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Dentin Degradation: From Tissue Breakdown to Possibilities for Therapeutic Intervention

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Abstract

Purpose of the Review Presently, dental materials science is driven by the search for new and improved materials that can trigger specific reactions from the affected tissue to stimulate repair or regeneration while interacting with the oral environment to promote or maintain oral health. In parallel, evidence from the past decades has challenged the exclusive role of bacteria in dentin tissue degradation in caries, questioning our understanding of caries etiopathogenesis. The goal of this review is to recapitulate the current evidence on the host and bacterial contributions to degradation, inflammation, and repair of the dentin-pulp complex in caries.

Recent Findings Contrasting findings attribute dentin breakdown to the activity of endogenous enzymes, such as matrix metalloproteinases (MMPs) and cathepsins, while the role of bacteria and their by-products in the destruction of dentin organic matrix and pulp inflammation has been for decades supported as an incontestable paradigm. Aiming to better understand the mechanisms involved in collagen degradation by host enzymes in caries, studies have showed that these proteinases are expressed in the mature dentin (i.e., after dentin formation) and become activated by the low pH in the acidic environment resulted by bacterial metabolism in caries. However, different host sources other than dentin-bound proteinases seem to also contribute to caries progression, such as saliva and pulp. Interestingly, studies evaluating pulp responses to bacteria invasion and inflammation in caries report higher levels of MMPs and cathepsins in inflamed tissue, but also showed MMP potential to resolve inflammation and stimulate wound healing. Notably, as reported for other tissues, MMPs exert dual roles in the dentin-pulp complex in caries, participating or regulating both degradative and reparative mechanisms.

Summary The specific roles of host and bacteria and their by-products in caries progression have yet to be clarified. The complex interactions between inflammation and repair in caries pose challenges to a clear understanding of the dentin-pulp complex responses and changes to bacteria invasion. However, it opens new venues for the development of novel therapies and dental biomaterials based on the modulation of specific mechanisms to favor tissue repair and healing.

Keywords Dental caries · Dentin · Dental pulp · Extracellular matrix · Matrix metalloproteinases · Cathepsins

Introduction: Caries Disease and the Evolution of Dental Restorative Materials

As one of the most prevalent non-communicable diseases, dental caries largely affects the global population. Current estimates indicate that 2 billion people have permanent teeth with caries lesions, and more than 500 million children present caries in primary teeth [1]. Over the last decades, scientific evidence led to a shift on our understanding of caries disease's mechanisms. These changes fundamentally impact how caries is diagnosed and managed (i.e., by assessment of patient's risks for development and/or progression of lesions) [2]. The concept of minimally invasive dentistry

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plays a central role in this novel approach, in which minimum intervention sets the basis for disease management [3, 4]. Accordingly, the operative parameters that guide cavity preparation and teeth restoration changed from a model of "extension for prevention," established by G.V. Black in 1891, to the selective carious tissue removal. This new model aims at preserving the tissue that can be still repaired/regenerated and only removing the necessary tissue to provide support for the final restoration [5]. Such an approach becomes feasible only because in parallel, the dental materials have evolved from being non-adhesive materials (i.e., dental amalgam), which require extensive tissue removal to ensure proper retention, to materials that can be bonded micromechanically to the tooth structure. Thus, other than proposing an esthetic revolution, the advent of adhesive dentistry allowed for more conservative management of tooth decay.

Interestingly, the advances in Cariology research that provided a better appreciation of the disease also inspired material research and development. For decades now, the dental materials science has been diligently focused on the design of materials that are not only capable of replacing the tissue lost by caries with mechanical and esthetic competence, but also of eliciting host-tissue biological responses. Novel and next generation of dental materials and restorative therapies are expected to promote tissue repair and/or trigger endogenous reactions that include the remineralization of dental hard tissues [6, 7], the strengthening of dentin organic matrix [8, 9], the control of biofilm formation and activity [10], the induction of pulp responses to promote dentin repair and resolve inflammation [11], etc. While a tremendous advancement in the development of dental restorative materials is already in course, the future of dental materials science and regenerative dentistry still requires clear understanding of the complexity of the tissue's responses to caries progression, particularly related to dental tissues' destruction, pulp inflammation, and reparative mechanisms.

As caries lesions progress into dentin leading to the destruction of its organic components and pulp inflammation, a significant constraint is posed to the more conservative (i.e., non-surgical) management of the disease. For example, carious dentin is mechanically compromised due to several modifications in its proteins' (including collagen and non-collagenous proteins) structure and organization [12]. Moreover, dentin is also dramatically affected by the mineral loss, which causes a gradient in its relative content and composition at the different zones of the lesion [13]. As we have deepened our comprehension on the responses of the dentin-pulp complex to bacterial invasion, less invasive techniques of tissue handling and removal associated with the use of materials that can trigger pulp repair and tertiary dentin formation have been proposed. Yet, more predictable results using the existing materials as well as the development of novel minimally invasive treatment options for dentin caries require a clear understanding of tissue degradation and reparative mechanisms.

Research from the past decades supports the hypothesis of an endogenous mechanism to explain dentin breakdown in caries, which contests the exclusive involvement of bacteria in caries disease/lesion development and progression. This review focuses on discussing the scientific evidence that show the host and bacterial contributions to dentin organic matrix destruction in caries progression as well as alludes to their role also in inflammation resolution and tissue repair. Interestingly, these three mechanisms — tissue destruction, inflammation, and repair — are intricately connected and should be taken into consideration in the design of new dental materials. Advancing our knowledge of exogenous and endogenous mechanisms taking place in caries progression will lead to exciting new venues in caries management and development of novel reparative and restorative dental materials to promote oral health.

Dentin Degradation in Caries: Enzymes and Mechanisms Driving Tissue Breakdown

The Role of Host and Bacterial Enzymes in Dentin Degradation

The breakdown of the dentin substrate in caries has been traditionally attributed to the metabolic activity of bacterial enzymes/by-products of dysbiotic biofilms. However, evidence from the last 25 years has challenged the concept of bacterial exclusivity in guiding caries progression. The exact contribution of host and bacterial enzymes and their roles in carious dentin degradation remain unclear, but there is growing evidence of their interplay. As early as in 1983, Dayan et al. proposed that a collagenase with latent activity would be present in the dentin, suggesting its involvement with a slow rate of degradation of collagen fibrils within the demineralized tissue [14]. In addition, the lack of correlation between levels of dentin degradation and gelatinolytic activity of bacteria in studies in vitro and in situ resulted in the first speculations that collagen could be initially cleaved by endogenous collagenases from macrophages or polymorphonuclear leukocytes [15]. The presence and activity of dentin proteinases were later confirmed and further characterized, with the identification of several matrix metalloproteinases (MMPs) in carious soft dentin and increased gelatinolytic activity in acidic conditions [16]. In addition, salivary MMPs showed potential gelatinolytic activity but only after acid activation. Those findings served to ground the hypothesis of a bacterial acid-activation of dentin MMPs followed by subsequent hydrolysis of dentin collagen when the pH of oral fluids

returns to neutral [16]. While most of the evidence from studies published in the 2000s point out the role of MMPs in caries progression came from in vitro investigations, a patent reduction in the rate of caries lesions' progression in rats that received MMP inhibitors firmly corroborates with such a hypothesis [17]. More recently, a decrease in MMP activity in both active and chronic lesions with an increase in the age of the subjects was reported for carious dentin excavated from human teeth, regardless of the extension of the lesions [18]. These in vivo findings confirmed the contribution of dentin-bound MMPs to caries progression. In addition, human studies showed changes in the expression of MMPs, type I collagen, and bone sialoprotein in carious dentin after restoration that strongly support the hypothesis of host enzymes' involvement in the progression of caries [19, 20].

In parallel, Cariology studies have associated dentin degradation with bacterial invasion. Nonetheless, the use of dentin samples (which likely contain endogenous enzymes) or single species of bacteria in many of these in vitro studies raises questions about the exclusive role of bacterial collagenases in promoting the dentin collagen proteolysis [21, 22]. For example, bacteria showed ability to degrade gelatin (denatured collagen), but did not exhibit collagenolytic activity against sound demineralized dentin [23]. A recent study demonstrated degradation of type I collagen mainly by secreted proteins from *S. mutans*, with an increased degradative potential in the late growth stage of this bacteria [24]. Another in vitro investigation reported that specific mutant strains of *S. mutans* can express proteins with affinity to collagen type I, but other studies failed to either exclude the contribution of host MMPs or demonstrate the role of *S. mutans* proteins in collagen binding and hydrolysis in animal caries models [25].

While these findings raise questions about the actual role of bacteria and host proteinases in dentin caries progression, they highlight the gap in the knowledge regarding the specific contribution of the different exogenous and endogenous enzymes in tissue breakdown. While controversial results exist in the literature, based on recent reports, it is wise to state that both contribute for tissue breakdown. Therefore, unanswered questions include when and how these proteinases work together to result in dentin proteolysis. Possibly, as previously speculated, endogenous enzymes could initiate collagen degradation after removal of extrafibrillar minerals due to their telopeptidase activity, but salivary or bacterial collagenases would contribute to complete destruction of the collagen triple-helix only after demineralization has advanced [26]. Therefore, future studies focused on the interplay between bacterial and host enzymes in carious dentin breakdown rather than attributing tissue degradation exclusively to one source of enzymes are encouraged.

Different Host Enzymes, Their Release and Activation in Caries Progression

The first family of host enzymes to be studied in the progression of dentin caries is the MMPs. MMPs comprise a family of 28 calcium- and zinc-dependent endopeptidases able to degrade a variety of proteins in the extracellular matrix and regulate different cellular and signaling pathways [27]. MMPs participate in physiological and pathological processes to promote cell proliferation, migration, differentiation, and apoptosis as well as are involved in tissue immune response, repair, and angiogenesis. Synthesized and secreted in an inactive form, MMP activity is finely regulated at the expression level, by their activation from zymogen to active form, and by the action of endogenous tissue inhibitors of MMPs (TIMPs) [27]. In addition, MMP activity is highly dependent on the tissular concentration of metal ions such as zinc and calcium [28].

Several MMPs were identified in dentin, pulp, and odontoblasts including mainly MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, and MMP-20 [29–34]. Evidence points out that MMPs are synthesized by the odontoblasts, released, and incorporated into the dentin mineralized matrix [33, 35]. MMPs are also present in the dentinal fluid and dentinal tubules, possibly a result of more recent odontoblast response to injuries or other stimuli and are considered odontoblast-derived instead of dentinal MMPs (immobilized into the mineralized matrix) [33]. The differential location of MMPs in mineral-bound and mineral-unbound protein extracts [35] as well as in the dentinal fluid [36] suggests specific roles for these enzymes, which is discussed in detail as follows. The specific proportion of these enzymes in dentinal fluid and dentin tissue bound and unbound to the minerals is still unknown. Most studies reported increased presence of some of these enzymes in carious than in sound tissue [18, 37–40]. Interestingly, there are very few studies reporting decreased presence or activity of MMPs in caries when compared to sound condition [36]. Table 1 compiles a number of in vitro, in situ, and in vivo studies that reported the presence, levels, and activity of MMPs in coronal dentin in caries.

Tjäderhane et al. [16] were the first to propose the acid-activation mechanism to explain the release and activation of MMPs in carious dentin. Accordingly, that classic study hypothesized that as dentin is demineralized by the solubilization of hydroxyapatite due to the release of organic acids by the bacteria, the organic components of dentin extracellular matrix (ECM), including matrix-bound MMPs, become exposed and prone to contribute for the tissue breakdown. Building upon this hypothesis, it has been further suggested that the decrease in the carious dentin milieu pH, caused by increased concentration of organic acids, could lead to a chemical activation of MMPs by disturbance of the

Table 1 Summary of in vitro, in situ, and in vivo studies reported herein that evaluated presence, levels, activity, and/or other mechanisms of MMPs in coronal dentin and saliva in caries and in pulp inflammation. The literature presented in the table shows MMPs involved in tissue degradation, remodeling, repair, and inflammation

References	Tissue/Source	MMPs	Expression/activity	Function/mechanism
[16]	Sound and carious dentin (permanent teeth)	MMP-2 MMP-8 MMP-9	Presence of MMPs in carious dentin Gelatinolytic activity in carious dentin MMP-9 was the predominant gelatinase in carious dentin	MMPs associated with caries progression in dentin
[37]		MMP-2 MMP-9	Increased levels of MMPs and less collagen detection in caries than in sound dentin	
[40]		MMP-2	Increased levels of MMPs in carious than in sound dentin, especially at deep areas of the lesion	
[17]	Caries lesions (animal study)	Not specified	Reduction of size of caries lesions when using broad-spectrum MMPs inhibitors	
[38]	Sound and carious dentin (permanent teeth)	MMP-2	Increased levels of MMPs in the dentinal tubules in carious tissue, but absence in sound dentin	MMPs associated with caries progression in dentin, but actively secreted by odontoblasts
[83]	Sound and carious dentin (permanent teeth)	MMP-2	Increased presence of MMP-2 in carious dentin with higher levels of this enzyme in superficial layer of the lesion Higher gene expression for MMP-2 in odontoblasts adjacent to carious lesions than in healthy teeth More contribution from MMP-2 to overall gelatinolytic activity in deep layers of the caries lesions (reactionary dentin)	MMP-2 might be involved in remineralization of tertiary dentin by cleaving DSP from DSPP MMP-2 might release and activate TGF-β1 from dentin to stimulate collagen synthesis by odontoblasts
[39]	Carious dentin (permanent teeth)	MMP-2 MMP-8 MMP-9 MMP-20	Same levels of MMP-20 when comparing inner and outer layers of the caries lesions, but more MMP-8 and MMP-9 in outer layers and more MMP-20 in inner layers of the lesions	MMPs associated with caries progression in dentin MMP-20 might be involved in early changes of the non-collagenous dentin matrix Contribution of saliva to the increased levels of MMPs in outer dentin in caries lesions
[19]	Carious dentin (permanent teeth)	MMP-2 MMP-8 MMP-9	MMPs present in intertubular and intratubular dentin Reduced levels of MMP-8 in carious tissue after removal and restoration	MMPs might be associated with dentin remodeling and healing processes
[20]	Carious dentin (primary teeth)	MMP-2 MMP-8 MMP-9	MMP-2 and MMP-9 localized around dentin tubules MMP-8 present throughout the organic matrix Increased MMPs expression after carious tissue removal and restoration	
[36]	Dentinal fluid from carious teeth	MMP-2 MMP-9	Increased amount of MMP-9 in dentinal fluid from deep lesions, same levels of MMP-2	MMPs (mainly MMP-9) could be considered a potential biomarker for pulp inflammation
[16] [17]	Saliva	Not specified	Increased gelatinolytic activity and dentin degradation by acid-activation saliva Activation dependent on the pH	Acid-activation of MMPs in caries
[69] [86]	Healthy and inflamed dental pulp (primary and permanent teeth)	MMP-1 MMP-8 MMP-9 MMP-13	Higher levels of MMPs in inflamed pulps	MMPs are involved in dental pulp matrix degradation in inflammation

Table 1 (continued)

References	Tissue/Source	MMPs	Expression/activity	Function/mechanism
[71]	Inflamed pulp	MMP-3	MMP-3 application to pulps with experimentally induced inflammation downregulated the expression of interleukins and nitric oxide, reducing inflammation, and also accelerates angiogenesis and wound healing	MMP-3 possesses anti-inflammatory functions, accelerates angiogenesis and wound healing
[72]				The anti-inflammatory effect of MMP-3 is seen in mild pulpitis, but not in severe pulpitis model
[73]				
[68]	Matrix proteins from sound dentin	MMP-1	Pulp capping with dentin matrix protein digested by MMPs resulted in tertiary dentin formation, mainly when digestion was performed with MMP-1, MMP-9, MMP-13, or MMP-20	Endogenous MMPs may be involved in the wound healing process of the dentin-pulp complex
[75••]		MMP-2		Even though MMPs partially digest dentin after demineralization, the released molecules (for example protein S100-A7) have bioactive functions and potential to induce pulp wound healing
[78]		MMP-3		
		MMP-8		
		MMP-13	Digestion of dentin matrix proteins with MMP-20 resulted in the most bioactive components for tertiary dentin stimulation	
		MMP-20		

cysteine-switch present in their active site [42]. Thus, once activated in an exposed demineralized dentin ECM, MMPs will be available not only to mediate collagen's degradation, but also contribute with the breakdown of other organic components of dentin (Fig. 1). While the acidic environment is not ideal for MMP proteolytic activity, the dentin buffering mechanism, which returns the pH to neutral, is thought to allow MMPs to act cleaving dentin organic components. The acid-activation mechanism of MMPs in dentin is supported by other in vitro studies [29, 41], and it is considered to be key for the activity of cysteine cathepsins as well.

Cathepsins comprise another clan of enzymes shown to be highly expressed in carious dentin [18, 37]. Similar to MMPs, cathepsins regulate several physiological and pathological processes, participating in tissue degradation in cancer, cardiovascular and bone diseases, and arthritis [42]. Cathepsin functions seem to go beyond tissue remodeling as they appeared to be prevalently involved in processing of inflammatory markers and signaling pathways that contribute to disease progression. Cathepsins are also synthesized as proenzymes, their activity is regulated by pH and endogenous inhibitors, and a mildly acidic pH is required for the activity of most cathepsins [42]. Identified in dentin, pulp, saliva, and odontoblasts [18, 37, 43, 44], cathepsins were shown to have their expression and activity remarkably augmented in carious dentin in comparison to healthy teeth [18, 37].

Evidence shows that MMPs can also be activated by other proteinases [45]. It has been suggested that cathepsins work together with MMPs in dentin destruction in caries in a mechanism where cathepsins can activate MMPs, cleave and inactive TIMPs, and/or directly degrade the extracellular matrix [18]. Therefore, one of the roles of cathepsins in caries lesion progression is amplifying the degradative process. Interestingly, evidence suggests that, even though cathepsins are present in the dentin, the main source of these enzymes is the pulp tissue in response to bacterial invasion in caries. Important data from Nascimento et al. [18] shows the greatest activity of cathepsins in deep caries lesions in teeth from young patients, suggesting that most of these enzymes are synthesized by the odontoblast in response to the bacteria invasion (Fig. 1).

Besides interaction between different families of host enzymes, the potential of bacterial proteinases to activate MMPs, which is reported in other diseases and conditions [46], is still underestimated in caries. A cysteine (thiol-) proteinase from *P. gingivalis* showed potential to activate MMP-1, MMP-3, and MMP-9 [47], while fibroblasts cultured in the presence of bacterial products from *P. gingivalis* exhibited increased ability to degrade both collagen and activated MMPs (i.e., MMP-1, MMP-2, MMP-3, and MMP-14) [48]. In addition, reduced levels of TIMP-1 expressed by fibroblast treated with by-products of *P. gingivalis* were

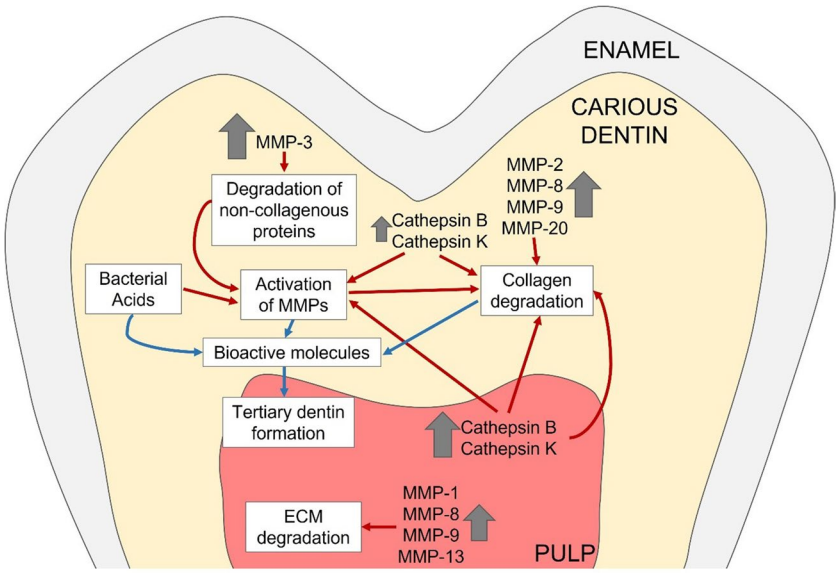

Fig. 1 Changes in the levels/activity of MMPs and cysteine cathepsins in dentin-pulp complex and the associated degradative (in red) and reparative (in blue) mechanisms. Caries progression into dentin exposes MMPs from the dentin matrix resulting in higher levels of these enzymes in carious tissue [37–40, 83]. Following demineralization, bacterial acids can activate MMPs to start degradation of the dentin organic matrix [16]. The acidic environment can also activate cysteine cathepsins present in the dentin [18], which in turn have the potential to both degrade collagen as well as activate other dentinal MMPs. In addition, the acids from bacteria, by promoting dentin demineralization, release bioactive components from the matrix that can stimulate pulp response and repair [57]. Other bioactive molecules can also be released from the dentin matrix after its digestion by some MMPs [75••]. In pulp, as bacteria invasion progresses, by-products stimulate different cells in the pulp tissue to express higher levels of MMPs, which are involved in pulp extracellular matrix degradation and tissue inflammation [69, 70]

reported [48], which indicated that MMP activity may be regulated at the level of TIMP-MMPs complexes by such bacteria metabolite. More recently, the effects of bacteria endotoxin on the activity of cathepsin K in dentin were also described. In effect, the contamination of dentin with bacterial lipopolysaccharides (LPS) presented an increased telopeptidase activity [49]. According to Bafail et al. [49], negatively charged LPS could interact with the positively charged residues of cathepsin K, modulating the activity of this enzyme in vitro. Therefore, additional studies are required to fully distinguish the interactions between MMPs and cathepsins in caries progression. In addition, a comprehensive characterization of the interactions between host and bacterial proteinases to degrade the dentin in caries is still missing and will be fundamental for the development of predictable clinical therapies to arrest lesion progression by reducing tissue degradation.

Mechanisms of Dentin Proteolytic Degradation

Interestingly, collagen structure, organization, and cross-linkage make it resistant to degradation by many proteinases. Specifically looking at collagen breakdown by proteinases (not necessarily dentinal collagen), only some MMPs and bacterial collagenases are able to degrade it in its helical structure and are considered true collagenases (MMP-1, MMP-8, MMP-13), while other enzymes (MMP-2 and MMP-9) can only hydrolyze collagen in its denatured form [50]. The main difference between bacterial collagenolytic enzymes and MMPs is that, while MMPs degrade collagen at a specific site to release three-quarter N-terminal and one-quarter C-terminal fragments, bacterial enzymes hydrolyze the collagen molecule into a mix of multiple peptides. However, some bacterial collagenases present a cleavage pattern of collagen that is similar to that of MMPs, at least in the first step of the degradation [50]. Among cathepsins, cathepsin K can cleave collagen at multiple sites, making this enzyme a potent collagenase [51]. Some MMPs and cathepsins also have the ability to cleave collagen at the terminal telopeptides.

As already mentioned in the previous section, once demineralized by the bacteria-derived acid, the dentin organic matrix becomes exposed, displaying collagen and MMPs, including acid-activated proteinases. Modifications in the organic components also take place in carious dentin, including molecular changes or possible denaturation of collagen, reduction in the levels of proteoglycans, and loss of collagen periodicity [37, 52]. In fact, loss of collagen periodicity [53] combined with reduction of auto-fluorescence from molecularly intact collagen in caries-affected dentin [37] suggests that collagen modifications occur even at early stages of caries development. As already discussed in the literature, changes

in collagen organization and mineralization (presence of extra- and intrafibrillar minerals) could dictate the action of different proteinases [26]. It has been speculated that demineralization-remineralization cycles in caries initially remove extrafibrillar minerals and might expose some intrafibrillar minerals [26]. Possibly, the removal of extrafibrillar minerals from collagen exposes the C-terminal telopeptides to the action of endogenous telopeptidases (such as MMP-2, MMP-9, and cathepsin K) resulting in loss of collagen periodicity. Then, as demineralization advances and more telopeptides are removed, intrafibrillar minerals are lost, facilitating the collagen breakdown by more telopeptidases (cleaving the N-terminal telopeptides) and resulting in gradual degradation of collagen. Bacterial collagenases would then be able to continue the collagen destruction at this stage, but only after disassembling or initial degradation of the helical part of collagen [26]. However, these mechanisms have yet to be proven.

Lastly, it is important to highlight that some MMPs and cathepsins cleave non-collagenous proteins or interact with other proteins, still contributing to lesion progression but either regulating other mechanisms and/or destroying extracellular components other than collagen. For example, the degradation of decorin, byglican, and other SIBLING (small integrin-binding ligand, N-linked glycoprotein) proteins by MMP-3 releases dentinal molecules capable of activating other MMPs [52, 54] (Fig. 1). Regarding activity of cathepsins in caries, specific functional mechanisms of tissue degradation by these enzymes remain elusive and rely on the description of the function of these proteinases in other tissues. While both cathepsin B and K can contribute to extracellular matrix destruction, including dentin in caries, cathepsin K is referred as the most potent collagenase in promoting bone resorption [55]. Cathepsins could have several roles in dentin breakdown by regulating MMP-TIMP complexes, activating MMPs, and interacting with other non-collagenous proteins in dentin, as proposed by Nascimento et al. [18]. Cathepsin K activity is regulated by proteoglycans possibly by binding to glycosaminoglycans attached to collagen and then recruiting another enzyme that will unwind the triple helical collagen to facilitate its cleavage [56]. As already mentioned, novel research in dentin degradation in caries should consider all these potential interactions between different enzymes from the same or different families and sources as well as their interactions with other proteinases to facilitate tissue breakdown in caries.

Contribution of Dentin Matrix Components and Host Enzymes to the Dentin-Pulp Degradation-Repair Interplay

Dentin shares similar composition with bone, while key differences in morphology and behavior of its cells, the odontoblasts, make dentin a unique tissue. Apart from the inorganic content, mainly hydroxyapatite crystals, dentin extracellular matrix components are mainly made of type I collagen (i.e., ~ 90 wt%) intermingled with non-collagenous proteins, which include phosphorylated proteins (i.e., SIBLINGs), non-phosphorylated proteins (i.e., osteocalcin), and proteoglycans, growth factors, cytokines, neuropeptides and neurotrophic factors, and serum or plasma proteins [57]. Many of these components of the dentin organic matrix are involved in tooth formation but also possess potential to trigger specific reparative mechanisms in the dentin-pulp complex. For example, SIBLINGs, osteocalcin, and proteoglycans regulate or participate in mineralization events, and dentin matrix protein-1 (DMP-1) is also involved in dentin mineralization by regulating nucleation and formation of hydroxyapatite crystals [58–60]. Growth factors also have key roles in stimulating mineralization and dentin-pulp regeneration, mainly promoted by transforming growth factor-β1 (TGF-β1) and bone morphogenetic proteins (BMPs) [61–63].

As caries lesions initiate and progress, bacteria and their components will trigger a variety of innate, immune, and inflammatory responses from the dentin-pulp complex [64•]. From morphological changes in odontoblasts seen in early-stage enamel lesions, continuous demineralization and breakdown will lead to cavitation. Once the dentin is exposed, demineralization following bacterial invasion and diffusion of their products into the dentin tubules results in hypermineralized dentin and tertiary dentin formation to stop the progression of the insult to the pulp and resolution of inflammation [65]. If left untreated, chronic inflammation can progress to pulp necrosis and loss of the tissue function and potential to respond to diverse stimuli.

Changes in MMPs levels have been also reported in inflamed pulp tissue in caries-affected teeth [66–68] (Table 1). Together with increase in interleukins (IL), increased levels of MMP-1, MMP-8, MMP-9, and MMP-13 have been reported in inflamed pulps in comparison to healthy tissue [69], with data suggesting that the nuclear factor kappa light chain enhancer of activated B cells ($NF\text{-}_{K}B$) signaling pathway controls the expression of some of these MMPs by odontoblasts [70]. However, MMP presence and activity have also been attributed to the pulp response to caries and bacteria invasion by triggering reparative mechanisms. For example, MMP-3 showed potential to act reducing inflammation in LPS-treated pulps in vivo [71]. In addition, in a mild pulpitis animal model, pulp treatment with MMP-3 resulted in absence of inflammatory cells, low levels of IL-6, and increased vascularization, confirming the role of this enzyme in pulp repair/regeneration [72, 73]. These studies shine light on the fact that MMPs can regulate both host defense and pathological processes in caries like in other inflammatory diseases, and their functions depend on the type of cell, substrate, and disease [74].

Interestingly, as proposed by Smith et al. in 1995, the traditional views of odontoblast stimulation by acid released by bacteria metabolism are likely to be overlooked by the idea of dentin solubilization by the acids from bacteria [65]. Following dentin demineralization, growth factors and other molecules are exposed and released and will in turn stimulate odontoblasts to secrete reactionary dentin [65]. Since then, several studies have proved the complexity of cellular and molecular events in tissue degradation and repair in caries progression, which are intricately interconnected [57, 75••, 76, 77]. In fact, inflammation and regeneration responses are linked in a way that many pro-inflammatory molecules are also implicated in signaling reparative processes [77]. In this context, studies showed that dentin demineralization by bacterial acid releases a complex cocktail of bioactive molecules capable of inducing different processes including cell migration and tissue mineralization [57, 78, 79]. A variety of in vitro and animal studies support the concept of the dentin as a reservoir of several bioactive molecules that can trigger reparative mechanisms of the odontoblasts (Table 1). These bioactive molecules released from the dentin showed potential to increase pulp and apical papilla stem cell proliferation and differentiation, and expression of genes related to odontogenic lineage [79, 80]. Therefore, dentin degradation and repair in caries progress should always be investigated as coordinating mechanisms that elicit the host response to bacterial invasion. The same concept should be applied to the investigation of the role of bacteria and MMPs in such responses.

Investigating in more detail the reparative potential of dentin matrix components, Okamoto et al. hypothesized that MMP-digested dentin proteins could stimulate reparative mechanisms in the pulp, without any benefits of using MMPs only or undigested dentin proteins [75••]. More specifically, to prove the reparative potential of MMP-digested dentin proteins, the authors used dentin-extracted protein mixtures embedded in gelatin sponges for pulp capping in an animal model and reported tertiary dentin formation when dentin proteins were digested by MMP-1, MMP-9, MMP-13, and MMP-20 (Table 1). In the same study, MMPs alone did not affect the cells response in vitro or the pulp repair in vivo, confirming the key role of MMP digestion of dentin components to stimulate tissue repair. Later on, using proteomic analysis, the same research group identified protein S100-A7 as the major bioactive molecule released from MMP-20 digestion of dentin matrix proteins with potential to induce tertiary dentinogenesis in a direct pulp capping model [78]. These results support the role of MMPs in the wound healing of the dentin-pulp complex and demonstrate that even though tissue degradation is a result of MMP activity in carious dentin, the by-products generated by some of these proteinases can have a positive impact on the tooth response to caries progression and inflammation. Similarly, cathepsins could also be involved in dentin-pulp complex repair; however, there is little evidence in the literature to support that. It has been suggested that cathepsins could release bioactive molecules from the dentin after the tissue degradation. For example, TGF-β1, which is known to be involved in the activity of the odontoblasts in tissue repair, upregulated the expression of cathepsin B in pulp cells, suggesting that this proteinase could be involved in dentin-pulp response in caries [81].

Besides the release of specific bioactive molecules from the degraded dentin, the activity of MMPs can also result in activation of some key proteins for tissue repair. For example, membrane-type 1 matrix metalloproteinase (MT1-MMP) can activate latent TGF-β complexes from the subendothelial extracellular matrix in a process that requires activities of other signaling pathways [82]. Higher levels of MT1-MMP were reported in response to caries, so it is possible that this mechanism takes place in the dentin-pulp complex. MMP-2 also seems to be greatly involved in tertiary dentin formation. Charadram et al. proposed that MT1-MMP could activate MMP-2 and, considering the high levels of MMP-2 and dentin sialoprotein (DSP) in reactionary dentin in carious teeth and other findings reported in the literature, it is possible that MMP-2 facilitates the release of dentin sialophosphoprotein DSPP into DSP to favor tissue mineralization [83].

This interesting knowledge of inflammation-repair interplay and the dual role of MMPs in these processes have inspired the development of novel restorative materials and should continue to open new venues for dental materials research, mainly bioactive materials to treat dentin and pulp in caries lesions. An already explored approach is to stimulate pulp wound healing and dentin repair by pulp capping materials that can modulate tissue's response to caries progression. Mineral trioxide aggregate (MTA) promotes release of bioactive molecules sequestered in dentin to stimulate cellular response in the pulp favoring dentin-pulp complex repair [84]. For tissue regeneration purposes, the use of scaffolds containing MMP-2 cleavage sites has been proposed recently and showed that MMP-2 facilitated binding of growth factors into the scaffold to promote their sustained release both in vitro and in vivo [85]. In terms of diagnostic purposes, measuring the levels of MMP-9 in dentinal fluid can be used to aid in the diagnosis of pulp vitality and level of inflammation for clinical decision-making [86].

Conclusion

Combined with the multifactorial etiology of caries, the specific contributions of host and bacterial proteinases and their by-products to dentin breakdown and lesion progress remain unclear. Therefore, further research is still needed to better understand the specific roles of proteinases from

different sources (bacteria, dentin, pulp, and saliva) in tissue breakdown. In addition, dentin-pulp inflammation and regeneration are intertwined in caries. These complex and intricate mechanisms linking inflammation and regeneration open new venues to explore therapeutic possibilities to accelerate natural tissue healing and resolve inflammation. While the ultimate goal would be to promote tissue repair and regeneration, these therapies are far from being translated into clinical practice for treating dentin caries. However, dental materials scientists should continue to focus on shifting from restoration after tissue removal to the stimulation of innate and immune dentin-pulp complex responses based on the modulation of key pathways that favor repair. This concept departs from the status quo as it pertains to targeting broad MMP inhibition to prevent dentin degradation to the idea of taking advantage of the properties of these enzymes to stimulate dentin repair while avoiding further tissue breakdown.

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Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent Not applicable.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Jain N, Dutt U, Radenkov I, Jain S. WHO's global oral health status report 2022: actions, discussion and implementation. Oral Dis 2023. https://doi.org/10.1111/odi.14516
2. Featherstone JDB, Chaffee BW. The Evidence for Caries Management by Risk Assessment (CAMBRA(R)). Adv Dent Res. 2018;29(1):9–14. https://doi.org/10.1177/0022034517736500.
3. Giacaman RA, Fernandez CE, Munoz-Sandoval C, Leon S, Garcia-Manriquez N, Echeverria C, et al. Understanding dental caries as a non-communicable and behavioral disease: management implications. Front Oral Health. 2022;3:764479. https://doi.org/10.3389/froh.2022.764479.
4. Slayton RL, Urquhart O, Araujo MWB, Fontana M, Guzman-Armstrong S, Nascimento MM, et al. Evidence-based clinical practice guideline on nonrestorative treatments for carious lesions: a report from the American Dental Association. J Am Dent Assoc. 2018;149(10):837-49 e19. https://doi.org/10.1016/j.adaj.2018.07.002.
5. Schwendicke F, Walsh T, Lamont T, Al-Yaseen W, Bjorndal L, Clarkson JE, et al. Interventions for treating cavitated or dentine carious lesions. Cochrane Database Syst Rev. 2021;7(7):CD013039. https://doi.org/10.1002/14651858.CD013039.pub2.
6. Yun J, Burrow MF, Matinlinna JP, Wang Y, Tsoi JKH. A narrative review of bioactive glass-loaded dental resin composites. J Funct Biomater 2022;13(4). https://doi.org/10.3390/jfb13040208
7. Bacino M, Girn V, Nurrohman H, Saeki K, Marshall SJ, Gower L, et al. Integrating the PILP-mineralization process into a restorative dental treatment. Dent Mater. 2019;35(1):53–63. https://doi.org/10.1016/j.dental.2018.11.030.
8. Trevelin LT, Alania Y, Mathew M, Phansalkar R, Chen SN, Pauli GF, et al. Effect of dentin biomodification delivered by experimental acidic and neutral primers on resin adhesion. J Dent. 2020;99:103354. https://doi.org/10.1016/j.jdent.2020.103354.
9. Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, et al. Dentin biomodification: strategies, renewable resources and clinical applications. Dent Mater. 2014;30(1):62–76. https://doi.org/10.1016/j.dental.2013.10.012.
10. Fugolin AP, Dobson A, Huynh V, Mbiya W, Navarro O, Franca CM, et al. Antibacterial, ester-free monomers: polymerization kinetics, mechanical properties, biocompatibility and anti-biofilm activity. Acta Biomater. 2019;100:132–41. https://doi.org/10.1016/j.actbio.2019.09.039.
11. Emara R, Elhennawy K, Schwendicke F. Effects of calcium silicate cements on dental pulp cells: a systematic review. J Dent. 2018;77:18–36. https://doi.org/10.1016/j.jdent.2018.08.003.
12. Marshall GW, Habelitz S, Gallagher R, Balooch M, Balooch G, Marshall SJ. Nanomechanical properties of hydrated carious human dentin. J Dent Res. 2001;80(8):1768–71. https://doi.org/10.1177/00220345010800081701.
13. Liu Y, Yao X, Liu YW, Wang Y. A Fourier transform infrared spectroscopy analysis of carious dentin from transparent zone to normal zone. Caries Res. 2014;48(4):320–9. https://doi.org/10.1159/000356868.
14. Dayan D, Binderman I, Mechanic GL. A preliminary study of activation of collagenase in carious human dentine matrix. Arch Oral Biol. 1983;28(2):185–7. https://doi.org/10.1016/0003-9969(83)90126-7.
15. van Strijp AJ, van Steenbergen TJ, de Graaff J, ten Cate JM. Bacterial colonization and degradation of demineralized dentin matrix in situ. Caries Res. 1994;28(1):21–7. https://doi.org/10.1159/000261615.
16. Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res. 1998;77(8):1622–9. https://doi.org/10.1177/00220345980770081001.
17. Sulkala M, Wahlgren J, Larmas M, Sorsa T, Teronen O, Salo T, et al. The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. J Dent Res. 2001;80(6):1545–9. https://doi.org/10.1177/00220345010800061301.
18. Nascimento FD, Minciotti CL, Geraldeli S, Carrilho MR, Pashley DH, Tay FR, et al. Cysteine cathepsins in human carious dentin. J Dent Res. 2011;90(4):506–11. https://doi.org/10.1177/0022034510391906.
19. Kuhn E, Reis A, Campagnoli EB, Chibinski AC, Carrilho MR, Wambier DS. Effect of sealing infected dentin with glass ionomer cement on the abundance and localization of MMP-2, MMP-8, and MMP-9 in young permanent molars in vivo. Int J Paediatr Dent. 2016;26(2):125–33. https://doi.org/10.1111/ipd.12167.
20. Chibinski AC, Gomes JR, Camargo K, Reis A, Wambier DS. Bone sialoprotein, matrix metalloproteinases and type I collagen expression after sealing infected caries dentin in primary teeth.

Caries Res. 2014;48(4):312–9. https://doi.org/10.1159/000355302.
21. Goldberg M, Keil B. Action of a bacterial Achromobacter collagenase on the soft carious dentine: an in vitro study with the scanning electron microscope. J Biol Buccale. 1989;17(4):269–74.
22. Nyvad B, Fejerskov O. An ultrastructural study of bacterial invasion and tissue breakdown in human experimental root-surface caries. J Dent Res. 1990;69(5):1118–25. https://doi.org/10.1177/00220345900690050101.
23. van Strijp AJ, van Steenbergen TJ, ten Cate JM. Bacterial colonization of mineralized and completely demineralized dentine in situ. Caries Res. 1997;31(5):349–55. https://doi.org/10.1159/000262417.
24. Huang B, Stewart CA, McCulloch CA, Santerre JP, Cvitkovitch DG, Finer Y. Streptococcus mutans proteases degrade dentinal collagen. Dentistry Journal. 2022;10(12):223.
25. Crowley PJ, Brady LJ, Michalek SM, Bleiweis AS. Virulence of a spaP mutant of Streptococcus mutans in a gnotobiotic rat model. Infect Immun. 1999;67(3):1201–6. https://doi.org/10.1128/IAI.67.3.1201-1206.1999.
26. Tjaderhane L, Buzalaf MA, Carrilho M, Chaussain C. Matrix metalloproteinases and other matrix proteinases in relation to cariology: the era of "dentin degradomics." Caries Res. 2015;49(3):193–208. https://doi.org/10.1159/000363582.
27. Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. Prog Mol Biol Transl Sci. 2017;147:1–73. https://doi.org/10.1016/bs.pmbts.2017.02.005.
28. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92(8):827–39. https://doi.org/10.1161/01.RES.0000070112.80711.3D.
29. Mazzoni A, Mannello F, Tay FR, Tonti GA, Papa S, Mazzotti G, et al. Zymographic analysis and characterization of MMP-2 and -9 forms in human sound dentin. J Dent Res. 2007;86(5):436–40. https://doi.org/10.1177/154405910708600509.
30. Mazzoni A, Maravic T, Tezvergil-Mutluay A, Tjaderhane L, Scaffa PMC, Seseogullari-Dirihan R, et al. Biochemical and immunohistochemical identification of MMP-7 in human dentin. J Dent. 2018;79:90–5. https://doi.org/10.1016/j.jdent.2018.10.008.
31. Mazzoni A, Papa V, Nato F, Carrilho M, Tjaderhane L, Ruggeri A Jr, et al. Immunohistochemical and biochemical assay of MMP-3 in human dentine. J Dent. 2011;39(3):231–7. https://doi.org/10.1016/j.jdent.2011.01.001.
32. Mazzoni A, Pashley DH, Tay FR, Gobbi P, Orsini G, Ruggeri A Jr, et al. Immunohistochemical identification of MMP-2 and MMP-9 in human dentin: correlative FEI-SEM/TEM analysis. J Biomed Mater Res A. 2009;88(3):697–703. https://doi.org/10.1002/jbm.a.31920.
33. Sulkala M, Larmas M, Sorsa T, Salo T, Tjaderhane L. The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth. J Dent Res. 2002;81(9):603–7. https://doi.org/10.1177/154405910208100905.
34. Palosaari H, Pennington CJ, Larmas M, Edwards DR, Tjaderhane L, Salo T. Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue. Eur J Oral Sci. 2003;111(2):117–27. https://doi.org/10.1034/j.1600-0722.2003.00026.x.
35 Martin-De Las Heras S, Valenzuela A, Overall CM. The matrix metalloproteinase gelatinase A in human dentine. Arch Oral Biol. 2000;45(9):757–65. https://doi.org/10.1016/s0003-9969(00)00052-2.
36. Ballal V, Rao S, Bagheri A, Bhat V, Attin T, Zehnder M. MMP-9 in dentinal fluid correlates with caries lesion depth. Caries Res. 2017;51(5):460–5. https://doi.org/10.1159/000479040.
37. Vidal CM, Tjaderhane L, Scaffa PM, Tersariol IL, Pashley D, Nader HB, et al. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. J Dent Res. 2014;93(3):269–74. https://doi.org/10.1177/0022034513516979.
38. Boushell LW, Nagaoka H, Nagaoka H, Yamauchi M. Increased matrix metalloproteinase-2 and bone sialoprotein response to human coronal caries. Caries Res. 2011;45(5):453–9. https://doi.org/10.1159/000330601.
39. Shimada Y, Ichinose S, Sadr A, Burrow MF, Tagami J. Localization of matrix metalloproteinases (MMPs-2, 8, 9 and 20) in normal and carious dentine. Aust Dent J. 2009;54(4):347–54. https://doi.org/10.1111/j.1834-7819.2009.01161.x.
40. Toledano M, Nieto-Aguilar R, Osorio R, Campos A, Osorio E, Tay FR, et al. Differential expression of matrix metalloproteinase-2 in human coronal and radicular sound and carious dentine. J Dent. 2010;38(8):635–40. https://doi.org/10.1016/j.jdent.2010.05.001.
41. Amaral SFD, Scaffa PMC, Rodrigues RDS, Nesadal D, Marques MM, Nogueira FN, et al. Dynamic influence of pH on metalloproteinase activity in human coronal and radicular dentin. Caries Res. 2018;52(1–2):113–8. https://doi.org/10.1159/000479825.
42. Vidak E, Javorsek U, Vizovisek M, Turk B. Cysteine cathepsins and their extracellular roles: shaping the microenvironment. Cells. 2019;8(3). https://doi.org/10.3390/cells8030264.
43. Carrilho MR, Scaffa P, Oliveira V, Tjaderhane L, Tersariol IL, Pashley DH, et al. Insights into cathepsin-B activity in mature dentin matrix. Arch Oral Biol. 2020;117:104830. https://doi.org/10.1016/j.archoralbio.2020.104830.
44. Scaffa PM, Breschi L, Mazzoni A, Vidal CM, Curci R, Apolonio F, et al. Co-distribution of cysteine cathepsins and matrix metalloproteases in human dentin. Arch Oral Biol. 2017;74:101–7. https://doi.org/10.1016/j.archoralbio.2016.11.011.
45. Nagase H, Suzuki K, Morodomi T, Enghild JJ, Salvesen G. Activation mechanisms of the precursors of matrix metalloproteinases 1, 2 and 3. Matrix Suppl. 1992;1:237–44.
46. Okamoto T, Akaike T, Suga M, Tanase S, Horie H, Miyajima S, et al. Activation of human matrix metalloproteinases by various bacterial proteinases. J Biol Chem. 1997;272(9):6059–66. https://doi.org/10.1074/jbc.272.9.6059.
47. DeCarlo AA Jr, Windsor LJ, Bodden MK, Harber GJ, Birkedal-Hansen B, Birkedal-Hansen H. Activation and novel processing of matrix metalloproteinases by a thiol-proteinase from the oral anaerobe Porphyromonas gingivalis. J Dent Res. 1997;76(6):1260–70. https://doi.org/10.1177/00220345970760060501.
48. Zhou J, Windsor LJ. Porphyromonas gingivalis affects host collagen degradation by affecting expression, activation, and inhibition of matrix metalloproteinases. J Periodontal Res. 2006;41(1):47–54. https://doi.org/10.1111/j.1600-0765.2005.00835.x.
49. Bafail A, Carneiro KMM, Kishen A, Prakki A. Effect of odanacatib on the release of NTX (amino terminal telopeptide) from LPS contaminated type I dentin collagen. Dent Mater. 2023;39(2):162–9. https://doi.org/10.1016/j.dental.2022.12.004.
50. Zhang YZ, Ran LY, Li CY, Chen XL. Diversity, structures, and collagen-degrading mechanisms of bacterial collagenolytic proteases. Appl Environ Microbiol. 2015;81(18):6098–107. https://doi.org/10.1128/AEM.00883-15.
51. Mazzoni A, Tjaderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res. 2015;94(2):241–51. https://doi.org/10.1177/0022034514562833.
52. Suppa P, Ruggeri A Jr, Tay FR, Prati C, Biasotto M, Falconi M, et al. Reduced antigenicity of type I collagen and proteoglycans in sclerotic dentin. J Dent Res. 2006;85(2):133–7. https://doi.org/10.1177/154405910608500204.

53. Deyhle H, Bunk O, Muller B. Nanostructure of healthy and caries-affected human teeth. Nanomedicine. 2011;7(6):694–701. https://doi.org/10.1016/j.nano.2011.09.005.
54. Stankoska K, Sarram L, Smith S, Bedran-Russo AK, Little CB, Swain MV, et al. Immunolocalization and distribution of proteoglycans in carious dentine. Aust Dent J. 2016;61(3):288–97. https://doi.org/10.1111/adj.12376.
55. Fonovic M, Turk B. Cysteine cathepsins and extracellular matrix degradation. Biochim Biophys Acta. 2014;1840(8):2560–70. https://doi.org/10.1016/j.bbagen.2014.03.017.
56. Aguda AH, Panwar P, Du X, Nguyen NT, Brayer GD, Bromme D. Structural basis of collagen fiber degradation by cathepsin K. Proc Natl Acad Sci U S A. 2014;111(49):17474–9. https://doi.org/10.1073/pnas.1414126111.
57. Smith AJ, Scheven BA, Takahashi Y, Ferracane JL, Shelton RM, Cooper PR. Dentine as a bioactive extracellular matrix. Arch Oral Biol. 2012;57(2):109–21. https://doi.org/10.1016/j.archoralbio.2011.07.008.
58. He G, Dahl T, Veis A, George A. Nucleation of apatite crystals in vitro by self-assembled dentin matrix protein 1. Nat Mater. 2003;2(8):552–8. https://doi.org/10.1038/nmat945.
59. Baht GS, Hunter GK, Goldberg HA. Bone sialoprotein-collagen interaction promotes hydroxyapatite nucleation. Matrix Biol. 2008;27(7):600–8. https://doi.org/10.1016/j.matbio.2008.06.004.
60. Goldberg M, Septier D, Rapoport O, Iozzo RV, Young MF, Ameye LG. Targeted disruption of two small leucine-rich proteoglycans, biglycan and decorin, excerpts divergent effects on enamel and dentin formation. Calcif Tissue Int. 2005;77(5):297–310. https://doi.org/10.1007/s00223-005-0026-7.
61. Kalyva M, Papadimitriou S, Tziafas D. Transdentinal stimulation of tertiary dentine formation and intratubular mineralization by growth factors. Int Endod J. 2010;43(5):382–92. https://doi.org/10.1111/j.1365-2591.2010.01690.x.
62. Six N, Decup F, Lasfargues JJ, Salih E, Goldberg M. Osteogenic proteins (bone sialoprotein and bone morphogenetic protein-7) and dental pulp mineralization. J Mater Sci Mater Med. 2002;13(2):225–32. https://doi.org/10.1023/a:1013846516693.
63. Goldberg M, Six N, Decup F, Buch D, Soheili Majd E, Lasfargues JJ, et al. Application of bioactive molecules in pulp-capping situations. Adv Dent Res. 2001;15:91–5. https://doi.org/10.1177/08959374010150012401.
64.• Galler KM, Weber M, Korkmaz Y, Widbiller M, Feuerer M. Inflammatory response mechanisms of the dentine-pulp complex and the periapical tissues. Int J Mol Sci. 2021;22(3). https://doi.org/10.3390/ijms22031480. (**Comprehensive review about inflammatory responses of the dentin-pulp complex including odontoblast response to bacterial by-products and formation of tertiary dentin. This review also discusses the healing mechanisms of the dental pulp and link between inflammation and repair.**)
65. Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch JV, Lesot H. Reactionary dentinogenesis. Int J Dev Biol. 1995;39(1):273–80.
66. Wan CY, Li L, Liu LS, Jiang CM, Zhang HZ, Wang JX. Expression of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases during apical periodontitis development. J Endod. 2021;47(7):1118–25. https://doi.org/10.1016/j.joen.2021.04.005.
67. Shin SJ, Lee JI, Baek SH, Lim SS. Tissue levels of matrix metalloproteinases in pulps and periapical lesions. J Endod. 2002;28(4):313–5. https://doi.org/10.1097/00004770-200204000-00013.
68. Gusman H, Santana RB, Zehnder M. Matrix metalloproteinase levels and gelatinolytic activity in clinically healthy and inflamed human dental pulps. Eur J Oral Sci. 2002;110(5):353–7. https://doi.org/10.1034/j.1600-0722.2002.21347.x.
69. Kritikou K, Greabu M, Imre M, Miricescu D, Ripszky Totan A, Burcea M, et al. ILs and MMPs levels in inflamed human dental pulp: a systematic review. Molecules. 2021;26(14). https://doi.org/10.3390/molecules26144129.
70. Yu F, Huo F, Li F, Zuo Y, Wang C, Ye L. Aberrant NF-kappaB activation in odontoblasts orchestrates inflammatory matrix degradation and mineral resorption. Int J Oral Sci. 2022;14(1):6. https://doi.org/10.1038/s41368-022-00159-3.
71. Takimoto K, Kawashima N, Suzuki N, Koizumi Y, Yamamoto M, Nakashima M, et al. Down-regulation of inflammatory mediator synthesis and infiltration of inflammatory cells by MMP-3 in experimentally induced rat pulpitis. J Endod. 2014;40(9):1404–9. https://doi.org/10.1016/j.joen.2014.04.001.
72. Eba H, Murasawa Y, Iohara K, Isogai Z, Nakamura H, Nakamura H, et al. The anti-inflammatory effects of matrix metalloproteinase-3 on irreversible pulpitis of mature erupted teeth. PLoS One. 2012;7(12):e52523. https://doi.org/10.1371/journal.pone.0052523.
73. Zheng L, Amano K, Iohara K, Ito M, Imabayashi K, Into T, et al. Matrix metalloproteinase-3 accelerates wound healing following dental pulp injury. Am J Pathol. 2009;175(5):1905–14. https://doi.org/10.2353/ajpath.2009.080705.
74. Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol. 2008;19(1):34–41. https://doi.org/10.1016/j.semcdb.2007.07.003.
75.•• Okamoto M, Takahashi Y, Komichi S, Cooper PR, Hayashi M. Dentinogenic effects of extracted dentin matrix components digested with matrix metalloproteinases. Sci Rep. 2018;8(1):10690. https://doi.org/10.1038/s41598-018-29112-3. (**This in vivo and in vitro study reported the potential of dentin extracted proteins digested by MMPs to induce dental pulp stem cell proliferation and migration, improve the angiogenic and mineralization potential, and induce formation of tertiary dentin.**)
76. Cooper PR, Takahashi Y, Graham LW, Simon S, Imazato S, Smith AJ. Inflammation-regeneration interplay in the dentine-pulp complex. J Dent. 2010;38(9):687–97. https://doi.org/10.1016/j.jdent.2010.05.016.
77. Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, et al. Dental pulp defence and repair mechanisms in dental caries. Mediators Inflamm. 2015;2015:230251. https://doi.org/10.1155/2015/230251.
78. Komichi S, Takahashi Y, Okamoto M, Ali M, Watanabe M, Huang H, et al. Protein S100-A7 derived from digested dentin is a critical molecule for dentin pulp regeneration. Cells. 2019;8(9). https://doi.org/10.3390/cells8091002.
79. Salehi S, Cooper P, Smith A, Ferracane J. Dentin matrix components extracted with phosphoric acid enhance cell proliferation and mineralization. Dent Mater. 2016;32(3):334–42. https://doi.org/10.1016/j.dental.2015.11.004.
80. Athirasala A, Tahayeri A, Thrivikraman G, Franca CM, Monteiro N, Tran V, et al. A dentin-derived hydrogel bioink for 3D bioprinting of cell laden scaffolds for regenerative dentistry. Biofabrication. 2018;10(2):024101. https://doi.org/10.1088/1758-5090/aa9b4e.
81. Tersariol IL, Geraldeli S, Minciotti CL, Nascimento FD, Paakkonen V, Martins MT, et al. Cysteine cathepsins in human dentin-pulp complex. J Endod. 2010;36(3):475–81. https://doi.org/10.1016/j.joen.2009.12.034.
82. Tatti O, Vehvilainen P, Lehti K, Keski-Oja J. MT1-MMP releases latent TGF-beta1 from endothelial cell extracellular

matrix via proteolytic processing of LTBP-1. Exp Cell Res. 2008;314(13):2501–14. https://doi.org/10.1016/j.yexcr.2008.05.018.
83. Charadram N, Farahani RM, Harty D, Rathsam C, Swain MV, Hunter N. Regulation of reactionary dentin formation by odontoblasts in response to polymicrobial invasion of dentin matrix. Bone. 2012;50(1):265–75. https://doi.org/10.1016/j.bone.2011.10.031.
84. Tomson PL, Lumley PJ, Smith AJ, Cooper PR. Growth factor release from dentine matrix by pulp-capping agents promotes pulp tissue repair-associated events. Int Endod J. 2017;50(3):281–92. https://doi.org/10.1111/iej.12624.
85. Huang CC, Ravindran S, Kang M, Cooper LF, George A. Engineering a self-assembling leucine zipper hydrogel system with function-specific motifs for tissue regeneration. ACS Biomater Sci Eng. 2020;6(5):2913–28. https://doi.org/10.1021/acsbiomaterials.0c00026.
86. Zehnder M, Wegehaupt FJ, Attin T. A first study on the usefulness of matrix metalloproteinase 9 from dentinal fluid to indicate pulp inflammation. J Endod. 2011;37(1):17–20. https://doi.org/10.1016/j.joen.2010.10.003.