules, including C1q and C7. Only C3, central molecule toward which all activation pathways converge, and C6, which is an essential component in the formation of MAC, were not expressed by pulp fibroblasts. However, after stimulation with lipoteichoic acid (LTA), which mimics an infection by gram-positive bacteria, pulp fibroblasts express all molecules necessary for complement effective activation, ie, all components from C1 to C9. Analysis of expression of these expressions revealed some interesting aspects. Expression of factor B, which is involved in the C3 convertase formation in the alternative pathway, significantly increased after stimulation with LTA. Expression of C4, which is a component necessary for activation by classical and lectin pathway, decreased after stimulation with LTA. This could promote the complement alternative pathway activation, the main complement activation pathway in case of infection.

Therefore, this study was the first to demonstrate that all complement molecules are expressed by a single cell type (10). Indeed, when extrahepatic and non-immune complement components production is reported, only a few molecules were detected to optimize the action of complement molecules produced by the liver (49).

Complement in Pulp Regeneration C5a and Pulp Regeneration

The ability of pulp progenitor cells to migrate to the injured site is a critical step in the dentin-pulp regeneration process (60–62). Recent data have clearly demonstrated that pulp progenitor STRO-1+ cells, known for their ability to generate the dentin matrix (2, 63), express C5aR. Indeed, co-expression of STRO-1 and C5aR marker was observed in the perivascular area of dental pulp *in vivo* (11), and this expression was confirmed *in vitro* by

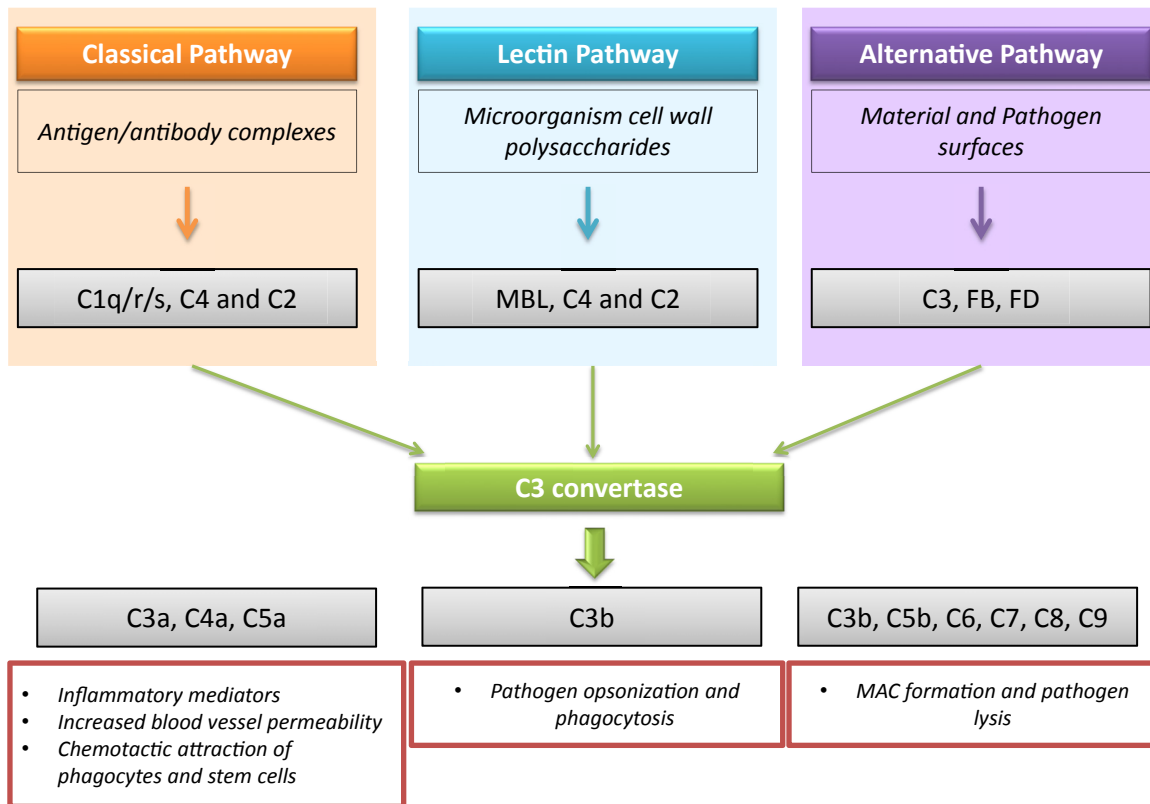


Figure 2. Overview of the complement system and its activation. The complement is activated by 3 pathways. The classical complement pathway requires antigen-antibody complexes for activation. The alternative pathway can be activated spontaneously or by pathogen/material surface. The mannose-binding lectin pathway can be activated by microorganism cell wall polysaccharides. All 3 activation pathways generate the protease C3-convertase. C3-convertase cleaves and activates C3 component, creating C3a and C3b, and leads to a cascade of further cleavage and activation events. C3b binds to the surface of pathogens, leading cells to phagocytose by opsonization. The anaphylatoxin C3a, C4a, and C5a directly trigger inflammation, blood vessel permeability, chemotactic attraction of phagocytes, and stem cells. The polymerization of C5b, C6, C7, C8, and C9 leads to the formation of a transmembrane channel called MAC, which induces pathogen lysis.

RT-PCR and double immunostaining on STRO-1+ pulp cells obtained by magnetic sorting.

It has been well-established that C5a induced the recruitment of MSCs via the C5aR through prolonged phosphorylation of ERK1/2 (64). This phosphorylation requires the interaction of C5a with C5aR located in the cell membrane. It has been demonstrated by immunofluorescence and enzyme binding assay that the C5aR expressed by STRO-1 pulp progenitor cells interacts specifically with C5a (11). Moreover, the use of microfluidic migration chambers demonstrated that soluble C5a specifically induced pulp progenitor cell migration. Indeed, although the pulp fibroblasts do not respond to C5a, a significant number of pulp progenitors migrated toward the C5a-containing chamber (11). This migration was not random but followed a gradient of C5a generated in the migration chamber.

Complement activation is known as a rapid, amplified, and localized event. One of the consequences of this activation is that it generates C5a locally and thus enables setting up a C5a gradient that guides progenitor cell migration (41). Investigating this event required specific adaptation of the microfluidic migration chambers to study the effect of C5a produced by LTA-stimulated pulp fibroblasts directly on pulp progenitor cell recruitment. The use of pulp fibroblasts in the reservoirs of microfluidic chambers allowed creating a dynamic cell-to-cell interaction response with or without LTA stimulation, corresponding to the dynamics of the progenitor cell migration process. With this system, pulp progenitor cells significantly migrated toward LTA-stimulated fibroblasts but not toward unstimulated cells, and this migration was

significantly inhibited by W54011 (C5aR antagonist). Further analysis demonstrated that this migration followed a C5a gradient. This clearly shows that pulp fibroblasts are capable of generating a C5a gradient only after LTA stimulation and that this gradient guides pulp progenitor cell recruitment (10).

These results suggest that activation of the complement system, generated by a carious lesion, leads to the establishment of a C5a chemotactic gradient in the pulp tissue. Progenitor pulp cells, which express C5aR, therefore migrate toward this gradient by specific interaction with C5a from their niches to the injured site to regenerate the dentin-pulp complex.

The nervous system of the tooth can also be altered under carious lesions. Recent studies have demonstrated that when pulp fibroblasts are stimulated with LTA, they express C5aR both *in vivo* and *in vitro* (65). The interaction between fibroblast-secreted C5a and its receptor on the same cells induced the production of brain-derived neurotrophic factor. This neurotrophic factor promotes nerve growth, including neurite formation. Indeed, when pulp fibroblasts were cultured in microfluidic chambers with human neurons, stimulation of fibroblasts with LTA induced sprouting and growth of human neurons toward LTA-stimulated fibroblasts but not toward unstimulated cells (9).

C3a and Pulp Regeneration

C3a is another complement component known through its effects in the inflammatory process. It is known to induce migration and activation of

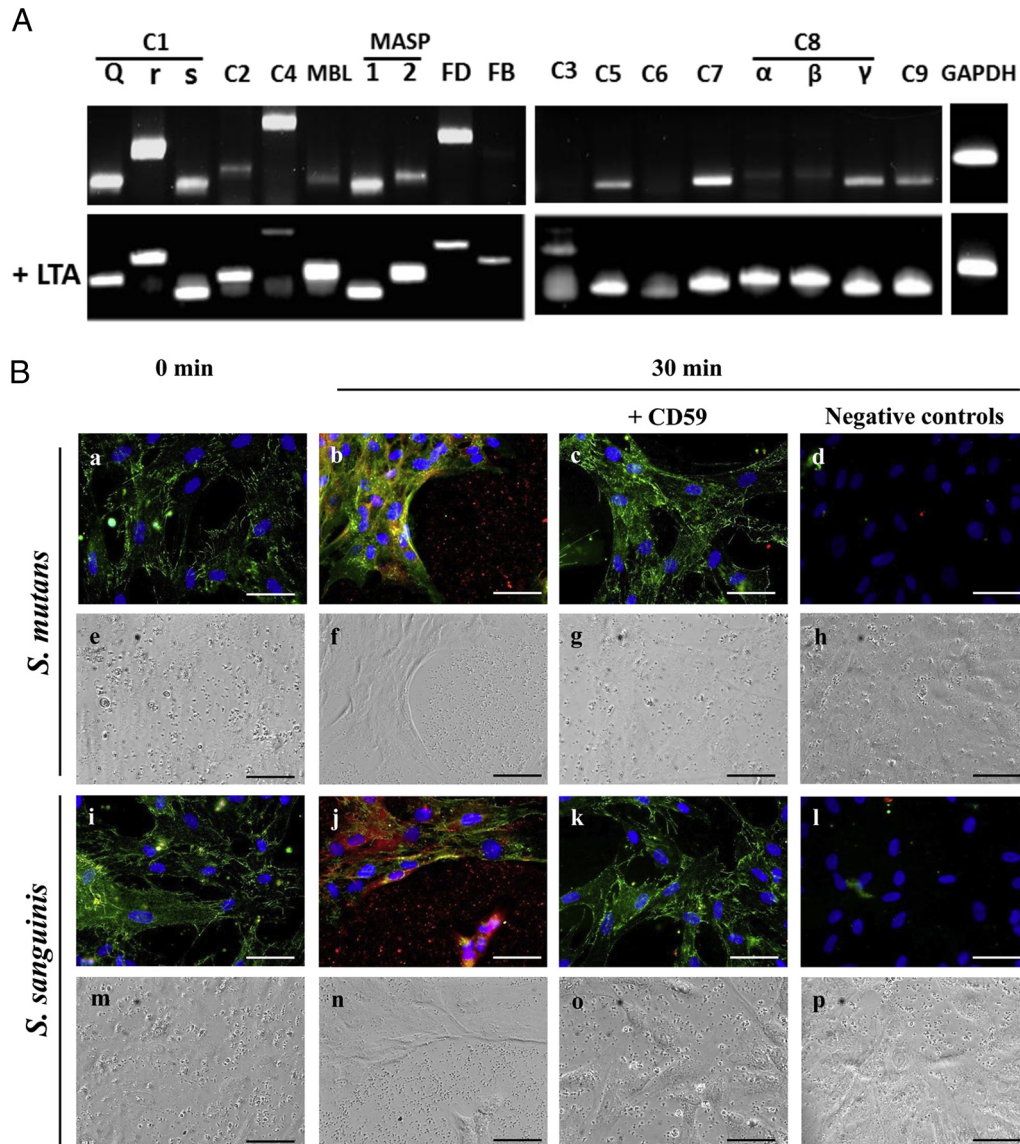


Figure 3. (A) Human pulp fibroblasts stimulated with LTA express all components required for complement system activation. RT-PCR product from unstimulated or LTA-stimulated human pulp cells for complement components C1q, C1r, C1s, C2, C4, MBL, MASP1, MASP2, FD, FB, C3, C5, C6, C7, C8 α , C8 β , C8 γ , and C9. GAPDH was used as a housekeeping control. In unstimulated pulp cells, C3 and C6 (2 components required for complement system activation) were not detected. By contrast, after LTA stimulation, all complement components were detected, including C3 and C6. (B) Cariogenic bacteria directly induce MAC production by human pulp fibroblasts. Immunofluorescence double-staining was used to visualize FSP-1 in green and MAC in red at 0 minute (*a, e, i, m*) and 30 minutes (*b, c, d, f, g, h, j, k, l, n, o, p*) of co-cultures of human pulp fibroblast and *S. mutans* or *S. sanguinis* in the absence (*a, b, e, f, i, j, m, n*) or presence (*c, g, k, o*) of CD59. Co-cultures of fibroblasts and bacteria can be observed in all conditions on phase-contrast images (*e-h, m-p*). No immunostaining was observed in control conditions (*a, e, i, m*) or with an isotype (*d, h, l, p*). An intense MAC red labeling was observed on bacteria after 30 minutes of co-culture (*b, j*). Formation of MAC on bacteria was confirmed by significant decrease in staining on addition of CD59 (a MAC fixation inhibitor) in the co-cultures (*c, k*). Scale bars: 500 μ m. (C) Fibroblast-secreted complement orchestrates dentin-pulp regeneration. Fibroblasts play a significant role in control of regeneration and bacterial progression during the inflammatory process. During complement activation, C3a is the first active fragment to be produced, and this is followed by C5a. Thus, whereas C3a first mobilizes stem cells and provides a chemotactic gradient for pulp fibroblasts migration, C5a provides a chemotactic gradient for stem cells recruitment and nerve sprouting. Moreover, complement activation is also involved in cariogenic bacteria growth inhibition through MAC formation.

leukocytes, trigger smooth muscle cell contraction, and increase endothelial permeability (45, 66–68). C3aR is widely expressed by many immune and non-immune cells (69). The large distribution of C3aR within most tissues allows C3a to act efficiently during tissue development and regeneration. It has been shown that complement C3a gradient can co-attract cohesive clusters of migrating MSCs during neural crest formation (70). Similarly, it has been reported that human MSCs are chemoattracted by C3a and C5a to injury sites, where mobilization and recruitment of

MSCs are required for wound healing (64). Thus, C3a/C3aR interactions represent one of the major pathways involved in tissue regeneration. Recent study in the dental pulp clearly demonstrated expression of C3aR *in vivo* on tooth sections and on pulp fibroblasts expressing FSP-1 and on DPSCs expressing STRO-1 by RT-PCR and immunofluorescence double-staining. The same study showed that the complement-derived C3a is involved in both DPSCs and pulp fibroblast proliferation and in pulp fibroblast recruitment following a C3a gradient (71).

C

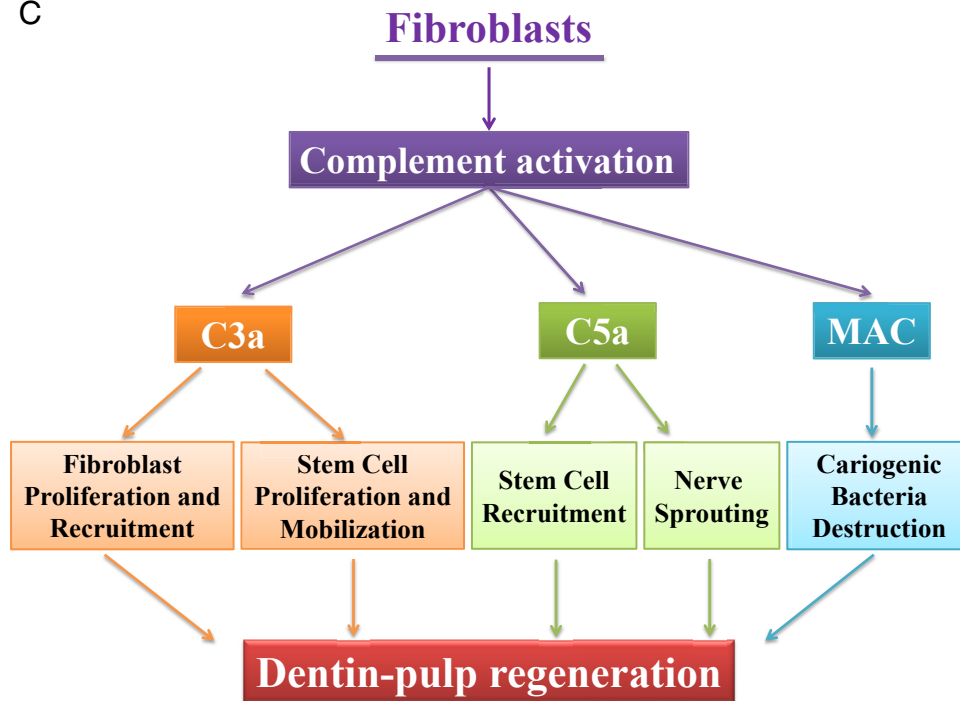


Figure 3. (continued).

During the regeneration process, proliferation of both fibroblasts and progenitor cells is required to regenerate the dentin pulp. Surprisingly, when both cell types were submitted to a C3a gradient, a random cell movement was observed with DPSCs, whereas pulp fibroblasts migrated following the C3a gradient. This mobilization of stem cells from their environment may be required as an initial step to subsequently follow a C5a gradient. Indeed, during complement activation, C3a is the first active fragment to be produced, and this is followed by C5a. Thus, although C3a first mobilizes stem cells, C5a provides the chemotactic gradient for guiding their migration (71).

MAC Plays a Significant Role in Destroying Cariogenic Bacteria

Beside C5a and C3a production, MAC formation is an end product of complement activation. This complex is known for its capacity to induce cytolysis via its direct fixation on bacteria walls (72, 73). In the context of dentin-pulp regeneration, it should be remembered that regeneration occurs after the elimination of cariogenic bacteria or, at least, after the arrest of their progression toward the pulp. A recent study examined whether complement activation through MAC formation by pulp fibroblasts is also involved in cariogenic bacteria growth inhibition (12). MAC (C5b-9/MAC) fixation on carious teeth was demonstrated for the first time *in vivo*. MAC was observed not only in the carious dentin but also in the pulp of deep carious lesions. This complement activation was correlated with the presence of both gram-positive and gram-negative bacteria in the dentinal tubules and in the pulp (4, 10, 74). Indeed, MAC formation was localized on *Streptococcus mutans* in carious tissue histologic sections. Furthermore, when pulp fibroblasts were stimulated with LTA, they produced MAC in the supernatant. When cariogenic bacteria were incubated with this supernatant, immunofluorescence revealed a direct fixation of MAC on both *S. mutans* and *S. sanguinis*. This fixation on cariogenic bacteria walls induced

growth inhibition and decreased bacterial viability. To further demonstrate this direct MAC fixation on bacteria walls and subsequent destruction of bacteria directly via complement activation, a specific eukaryote/prokaryote culture technique was developed (Fig. 3B). For this purpose, pulp fibroblasts were plated in serum-free medium, and cariogenic bacteria were then added to these cells, followed by immunofluorescence labeling of FSP-1 and MAC specific antibodies. MAC formation was detected on control co-cultures fixed immediately after adding bacteria on fibroblasts (Fig. 3Ba, Be, Bi, and Bm). However, after co-culture for 30 minutes, intense MAC labeling was observed on bacteria (Fig. 3Bb, Bf, Bj, and Bn).

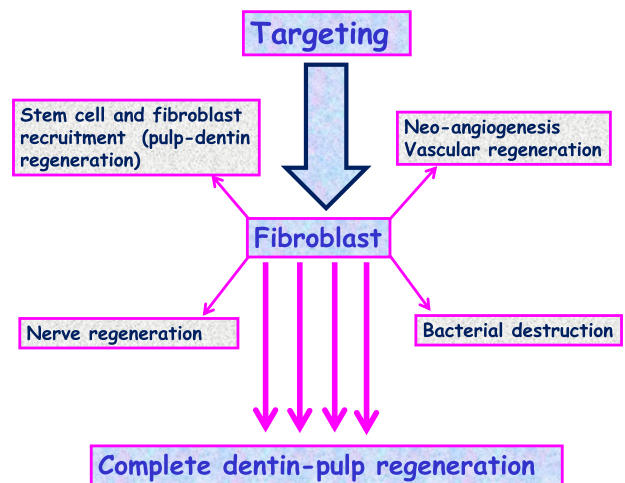


Figure 4. Targeting pulp fibroblast for complete dentin-pulp regeneration. Pulp fibroblasts secrete multiple growth factors and complement active fragments that orchestrate vascularization, innervation, and anti-cariogenic effects leading to complete dentin-pulp regeneration. Thus, targeting pulp fibroblast represents a good strategy in dentin-pulp regeneration.

The formation of MAC on bacteria was confirmed by a significant decrease in MAC labeling when CD59, a MAC formation inhibitor, was added to the co-culture (Fig. 3Bc, Bg, Bk, and Bo). No labeling was observed with control isotypes (Fig. 3Bd, Bb, Bl, and Bp). Although the production levels of all complement components were not determined directly, this co-culture system indicates that when pulp fibroblasts are subjected to cariogenic bacteria, they produce the complement components required for direct MAC fixation on cariogenic bacteria (12).

Conclusion

This work clearly shows that pulp fibroblasts play a significant role in the control of bacterial progression during the inflammatory process. As such, it partly elucidates why dentin regeneration can be observed directly under carious lesions and how arrested caries can be frequently observed *in vivo*. Overall, the anticariogenic role of complement produced by pulp fibroblasts and its involvement in complete dentin-pulp regeneration are illustrated in Figure 3C.

Thus, through the secretion of multiple growth factors and complement active fragments, the pulp fibroblast plays a major role in orchestrating the pulp regeneration process by inducing vascularization, innervation, and dentin-pulp regeneration. Overall, in addition to odontoblasts and pulp stem cells, pulp fibroblast should be considered as a central cell that represents a real target in strategies to induce the dentin-pulp regeneration process (Fig. 4).

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The authors deny any conflicts of interest related to this study.

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