Dynamic Balance of Non-collagenous Proteins in Dentine Mineralisation

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Dentine, the predominant structural element of the tooth, exhibits varying structural components, properties and mineralisation patterns across different regions. During dentinogenesis, diverse non-collagenous proteins (NCPs) play essential and varied roles in the mineralisation process. This paper systematically reviews the spatial distribution of NCPs across different dentine substructures and highlights their multifarious functions and collaborative interplay in governing the intricate mineralisation process. Specifically focusing on phosphorylated and glycosylated proteins, this review underscores their precisely programmed dynamic balance in orchestrating a series of distinct morphological patterns of dentinal substructures with varying degrees of mineralisation. By discussing the collaboration and balance of NCPs in dentine mineralisation, this paper also aims to advance the understanding of biomineralisation and provide valuable insights into developing highly biomimetic remineralisation strategies for dental applications.

Keywords: *biomineralisation, collagen matrix, dentine, hydroxyapatite, non-collagenous proteins*

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Dentine, which constitutes the major portion of the tooth, is deemed one of nature's masterpieces that comprises highly hierarchical inorganic-organic components. It is a mineralised collagen-based hard tissue encompassing 20% organic matrix and 70% inorganic hydroxyapatite (HAP) crystals. Unlike the highly uniform arrangement of HAP rods in enamel, the internal mineralisation of dentine is uneven, and distinct substructures are observable under the microscope due to varying degrees of mineralisation.[1] From the interior pulp chamber to the exterior dentinoenamel junction (DEJ), these structures include predentine, a translucent narrow band adjacent to the pulp consisting of unmineralised collagen matrix; dentinal tubules, minute channels extending from the pulp to the DEJ, traversing almost the entirety of the dentine; peritubular dentine (PTD), a concentric layer around the tubules that appears relatively darker under the microscope due to its higher mineral content; intertubular dentine (ITD), the substance between PTD with lower mineral density; globular dentine, a peripheral layer characterised by void nonmineralised spaces between globular mineralised masses; and mantle dentine, the outermost layer adjacent to enamel (Fig 1). Due to its hierarchical architecture, dentine emerges as a multifaceted entity with a unique combination of strength, flexibility and resistance to fracture, ensuring its vital role in supporting tooth structure and function.[2]

Within the well-organised architecture of the dentine matrix, a small yet significant amount (~ 10%) of non-collagenous proteins (NCPs) is present alongside the

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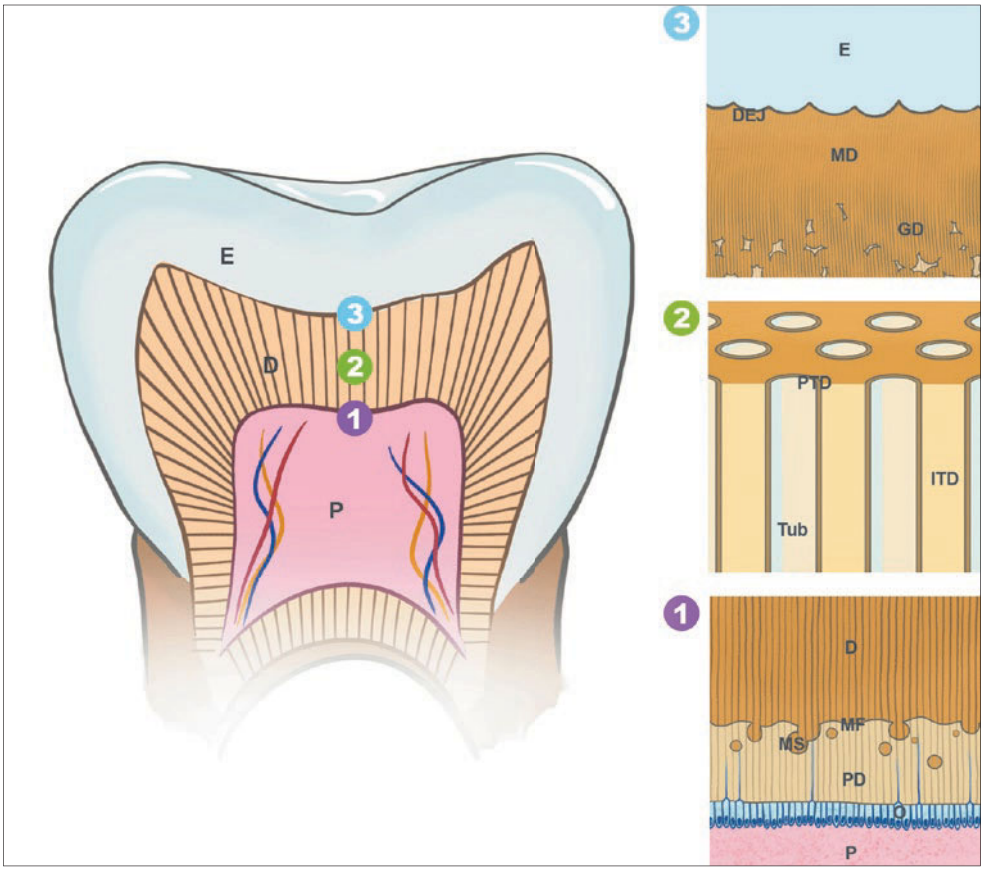

Fig 1 Schematic representation of the structure of dentine. Three typical subregions marked by numbers on the left are magnified on the right. From the interior pulp chamber to the exterior DEJ, these typical subregions include the predentine and mineralisation front (marked as 1); circumpulpal dentine, including PTD and ITD (marked as 2) and mantle dentine and globular dentine (marked as 3). D, dentine; DEJ, dentinoenamel junction; E, enamel; GD, globular dentine; ITD, intertubular dentine; MD, mantle dentine; MF, mineralisation front; MS, mineralised spherules; O, odontoblasts; P, pulp; PD, predentine; PTD, peritubular dentin; Tub, tubules.

predominant type I collagen matrix (~ 90%).[3] Previous studies have underscored the crucial and indispensable role played by various NCPs in governing dentinogenesis, such as dentine sialophosphoprotein (DSPP) and dentine matrix protein 1 (DMP1).[4] These NCPs, which can be divided into the phosphorylated group and the glycosylated group according to their post-translational modifications and molecular structures, are actively engaged in dentine matrix synthesis and mineralisation. Once the expression of these NCPs in dentine is disturbed, mineralisation-related disorders such as dentinogenesis imperfecta and dentine dysplasia emerge.[5] A crucial but as yet unanswered question pertains to how these proteins function collaboratively to precisely control the mineralisation of the collagen matrix to varying degrees. It is therefore imperative to investigate the spatial distribution, molecular structure and expression patterns of these NCPs, as well as the molecular regulation of the biosynthetic and proteolytic events accompanying the mineralisation processes. This is vital in order to comprehend the mechanisms involved in hard tissue formation.

Considering temporal and spatial distribution, this article systematically reviews the roles and interactions of NCPs in dentine mineralisation and draws a distribution map of their configuration patterns. By unravelling the intricate interplay between NCPs and mineral deposition, it aims to provide a comprehensive understanding of the balance and collaboration of NCPs in the dentine formation and maturation processes. Deciphering the intricacies of dentine hierarchical mineralisation and its well-programmed regulatory molecular components offers valuable insights for developing highly biomimetic strategies in hard tissue regeneration.

Predentine and mineralisation front

Dentine continues to grow throughout the entire lifespan of a tooth. Between the bulk of mineralised dentine and the central pulp chamber lies a narrow layer of nonmineralised collagen matrix, which is termed predentine.[1] Predentine is visible in all stages of tooth development, arising from a subtle time delay between the initial collagen matrix secretion and subsequent mineral deposition. Structurally, predentine is predominantly composed of randomly scattered collagen fibres, primarily type I collagen. Its thickness is approximately 20 to 60 µm, depending on age, site and systemic

conditions.[6] On the interior side, collagenous and noncollagenous proteins are secreted by odontoblasts and transported along the odontoblast processes to form the dentine matrix. On the exterior side, collagen fibres become more tightly packed, and minerals are deposited as calcified globules (also termed mineralised spherules[7]) under the regulation of NCPs. Numerous mineralised spherules assemble and construct the distinct continuous wavy line, termed the mineralisation front, indicating the border between nonmineralised predentine and mineralised dentine.

Decorin (DCN) and biglycan (BGN)

Predentine, interacting dynamically with odontoblasts, serves as an active interaction site for various NCPs during the continuous processes of collagen synthesis and mineral deposition. Proteoglycans, including DCN and BGN, are secreted into the sub-odontoblastic layer, accompanying collagen fibrils. DCN consists of a protein core with leucine repeats and a glycosaminoglycan chain, which may be either chondroitin sulphate or dermatan sulphate. BGN has a homologous structure and features two glycosaminoglycan chains.[8] Both BGN and DCN interact with type I collagen and serve as structural components in connective tissues, regulating the fibrillogenesis process during dentinogenesis.

In situ immunohistochemistry analysis revealed a significantly higher density of both DCN and BGN in predentine compared to mineralised dentine.[9] While BGN is uniformly distributed in the predentine layer, DCN content is increased in the distal region near the mineralisation front, where collagen fibres become more compact and minerals begin to deposit. In circumpulpal dentine, both DCN and BGN can be found in the dentinal tubules and ITD in relatively low amounts, which is supposed to be vital in maintaining the mechanical properties and homeostasis of the mature collagen matrix of the mineralised dentine.[10]

Analysis of the phenotype of *Dspp/Dcn* and *Dspp/Bgn* double knockout mice[11] indicated that DCN expression contributed to the exacerbation of enlarged predentine and hypomineralisation in *Dspp*-knockout mice, and BGN expression reduced the defect in the coalescence of mineralised spherules. With a high affinity for apatite that is facilitated by the glycosaminoglycan chains, BGN is able to initiate HAP nucleation and regulate crystal proliferation at a low concentration in vitro.[12,13] It is suggested that BGN can promote dentinogenesis from both fibrillogenesis and mineralisation.

In dentinogenesis, DCN is believed to be primarily associated with the fibrillogenesis process, predominantly regulating collagen fibril diameter and orientation.[14,15] Upregulation of DCN accelerates the assembly of collagen fibrils into hierarchical fibre-like structures both in vitro and in vivo. DCN possesses a higher affinity for type I collagen than other mineralisation regulators, such as DMP1, bone sialoprotein (BSP) and osteopontin (OPN).[16] It can easily crosslink with fibrils, possibly contributing to the prevention of intrafibrillar mineral deposition. Analysis based on the knockout mice revealed that DCN inhibits mineral deposition, hindering the conversion from predentine into dentine. The temporal expression pattern of DCN in osteoblastic cells shows a significant downregulation at the onset of mineralised spherule deposits, with content level remaining relatively low throughout the mineralisation process.[14] In vitro, the intrafibrillar mineralisation and mineralised spherule formation are severely delayed by DCN.[15,16] Based on the aforementioned evidence, DCN primarily acts as a promoter in collagen maturation and potentially serves as an inhibitor in mineral deposition. Given the partially collaborative promoting effect in fibrillogenesis and potential opposite effect in mineralisation, it is reasonable to speculate that there is a dynamic balance of DCN and BGN in regulating fibril assembly and maintaining the unmineralised status of predentine, as well as governing the predentine-dentine transformation.

Interestingly, in *Bgn*-knockout mice, the hypomineralisation in dentine was evident in the newborn mice, but no significance was detected in adult mice. In the absence of BGN, glycosylated proteins, such as dentin sialoprotein (DSP), BSP and OPN, exhibited overexpression.[17] Accordingly, a potential compensatory effect among these structurally assembled proteins is suggested in dentinogenesis.

DMP1

DMP1 is an osteo- and dentinogenesis-related protein that is widely distributed in hard tissues like bone and teeth.[18] In dentine, it presents primarily in predentine, dentinal tubules, PTD and mantle dentine.[18,19] DMP1 has an intrinsically highly acidic nature, with a large number of glutamic acid (Glu) and aspartic acid (Asp) residues interspersed in its amino acid sequence. It undergoes various post-translational modifications, including phosphorylation and glycosylation to different extents.

DMP1 primarily functions as fragments: a 37 kDa fragment from the N-terminal region, a 57 kDa fragment from the C-terminal region and a chondroitin-sulphate-linked N-terminal fragment, which is also known

as the proteoglycan form of DMP1 (DMP1-PG). In situ immunolocalisation[20] showed that the N-terminal fragment and DMP1-PG are mainly distributed in the nonmineralised predentine, whereas the C-terminal fragment localised at the mineralisation front and in mineralised circumpulpal dentine. The C-terminal fragment contains 41 phosphorylated serine (Ser) residues, which endow the fragment with a high affinity for calcium ions. It is suggested that the 57 kDa C-terminal fragment acts as a mineralisation promoter, which can initiate de novo HAP formation in vitro.[21] Meanwhile, DMP1-PG not only contains 12 phosphate groups but also a chondroitin 4-sulphate glycosaminoglycan chain. Based on in vitro and in situ evidence, it is supposed to be a mineralisation inhibitor.[22] Although the function of 57 kDa C-terminal fragment and DMP1-PG is relatively clear, the role of 37 kDa N-terminal fragment remains poorly understood. There is speculation that DMP1-PG is the functional form of this 37 kDa fragment after glycosylation, or the 37 kDa fragment is the remnant after DMP1-PG proteolytical degradation.[20] This may offer a reasonable explanation of the different outcomes of in vitro mineralisation experiments that do not take post-translational modification into consideration.

In addition to these fragments, a small amount of full-length isoform of DMP1 is identified in dentine,[23] yet whether it has a certain biological function or merely exists as a precursor to the functional fragments remains to be established. In vitro studies have shown that the full-length DMP1 regulates mineral deposition in a phosphorylation- and concentration-dependent manner.[24,25] *Dmp1*-knockout mice exhibited notable dental phenotypic alterations during the postnatal period, including increasing predentine width, failed maturation from nonmineralised predentine to mineralised dentine, and hypomineralisation of circumpulpal dentine.[26] In situ hybridisation analysis revealed that DMP1 mRNA could be detected as early as E20 in the preodontoblasts of rats, and continues to be expressed during dentinogenesis in polarised secretory odontoblasts.[27] The coincidence of the initial expression of DMP1 with the onset of mineral deposition in the dentine matrix indicates that DMP1 is the promoter of dentine mineralisation.[28]

DSPP and dentine phosphoprotein (DPP)

DSPP-derived proteins are dominant among all the NCPs in dentine. In contrast to DMP1, they are highly tooth-specific, with their presence in bone being approximately one-four hundredth of their concentration in dentine.[29] After being secreted by odontoblasts, DSPP is immediately proteolytically cleaved by metalloproteinases (MMPs) into three segments: DPP, located in the C-terminal region of DSPP, which is a highly phosphorylated intrinsically disordered protein (IDP) with genetic polymorphisms; DSP, found in the N-terminal region of DSPP, which is richly glycosylated with N-linked asparagine; and dentine glycoprotein (DGP), which is a phosphorylated and glycosylated segment in the middle region of DSPP in pigs that lacks reported occurrences in other species, and the function of which in dentinogenesis has remained unexplored.[30]

DPP constitutes over 50% of dentine NCPs in most species and is notably prevalent in the mineralisation front and mineralised circumpulpal dentine, yet absent in nonmineralised predentine.[31] It is a highly phosphorylated protein containing multiple Ser, Asp and threonine (Thr) residues, conferring hydrophilicity and capability to interact with calcium ions.[32] Among toothed mammalian species, although the general molecular weight of DPP differs dramatically among species, the repetition of the elemental SerSerAsp (SSD) motifs is highly conserved.[33] The phosphorylation status of the SSD motif serves as a mediator for DPP's function in dentine mineralisation. Dephosphorylation leads to the loss of its effectiveness in guiding mineralisation.[34] In *Dspp*-knockout mice, the transgenic expression of DPP partially restored the reduced dentine volume and mineral density.[35] The mineralisation process was actively promoted by DPP, accelerating the transformation and maturation from nonmineralised predentine to mineralised dentine. In vitro, DPP induces mineral nucleation at low concentrations and inhibits crystal growth at elevated concentrations.[36] Within the collagen matrix, DPP primarily binds to the gap zone of the collagen fibrils, initiating the mineralisation process.[37]

Interestingly, recent studies have reported that the DPP domain of DSPP is mutated in truly toothless mammals, such as the anteater, while DSP is normally expressed.[38] This indicates the physiological significance of DPP in dentine mineralisation and tooth development, suggesting that it plays a more indispensable role compared to DSP. However, DSP is not redundant, as it contributes to the regulation of HAP morphology,[39] facilitating the coalescence of mineralised spherules at the mineralisation front. The balance and synergy of DPP and DSP drive the serial key events of dentine mineralisation, including mineral nucleation, mineralised spherule growth and coalescence, as well as HAP morphology regulation and alignment. Further details about DSP will be discussed in the following section.

PTD and ITD

Circumpulpal dentine can be divided into two types: PTD and ITD. Gilding the inner surface of dentinal tubules, PTD forms a protective sheath that envelops the odontoblast processes, clearly delineating the boundary between dentinal tubules and interstitial dentine, whereas ITD, the rest of the tissue between the tubules, constitutes the majority of the dentinal volume. These two types of dentine exhibit distinct differences in composition and structure. ITD consists of approximately 90% type I collagen, whereas PTD is nearly devoid of fibrils.[40] In ITD, needle-like HAP crystals deposit intra- and extra-fibrillarly within the dense network of collagen matrix. In PTD, mineral content is approximately 40% higher than in ITD, and the crystals exhibit a more ordered and compact arrangement.[41] Recent studies have revealed the porous nature of PTD, demonstrating a transient or even homogeneous state in the arrangement of HAP crystals and collagen fibrils between PTD and ITD.[42] Thus, these two structures should be regarded as a communicable and successive entity rather than separate motifs.

DSP

DSP constitutes approximately 5% to 8% of NCPs in dentine, predominantly situated in PTD, with minor expression in predentine.[43] The primary structure of DSP is rich in Asp and Ser, with fewer phosphorylated sites but more glycosylated residues in comparison with DPP.

In vitro, Boskey et al[39] observed that DSP at low concentrations modestly enhanced HAP formation, whereas at higher concentrations, it exhibited a slight inhibition of HAP accumulation. Compared to other mineralisation-related glycosylated proteins, such as OPN or BSP, DSP shows a relatively lower calcium binding affinity, suggesting its limited impact on mineral nucleation. Ex vivo, Jaha et al[44] reported that DSP induces type I collagen production, highlighting its potential role in dentine fibrillogenesis.

In vivo, Fang et al[45] observed that a large portion of the circumpulpal dentine matrix of *Dspp*-knockout mice remained nonmineralised, featuring numerous spherulitic mineralised inclusions. Further, based on the conditional *Dpp*-knockout mice that only express DSP in a DSPP null background, Suzuki et al[46] demonstrated that DSP expression can partially mitigate the decreased dentine matrix volume and irregular mineral deposition resulting from the absence of DSPP. DSP expression restored the dentinal collagen matrix volume to levels equivalent to the wild type and eliminated the abnormal sporadic mineralisation phenomenon, with an absence of irregular unmineralised areas in the circumpulpal dentine; however, the width of predentine and mineral density remained abnormal. In this situation, it is postulated that following the initial mineral deposition guided by alternative NCPs like DMP1, DSP plays a pivotal role in regulating HAP alignment and mineralised spherule coalescence within the collagen matrix. In comparison, transgenic expression of DPP in *Dspp*-knockout mice accelerated the transformation from nonmineralised predentine to mineralised dentine, by which the width of predentine and the mineral density of circumpulpal dentine were restored.[35]

In dentine, some NCPs like DPP exhibit high affinities for calcium and promote mineral nucleation, and some NCPs like DSP preferentially bind to HAP and collagen fibrils, regulating crystal morphology and alignment. Besides, a certain NCP can perform different functions depending on its varying post-translational modifications and concentrations. Such cooperation and balance of NCPs are fundamental in dentinogenesis, where every up- or downregulation of each molecule can influence the dynamic equilibrium between the nonmineralised and mineralised states, or alter the degree and pattern of mineralisation of specific regions.

BSP

BSP is a sulphated, glycosylated and phosphorylated protein with high affinities for selectively binding to both HAP and collagen fibrils.[47] In dentine, BSP is primarily distributed in the PTD and mantle dentine.[48] Building on the work of Hunter et al[49] and Baht et al,[50] BSP is considered a potential HAP crystal nucleator; however, its exact function in tooth development remains up for debate. Studies on *Bsp*-knockout mice showed that BSP deficiency impairs new bone formation, leading to hypomineralisation of cementum and alveolar bone.[47] However, no evident defects were observed in dentinogenesis when compared to the wild type.[51] Accordingly, though capable of regulating mineral deposition, BSP has a limited effect in dentine mineralisation. Considering its relatively low content (approximately 1% of the total NCPs in dentine),[52] BSP may serve as a subordinate regulator in dentinogenesis.

BSP belongs to the small integrin-binding ligand N-linked glycoprotein (SIBLING) family, which also includes DSPP, DMP1 and OPN. These proteins are genomically related, showing similarity in amino acid sequences and biochemical features that are crucial for their functions, such as calcium ion binding.[3] It is rational to speculate a

possible compensatory effect among these structurally resembled proteins, in which the overexpression of one protein can partially compensate for the absence of another. Gene expression studies of *Opn*-knockout mice revealed an increased expression of BSP transcripts, providing validation for this speculation.[53]

DMP1

In situ immunohistochemistry studies also localised DMP1 in PTD,[18] but further validation is required to determine whether it is present in the fragment or full-length form. It is reasonable to speculate that phosphorylated and glycosylated DMP1 plays a vital role in PTD formation, as it can not only initiate mineral deposition but also guide HAP crystals into ordered alignment in extrafibrillar spaces.

In vitro experiments have shown that recombinant DMP1 (rDMP1) can initiate HAP nucleation and mediate crystallisation in a sequential and stepwise process with or without collagen fibrils.[54] After chelating supersaturated calcium and phosphorous ions, rDMP1 nucleated the minerals in an amorphous shape, then restructured into a porous structure, and gradually transformed into highly ordered plate-like HAP aggregates. The mature form of HAP guided by rDMP1 in vitro resembles the physiological structure of PTD in vivo,[18,54] which offers evidence for the aforementioned speculation; however, further exploration is required for a comprehensive understanding of the specific role of DMP1 in PTD.

Studies indicate that DMP1, DSPP and other SIBLING family proteins closely interact with each other in dentinogenesis. DMP1 can directly induce DSPP gene expression.[55] Both DSPP and DMP1 contribute to hard tissue mineralisation, but the tissues affected by DSPP and DMP1 are different, presumably due to variations in their expression levels and distinct biochemical properties.[56] Accordingly, DMP1 and DSPP may serve complementary roles in mineralisation.

Interglobular dentine and mantle dentine

Globular dentine, located at the peripheral outer layer of the circumpulpal dentine, features shallow void spaces (known as interglobular dentine) within dense globular masses (coalescing mineralised spherules) when observed under a microscope.[3] This is a physiological developmental phenomenon resulting from deficient mineralisation during the final maturation of odontoblasts; however, the function of this layer has not been well demonstrated. It is widely acknowledged that the microstructure and development of dentine resemble the lamellar bone. Inspired by the crossfibrillar mineral tessellation pattern of bones, it is suggested that the globular dentine layer has similar physical functions of resistance to compression and bending at the globular-interglobular interfaces[57]; however, this presumption needs further validation.

Between the globular dentine and the DEJ lies the mantle dentine. Compared to the inner bulk of circumpulpal dentine, the mantle dentine features a relatively irregular arrangement of collagen fibrils and an atubular structure with fewer, thinner and more curved dentinal tubules.[58] The matrix primarily comprises type III collagen fibrils extending from enamel. Although thinner in diameter, these fibrils weave into a more stable network that offers potential binding sites. The mineral content of mantle dentine shows a gradual increment from the outer DEJ to the inner circumpulpal dentine.[59] Based on the features of collagen alignment and mineralisation gradient, mantle dentine is proposed to act as a resilience zone capable of transforming stress and dissipating tensile forces from oral physiological activities, which would otherwise induce enamel fissuration and detachment from the outer DEJ.

Immunolabelling reveals an absence of the highly phosphorylated DPP in mantle dentine,[60] indicating the limited influence and significance of protein phosphorylation in the formation and function of mantle dentine. It is further supported by the dentinal phenotype of *Dspp*-knockout mice[44] and patients with hypophosphatemic vitamin D-resistant rickets,[61] in which the mantle dentine remains unaffected whereas the interstitial dentine is hypomineralised with an abundance of calcified globules that fail to coalesce. Instead, OPN, an NCP characterised by both glycosylation and phosphorylation, was found to be prominent in mantle dentine.[62]

OPN

OPN is a phosphorylated and glycosylated protein widely distributed in mineralised tissues.[63] In teeth, OPN primarily abounds in the cementum lining root surfaces and at the junction of periodontal ligament fibre attachment. In dentine, the majority of OPN resides at the mantle dentine,[62] with a small amount present in the PTD, possibly originating from the odontoblast processes in dentinal tubules.[64]

Though OPN has been widely found to be involved in modulating mineralised tissue formation, its function in dentinogenesis still remains a matter of debate. Rittling et al[65] reported that the dental phenotype of *Opn*-

knockout mice was hardly affected, indicating that OPN plays a trivial role in primary dentine growth; however, Foster et al[66] found that the lack of OPN promoted the increment of dentine volume and mineral density at an early age; simultaneously, the volume of unmineralised periodontal ligament decreased due to ectopic calcification of cellular cementum and alveolar bone. In vitro, Boskey et al[67] evaluated the mineral binding affinity of OPN using a gel diffusion system. OPN showed a dose- and phosphate-dependent inhibitory effect on HAP formation and crystal proliferation. These observations suggest that the distribution of OPN in mantle dentine contributes to its unique gradient of mineral density. Again, this speculation reflects the core of this review; that is, that the intricate distributions of NCPs precisely regulate the mineralisation patterns of different substructures in dentine, which endow the entity with excellent mechanical properties and multifarious functions. Nevertheless, this still requires further validation.

In addition, OPN is significant in reparative dentine formation. In situ hybridisation analysis demonstrated that OPN participates in the reparative dentinogenesis process, as an elevated expression level of OPN can be detected at the boundary between the tertiary and preexisting dentine.[68] In contrast, the process of reparative dentinogenesis cannot be detected in *Opn*-null mice. The administration of recombinant OPN partially recovered the secretion of type I collagen for reparative dentine formation.[68] In conclusion, OPN plays a nonredundant role in dentinogenesis, but its exact regulating mechanism needs further exploration.

Conclusion and prospects

This review delves into the sophisticated landscape of dentine mineralisation, highlighting the synergy of multiple NCPs as well as their individual roles in controlling the formation and governing the function of multifaceted dentine substructures. The intricate organisation and mineralisation of dentinal substructures are precisely governed by a dynamic collaboration and balance of these NCPs, particularly phosphorylated and glycosylated proteins. Any subtle alterations in the content or post-translational modification of these NCPs can impact dentinogenesis significantly.

At the predentine layer, glycosylated DMP1-PG, DCN and BGN are secreted at relatively higher concentrations to promote fibrillogenesis. These glycosylated proteins bind to collagen fibrils, maintaining the nonmineralised state of predentine and preserving the matrix flexibility. At the mineralisation front, these glycosylated proteins undergo metabolism, proteolysis or removal; simultaneously, the secretion of DPP and other phosphorylated proteins promotes mineral deposition and nucleation, initiating the mineralisation process of the dentinal matrix. The dynamic balance between these glycosylated and phosphorylated proteins elaborately orchestrates the transformation from nonmineralised predentine to mineralised dentin. In the circumpulpal dentine, DSP, BSP and other NCPs distributed in specific locations further participate in the HAP maturation and alignment, endowing dentine with intricate and well-defined substructures. These observations suggest that in dentine mineralisation, each NCP with diverse molecular structures and post-translational modification patterns is distributed in specific places with varying concentrations, playing various functions. Together, they maintain a dynamic balance and synergistically orchestrate the sophisticated process of dentinogenesis. Having a comprehensive understanding of the spatial and temporal distribution of NCPs and their synergetic roles in precisely regulating mineralisation, as highlighted in this study (Table 1), sheds light on their crucial involvement in dentine formation and maturation.

This review not only enhances understanding of dentine mineralisation but also opens up avenues for clinical applications. The potential utilisation of the dynamic balance of NCPs serves as an innovative strategy for the effective treatment of dentinal caries and dental hypersensitivity. By biomimetic remineralisation of demineralised regions, such a strategy may hold promise for restoring damaged dentine with high biocompatibility and ideal mechanical properties.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Min Juan SHEN, Yang Yang ZHANG and Meng Qi ZHU drafted the manuscript; Drs Chun Yan ZHANG and Zhi Yong WANG made the figure and the table; Prof. Qian Ming CHEN contributed to the conceptualization and revision of the manuscript. All authors read and approved the final manuscript.

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Table 1 Distribution map of NCPs across dentine.

Region	Main NCPs	Molecular characteristics	Relative concentrations	Functions	Studies
Predentine	DCN	Contains one glycosaminoglycan chain	+++	Promotes fibrillogenesis, potentially inhibits mineral deposition	8-11,14-16
	BGN	Contains two glycosaminoglycan chains	+++	Promotes fibrillogenesis, potentially promotes mineral deposition	8-13,17
	DMP1-PG	Contains a chondroitin 4-sulphate glycosaminoglycan chain	++	HAP nucleation inhibitor	18-22
	DSP	Both glycosylated and phosphorylated	+	Promotes fibrillogenesis, possibly serves as a compensatory reservoir	29,39,43
Mineralisation front	DPP	Highly phosphorylated, contains abundant SSD motifs	+++++	Initiates nucleation, promotes HAP maturation	31-37
	57 kDa DMP1 C-terminal fragment	Multiple phosphorylated Ser residues	++	HAP nucleation promoter	24-28
	DSP	Both glycosylated and phosphorylated	+	Promotes fibrillogenesis, regulates the coalescence and morphology of mineralised spherules	29,39,42-46
PTD and ITD	DPP	Highly phosphorylated, contains abundant SSD motifs	++++	Initiates nucleation, promotes HAP maturation	31-37
	DSP	Both glycosylated and phosphorylated	+++	Regulates HAP alignment, promotes mineralised spherule coalescence	39,43-46
	BSP	Sulphated, glycosylated, and phosphorylated	++	Potential HAP nucleator, possibly serves as a compensation of the SIBLING family	47-53
	DMP1	Exact functional form needs further validation	+	Potentially promotes HAP nucleation and regulates HAP alignment	18,54-56
	DCN	Contains one glycosaminoglycan chain	+	Promotes fibrillogenesis	8-11,14-16
	BGN	Contains two glycosaminoglycan chains	+	Promotes fibrillogenesis, promotes coalescence of mineralised spherules	8-13,17
Globular dentine and mantle dentine	OPN	Both glycosylated and phosphorylated	+++	Inhibits mineralisation, possibly helps maintaining the gradient mineral density of mantle dentine	63-68

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