Scaffolds to Control Inflammation and Facilitate Dental Pulp Regeneration

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Abstract

In dentistry, the maintenance of a vital dental pulp is of paramount importance because teeth devitalized by root canal treatment may become more brittle and prone to structural failure over time. Advanced carious lesions can irreversibly damage the dental pulp by propagating a sustained inflammatory response throughout the tissue. Although the inflammatory response initially drives tissue repair, sustained inflammation has an enormously destructive effect on the vital pulp, eventually leading to total necrosis of the tissue and necessitating its removal. The implications of tooth devitalization have driven significant interest in the development of bioactive materials that facilitate the regeneration of damaged pulp tissues by harnessing the capacity of the dental pulp for self-repair. In considering the process by which pulpitis drives tissue destruction, it is clear that an important step in supporting the regeneration of pulpal tissues is the attenuation of inflammation. Macrophages, key mediators of the immune response, may play a critical role in the resolution of pulpitis because of their ability to switch to a proresolution phenotype. This process can be driven by the resolvins, a family of molecules derived from fatty acids that show great promise as therapeutic agents. In this review, we outline the importance of preserving the capacity of the dental pulp to self-repair through the rapid attenuation of inflammation. Potential treatment modalities, such as shifting macrophages to a proresolving phenotype with resolvins are described, and a range of materials known to support the regeneration of dental pulp are presented. *(J Endod 2014;40:S6–S12)*

Key Words

Biomaterials, dental pulp, endodontics, inflammation, pulp regeneration, resolvins, tissue engineering

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One of the most significant challenges in modern dentistry is the maintenance of a vital dental pulp. Due to the fact that root canal therapy results in a permanently devitalized tooth more susceptible to structural failure, there is an increasing desire to provide better options for endodontic therapy, effectively expanding the tools available in clinical dentistry. It is the desire to drive regeneration in the dental pulp that has prompted research into bioactive materials for use in regenerating dental pulp. True innovation in developing novel endodontic therapies will involve using materials that facilitate the regeneration of the dental pulp from any remaining, vital pulp tissues, effectively harnessing the innate capacity of the tooth for self-repair. This review explores the most prevalent cause of pulpal necrosis, bacterially induced tissue inflammation, and highlights the importance of attenuating this inflammation in order to support and facilitate dental pulp regeneration.

The Dental Pulp

The dental pulp itself is a unique and complex tissue serving to support the dentin, which provides the main structural component of the tooth organ. Its basic structure is well characterized and has been widely described (1–3). The pulp tissue itself is composed of collagen type I and type III along with a variety of noncollagenous proteins, including a large proteoglycan component (4). There are a variety of cell types present in the pulpal tissue, including immune cells, fibroblasts, mesenchymal progenitor cells, vascular cells, and nerve cells (5–11). Lining the pulp chamber is a layer of columnar odontoblasts, which have cellular projections associated with a system of fluid-filled tubules running through the dentin to the dentinoenamel junction (1, 12). These are cells directly responsible for the maintenance of the mineralized dentin during routine loading of the tooth. Fibroblasts are the most numerous cells in the dental pulp and maintain the collagen matrix of the pulpal tissue, and a population of immune cells, in particular tissue macrophages, hold themselves ready to respond to microbial incursion (2, 3, 5). A network of blood vessels runs throughout the pulp, perfusing the tissue and providing respiratory support (2, 3). Networks of nerve fibers provide enervation to the tissue, linking it with the central nervous system and providing sensory output (2, 3). This complex and dynamic environment is preserved in a delicate balance, with the odontoblasts maintaining the mineralized tissues and the other cell types effectively positioned to support the activity of the odontoblasts. A resident population of mesenchymal progenitor cells provides a reservoir of pluripotent cells capable of differentiation into a variety of cells as required for tissue maintenance and repair (13). The progenitor cells in particular are critical to the long-term function of the whole tooth and are therefore a critical target when considering the design of bioactive materials intended to encourage dental pulp regeneration.

The Capacity of the Dental Pulp for Self-repair

It is widely accepted that the tooth organ has an innate capacity for self-repair and contains all the necessary components to regenerate both the mineralized dentin and the soft tissues of the pulpal matrix. It is established that odontoblasts respond to microbial colonization of the dentinal tubules by producing sclerotic "reactionary" dentin in an attempt to block infected tubules, thus creating a barrier between the invading microbes and the pulp tissue (14, 15). In more advanced carious lesions, the inflammatory response causes cell death among odontoblasts and other cells of the

pulp. Mesenchymal progenitor cells are subsequently recruited to the site of cell death and are driven by a cascade of signals including degradation products from the dentin matrix to differentiate into odontoblasts and begin synthesizing "reparative" dentin (7, 10, 11, 16, 17). Dental pulp also contains a population of multipotent cells capable of responding to injury. *In vitro*, isolated human dental pulp progenitor cells have also been shown to be capable of differentiation into a variety of cell types, including osteoblasts, adipocytes, and chondrocytes (17, 18). Dental pulp–derived progenitor cells have also been shown to have vasculogenic potential, both *in vitro* and when implanted into a murine model of hind limb ischemia (19). *In vivo*, the capability of the dental pulp to respond to damage has been elegantly modeled by Six et al (20), who showed that exposing rat molar pulp results in increased proliferating cell nuclear antigen (PCNA) expression and reparative dentin formation around the site of injury, indicating increased cell proliferation and differentiation in response to dental tissue damage (20). Furthermore, progenitor cells isolated from dental pulp and selected on the basis of their vasculogenic potential are capable of regenerating functional dental pulp with normal blood flow when encapsulated into collagen gels containing granulocyte colony-stimulating factor and implanted into pulpectomized canine teeth (21). Embryonic mesenchymal progenitor cells from a developing tooth germ, when combined with human gingival epithelial cells in a renal capsule system, are capable of forming whole teeth complete with developing roots and pulp structures (22).

This work clearly shows that the dental pulp has the theoretic capacity to regenerate both mineralized tissues and functional, complex pulp tissues. The ability of the pulp to synthesize reparative mineralized tissue in response to the destruction of the dentin matrix is of critical importance clinically. This matrix is required for the formation of a dentin bridge between dental restorations and surrounding dentin, preventing the continuum of microleakage, reinfection, and ultimate failure of the restoration (23–25). In terms of pulpal soft tissue regeneration, the diversity of cell types that can arise from pulpal mesenchymal progenitor cells represents an opportunity to repair and restore functional dental pulp, even in cases in which significant amounts of necrotic tissue have been extirpated.

In healthy and intact teeth, the dental pulp is highly effective at maintaining the structure of the tooth over relatively long periods of time because of a capacity to respond to damage and initiate repair. It is when this equilibrium is disrupted by damage to the pulp tissue caused by trauma or the formation of a carious lesion that the integrity of the tooth is undermined.

Inflammation in the Dental Pulp

Inflammation resulting from the formation of infected dental caries is a factor that commonly causes a disruption to the dynamic equilibrium of the dental pulp (Fig. 1). Bacteria from the oral cavity (eg, *Streptococcus mutans*), a key organism identified in the formation of carious lesions, attach to the enamel surface, eventually forming a biofilm that may consist of an entire mixed-population ecosystem of organisms including, lactobacilli, non–mutans streptococci, and *Actinomyces* (26, 27). The species found in these biofilms are acidogenic and are fed by fermentable carbohydrates from the oral cavity, causing acid erosion of the mineralized enamel as a result of their metabolic processes, damaging the enamel matrix, and enlarging the lesion (26–28). Once the bacteria erode the enamel and reach the dentin, they readily spread through the fluid-filled dentinal tubule system and rapidly cause an enlargement of the tooth area affected by the lesion. As bacteria colonize the tubules and draw nearer to the dental pulp, lipopolysaccharide (LPS) from bacterial cell walls penetrates the pulp and stimulates an inflammatory response from a variety of cells resident in the tissue (14, 29). Macrophages and neutrophils, as in many tissues, are important mediators of the innate inflammatory response in the dental pulp (5, 30). As inflammation progresses, B and T cells of the acquired immune system also infiltrate the pulp and contribute to the inflammatory response from the tissue (30). Once activated by LPS, these immune cells mediate the destruction of the pulpal tissues by secreting a range of proinflammatory cytokines; prime examples are interleukin (IL)-1 beta and tumor necrosis factor alpha (TNF-α) and tissue-degrading enzymes such as matrix metalloproteinases (31–33). Initially, inflammatory mediators are critical drivers of the repair process and stimulate reparative dentin formation by odontoblasts and the differentiation of progenitor cells into a repair phenotype (6, 34, 35). However, if expression of these mediators persists, inflammation becomes sustained in the pulp, creating a maelstrom of cytotoxic and tissue-disrupting effects and ultimately leading to tissue necrosis (8, 29, 31, 32, 36, 37).

The process of pulpal inflammation has been modeled in a number of experimental systems. Interstitial fluid isolated from rat dental pulp stimulated with LPS contains increased levels of locally produced IL-1α, IL-1β, and TNF-α (38). In a study using an organotypic rat mandible section model, Roberts et al (39) showed that the addition of *Streptococcus anginosus* group bacteria to whole dental pulp tissues up-regulates the expression of IL-1β and TNF-α by resident cells, leading to the destruction of pulpal tissues. Interestingly, odontoblasts themselves are also capable of initiating an inflammatory response (40). LPS stimulation of odontoblasts in a tooth crown organotypic culture model caused an up-regulation of IL-1β, TNF-α, and IL-8 (41). When the same culture model was prepared using teeth with advanced caries, up-regulation of IL-1β and TNF-α by native odontoblasts was observed (41). Odontoblasts have also been shown to induce neutrophil migration via IL-8 secretion in response to LPS stimulation (42).

In clinical terms, if there is any chance of regenerating pulpal tissues, it is clear that the lesion must be cleared of any microbial presence and pulpitis must be efficiently attenuated, preventing the destruction of the tissue and thereby facilitating the recruitment and differentiation of mesenchymal progenitor cells into repair phenotypes. The first battle is clearly against the microbial presence in a carious lesion. However, there is a second, equally critical struggle against the proinflammatory environment in the pulp created by these microbes, which cannot only damage the tissue but also interfere with its ability to self-repair.

Controlling Pulpitis to Create a Regenerative Environment

As acute, reversible inflammation in response to a microbial presence becomes established in the pulp, it enters a chronic phase, which can become self-sustaining and irreversible. Sustained pulpal inflammation not only damages the pulp tissue but also actively prevents the repair response by down-regulating the recruitment and differentiation of mesenchymal progenitor cells (8, 29, 31, 32). Macrophages play a pivotal role in the innate immune response, and it is now well established that they are capable of responding to various complex stimuli by polarizing into either proinflammatory (M_1) or proresolution (M_2) phenotypes, with many subtle variations on these 2 broad classifications (43–46). Macrophages arise from monocytes, homing to sites of tissue damage or infection and polarizing into an M_1 or M_2 phenotype (45). This polarization has been shown to be reversible (46), meaning that it is quite possible for macrophages to switch between these 2 states given the appropriate conditions (Fig. 2). Macrophages stimulated with IL-10 and transforming growth factor beta (TGF-β) decrease the production of inflammatory cytokines