# Fluoride Effects on the Dentin-Pulp Complex

# 14

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## 14.1 Introduction: Facts of Fluoride

### 14.1.1 Fluoride and Dental Uses

Fluoride is known to protect against dental caries by being incorporated into demineralized hydroxyapatite crystals to make them less soluble to acid dissolution. Fluoride incorporation into biofilms has also been related to the inhibition of lactate production, possibly related to its incorporation into bacterial biofilms where it inhibits enolases [1, 2]. Fluoride has been administered to inhibit dental caries by adding it to drinking water or in topical forms including in toothpastes, gels, or varnishes. Ingestion of excess amounts of fluoride during tooth formation results in the formation of dental fluorosis.

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### 14.1.2 Dental Fluorosis

Dental fluorosis generally refers to the obvious effects of excess fluoride ingestion on tooth enamel formation [1]. Enamel fluorosis ranges from mildly fluorosed teeth, which have more accentuated perikymata, to more severe fluorosis where teeth have a white opaque appearance and more severely affected teeth which are characterized by pitting and loss of enamel, with increasing porosity relative to the severity of fluorosis [3, 4]. Pitting can lead to secondary staining causing a yellow to brown discoloration [5]. The severity of fluorosis in mineralized tissue is related to the concentration of fluoride in the serum [6].

All degrees of severity of enamel fluorosis are characterized by subsurface enamel hypomineralization. In the maturation stage, this hypomineralization may be in large part due to the effects of fluoride to enhance mineral precipitation in the highly mineralized enamel matrix, with a resulting increased formation of protons leading to acidification and hypomineralization [7–10]. This cycle of fluoride-mediated mineralization may explain the bands of hypo- and hypermineralized mineral that are found in fluorosed enamel.

The effect of fluoride on mineralized tissues may be due to fluoride-related changes in the mineralizing extracellular matrix, which then influences cell function. However, it is also possible that fluoride may have direct cellular effects which cause alterations in the formation of the extracellular mineralizing matrix.

Examination of the effects of fluoride on dentin formation may provide better understanding of the effects of fluoride on mineralizing tissues.

## 14.2 Dentin Fluorosis

### 14.2.1 Hypomineralization and Dentin Fluorosis

The effects of chronic fluoride exposure on human and rodent tooth formation were first compared by Fejerskov et al. [11]. They noted that while dentin is generally considered to be affected in dental fluorosis, consistent with previous studies, human dentin samples collected from an area with chronic endemic dental fluorosis (with 3.5 ppm F in drinking water) exhibited inhibition of mineralization, similar to the incisor dentin from rats receiving chronic high dose of fluoride (56.5 and 113 ppm F in drinking water for 1–3 months). The dentin-related changes included striations, hypoplastic defects, and hypomineralized interglobular spaces, which increased with increasing levels of fluoride and time of exposure. They further stressed that dental fluorosis is characterized by changes in both enamel and dentin [4, 12] (Fig. 14.1).

Moreover, numbers of studies have found that the severity of dentin fluorosis is related to fluoride levels in the drinking water, dentin fluoride concentration, and dentin microhardness [13–15]. Studies by Vieira et al. [13] measured microhardness and dentin mineralization in tooth samples from Montreal and Toronto, Canada, and Fortaleza, Brazil, where water fluoride levels were 0.2, 1.0, and 0.7 ppm, respectively. They found that dentin fluoride concentrations correlated with dental fluorosis severity. Dental fluorosis severity correlated with dentin microhardness, while enamel fluoride concentrations correlated with both dentin microhardness and dentin mineralization. Interestingly, the teeth from Brazil exposed to 0.7 ppm fluoride in the drinking water presented with the highest dentin fluoride concentration values and were also harder and less mineralized than the Montreal (0.2 ppm) and Toronto (1.0 ppm) teeth. The Montreal (0.2 ppm)
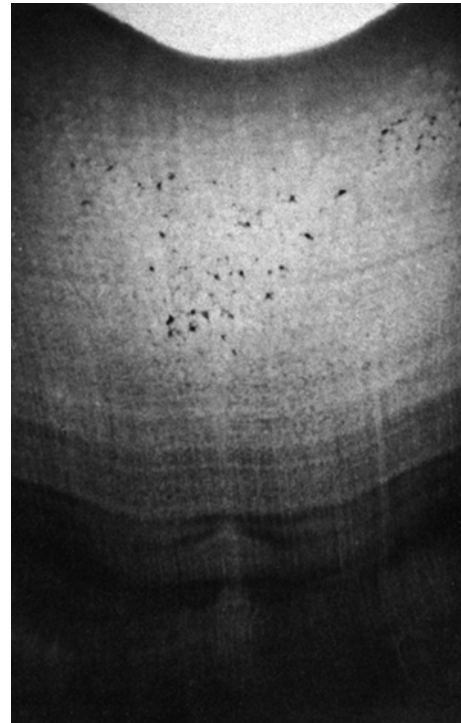


**Fig. 14.1** Micrograph of dentin from a severely fluorosed tooth showing distinct layering of hypomineralized lines (Used with permission of John Wiley and Sons from Fejerskov et al. [4])

teeth had lower levels of dental fluorosis when compared with teeth from Toronto (1 ppm) and Fortaleza (0.7 ppm).

Severely fluorosed deer [15] have a disrupted dentin crystal structure. The disrupted crystal formation may relate to the findings in a recent *in vitro* study by Waidyasekera and co-workers [16], who described crystallites in fluorosed human dentin as larger, but not homogenously arranged, and found a lower density of crystallites in fluorosed dentin.

### 14.2.2 Alteration in Extracellular Matrix Properties

Similar to the enamel matrix, the physicochemical effect of fluoride incorporated into the crystal lattice could directly regulate the growth of hydroxyapatite crystals in dentin. However, the molecular mechanism of hypomineralization in

fluorotic dentin is not yet fully elucidated. Several studies have suggested the changes in the inorganic phase of mineralization in dentin are due to structural alteration in the extracellular matrix resulting from changes in synthesis or modification of collagenous and noncollagenous extracellular matrix proteins.

Type I collagen is the major organic component of dentin extracellular matrix constructing structural basis of the dentin matrix. Moseley et al. reported that 6 mM sodium fluoride supplemented in dentin organ culture inhibited collagen synthesis [17]. A further *in vivo* study by Maciejewska et al. showed that type I collagen expression was downregulated in the early stage of tooth germs from pulps (P1 or P5) from dams that ingested 110 ppm fluoride [18].

Proteoglycans are a class of glycosylated proteins consists of sulfated glycosaminoglycans (i.e., chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate) covalently linked to core proteins. In mineralized tissues like dentin, proteoglycans are essential for maintenance of the stabilization of collagen fibers and act as a framework for mineralization in association with type I collagen. The proteoglycan extracted from rat teeth received 20 ppm fluoride for 10 weeks exhibited a more anionic profile due to changes in glycosaminoglycan characteristics and the presence of additional dermatan sulfate and heparin sulfate [19]. Changes in production of proteoglycans have also been demonstrated *in vitro*. Tooth organs cultured for 14 days in media supplemented with higher levels of fluoride than would be found in serum *in vivo* (1–6 mM) showed a reduction in biglycan, but no change in decorin or versican. In this same study, the levels of dermatan sulfate and sulfation of glycosaminoglycans in predentin and pulp extract were reduced by supplementation with 6 mM fluoride [20].

It is known that the each proteoglycan has different roles in matrix formation and mineralization. Chondroitin sulfate-substituted small leucine-rich proteoglycan/SLRP (including decorin and biglycan) within dentin may function to regulate the mineralization process, while dermatan sulfate-substituted SLRPs inhibit collagen

fibril formation [21]. The functions of these different proteoglycans are partially explained by the different binding abilities of these molecules to hydroxyapatite [22, 23]. Septier et al. have demonstrated that the expression of glycosaminoglycan and dermatan sulfate/chondroitin sulfate in predentin decreases toward the predentin (unmineralized)/dentine (mineralized) interface, indicating regulation of the mineralization status of dentin as one of the roles of proteoglycans [6]. If fluoride at physiological levels (micromolar) can alter proteoglycans in the dentin matrix, it would certainly alter dentin mineralization.

### 14.2.3 Alteration in Mineralization Front

Dentin phosphoprotein (DPP, a protein hydrolysis product of dentin sialophosphoprotein/DSPP) is the most abundant noncollagenous protein in the dentin matrix [24, 25] and is reported to have diverse effects on mineralization [26–30]. Because it is highly phosphorylated [31, 32], DPP has very high binding capacity for calcium ions and an affinity for crystalline hydroxyapatite surfaces, suggesting its role in nucleating mineral initiation at the mineralization front [33]. The phosphate content of DPP has been reported to be significantly reduced in dentin of rats receiving 20 ppm fluoride in drinking water for 17 weeks [34]. The possible mechanism of this finding was suggested by an *in vitro* study [35] showing that physiological concentration of fluoride (0.04–4 µM) inhibits casein kinase II, a potent kinase that phosphorylates DPP [24, 25].

Appositional mineralization of circumpulpal dentin has been shown to progress rapidly at the mineralization front. A lightly mineralized layer (LL) is observed in predentin immediately adjacent to the mineralization front of dentin [36, 37]. Crystal alignment in LL is within narrow spaces between the individual non-mineralized collagen fibers, thereby making LL distinct from the bulk of fully mineralized dentin where crystals are deposited in and around collagen fibers. This indicates that LL is where dentin mineralization initiates before more rapid mineralization of the
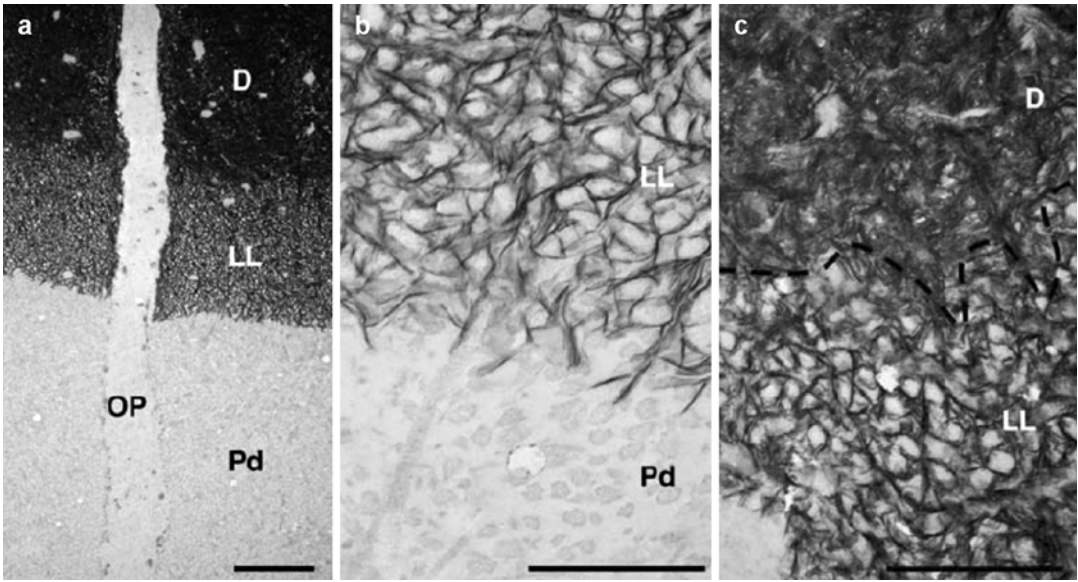
**Fig. 14.2** Lightly mineralized layer (*LL*). (**a**) LL along the mineralization front of normal rat dentin. (**b**, **c**) LL at high magnification (Used with permission of Oxford University Press from Ahmad et al. [36] *OP* odontoblast process, *PD* predentin)
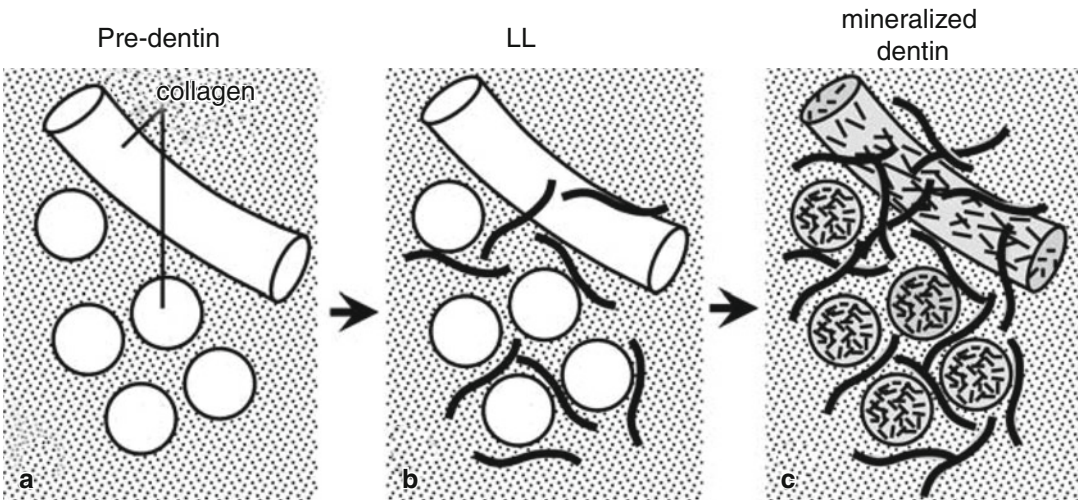


**Fig. 14.3** Diagrammatic model of stepwise mineralization at the mineralization front of dentin. (**a**) Predentin without mineral deposits. (**b**) LL where mineral crystals deposited in noncollagenous matrix between collagen fibers. (**c**) Mineralized dentin after massive mineral deposition in and around collagen fibers (Used with permission of Oxford University Press from Ahmad et al. [36])

matrix occurs (Figs. 14.2a–c and 14.3a–c). The LL along the mineralization front is clearly recognized at the crown-analogue dentin of the continuously erupting mouse and rat incisors as compared to root-analogue dentin. This is likely due to the differences in both organic and inorganic components between the crown- and root-analogue dentin matrix [36, 38–42].

DPP is present in the crown-analogue dentin at about four times greater levels than in the root-analogue dentin [39]. DPP has been reported to delay the mineralization of collagen fibers in the rodent incisor crown-analogue dentin, though at lower concentrations DPP promotes the nucleation of hydroxyapatite [36, 42–44]. These findings are consistent with those
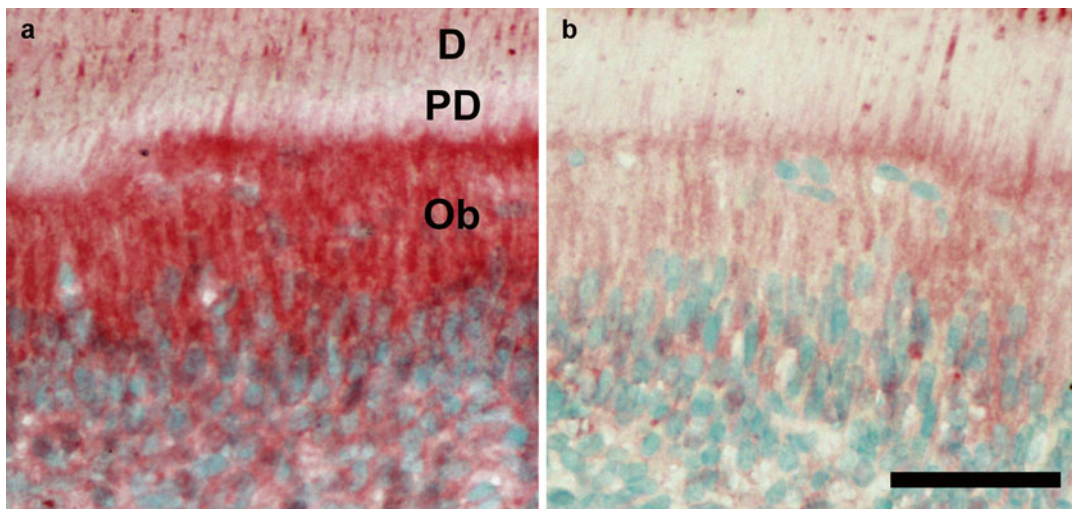
**Fig. 14.4** (**a**, **b**) Immunostaining of DSPP/DSP. DSPP/DSP is expressed in cytoplasm of odontoblasts and adjacent pulp cells. DSPP/DSP expression in cytoplasm of odontoblasts is downregulated in fluoride (50 ppm)-exposed A/J mice. *Ob* odontoblast, *D* dentin, *PD* predentin, bar 100 μm

of Boskey et al., who showed that high concentrations of DPP can inhibit the growth of nucleated crystals.

Mice ingesting 50 ppm fluoride in drinking water appear to lose LL in dentin from mice as compared to dentin from control mice (our unpublished data, Takano Y. 2014), with an abrupt change in the mineralization status of collagen fibers at the border between predentin and mineralized dentin under the influence of fluoride. These results, combined with our findings of reduced immunostaining for DSP (another hydrolysis product of DSPP to create DPP) in odontoblasts of the fluoride treated mice, further support the possibility that the loss of LL is caused by a reduction in the synthesis of DPP (Fig. 14.4a, b) (unpublished data, Nakano Y. 2014), allowing more rapid mineralization at the dentin mineral initiation front. These results support the possibility that the fluoride can have a direct effect on odontoblasts to reduce either the synthesis of DSPP or the hydrolysis of DSPP to form DPP.

## 14.3    Effects of Fluoride on the Dental Pulp

Few studies have specifically looked at the effects of fluoride on dental pulp. However, the changes that have been identified are consistent with those found in odontoblasts. *In vitro* studies of human dental pulp cells exposed to fluoride show that below 5 ppm fluoride, cell proliferation and alkaline phosphatase synthesis increase [45, 46]. However, at higher fluoride concentrations, alkaline phosphatase and type 1 collagen synthesis decrease, with no similar effects on fibronectin synthesis [47] or change in cell morphology [17]. Fluoride incorporation into pulp capping agents has been shown to reduce thermal conductivity and dentin acid solubility, with no irritating effects on the pulp [48]. At high concentrations, fluoride can also inhibit lysosomal enzymes found in the pulp, with a possible application of limiting cellular destruction in damaged pulp tissue [49].

## 14.4    Clinical Significance of Dentin Fluorosis

These relatively few studies showing that fluoride can alter dentin and pulp formation suggest that more attention should be given to possible changes in fluorosed dentin. Possible effects of fluorosed dentin include alterations in caries susceptibility, alterations in the dentin structure to affect bonding of composite based dental

materials, the capability of dentin to remineralize, secondary dentin formation, and dentin bridge formation. One aspect of fluoride-related effects on dentin formation has been explored in analyzing the effects of fluoride in drinking water on the so-called hidden caries.

### 14.4.1 Fluorosis and Dentin Caries

In the 1980s the term "hidden caries" was introduced to dentistry. This term is used to describe occlusal pit and fissure-type carious lesions seen in the dentin on a bitewing radiograph, where clinically the occlusal enamel appears intact or is minimally demineralized [50] (Fig. 14.5). These hidden caries create the risk that after cariogenic bacteria penetrate into the enamel through a minimal opening in the enamel surface, when they reach the less mineralized dentin, caries progresses at an increased rate. Meanwhile the fluoridated enamel undergoes remineralization, while caries progresses at an increased rate in the dentin. Hidden carious lesions present a challenge to dentists in diagnosis, treatment planning, and



**Fig. 14.5** Hidden caries. Radiolucency in the dentin (*arrow*) under an intact enamel surface suggests hidden caries

research, due to their "hidden" nature, resulting in misdiagnosis and treatment of dental caries [51].

In a clinical study to investigate the possible role of fluoride in the formation of hidden caries, Weerheijm et al. showed that children ingesting "optimally" fluoridated water at a level of 1.1 ppm from birth until the day of data collection had significantly fewer hidden caries than the group of children drinking water containing 0.1 ppm. Hashizume et al. examined the role of public water fluoridation and fluoride dentifrice on the prevalence of hidden caries in 8–10-year-old children and, similar to the study by Weerheijm et al., found a statistically significant reduction in the prevalence of hidden caries by water fluoridation at currently recommended level (0.7 ppm) [52]. These results suggest that the ingestion of fluoride at optimal levels can reduce the presence of radiographically detectable hidden caries.

Epidemiological data are not available to determine effect of dentin fluorosis on correlating hidden caries. Nevertheless, there is *in vitro* evidence to indicate that fluorosed dentin demineralizes more rapidly than normal dentin. Waidyasekera and colleagues grouped 33 human molars according to the modified Thylstrup-Fejerskov index (TFI) into normal (N, TFI 0), mild fluorosis (ML, TFI 1–3), and moderate fluorosis (MD, TFI 4–6) and exposed the cut enamel and dentin surfaces to acid to create artificial caries. They found that dentin from either mildly fluorosed or moderately fluorosed teeth demineralized to a significantly greater depth than normal teeth, while moderately fluorosed enamel was more acid resistant [53]. Further ultrastructural studies on fluorosed dentin showed large more randomly formed crystals with a more loosely formed collagen network in fluorosed as compared to normal dentin [54]. Differences were also found in optimal dentin bonding systems in fluorosed as compared to control dentin.
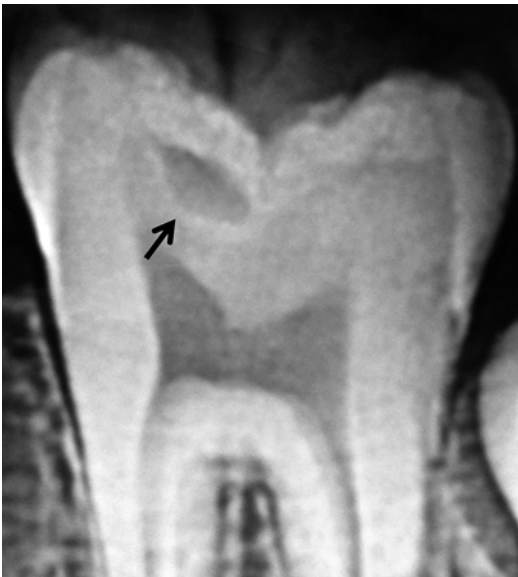
**Conclusion**

Dentin formation is regulated by signaling molecules, which direct matrix formation and mineralization. The effects of fluoride on the formation of collagenous and noncollagenous

dentin and pulp proteins show that, at high levels, fluoride can inhibit matrix formation resulting in hypomineralized dentin, possibly related to a disruption in initial dentin mineralization. Studies of *in vitro* organ culture systems with very high (millimolar) levels of fluoride suggest that fluoride mediates changes in matrix protein synthesis. Further studies of fluoride effects on dentin formation at lower, more physiological fluoride concentrations are needed to better understand the mechanisms by which fluoride alters dentin formation. Though fluoride at optimal levels in drinking water reduces the presence of hidden caries, *in vitro* studies show increased demineralization of dentin from even mildly fluorosed enamel as compared with nonfluorosed dentin. Strategic research approaches to combine *in vivo* and *in vitro* studies using human fluorosed teeth and mice/rat incisor models will allow us to examine the effect of fluoride on odontoblasts at different stages of odontoblast differentiation. *In vitro* and organ culture of dentin-pulp complex with physiological level of fluoride supplementation will enable us to analyze detailed pathways to provide further understanding of dentin fluorosis and its relevance to oral health.

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