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

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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

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.

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 synthetize 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

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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