The protective effect of topical fluoride treatments in dentine lesions

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Chapter 1

General Introduction
Dentine comprises 47% mineral, 33% organic matrix and 20% water by volume, in contrast to enamel that consists of 85% mineral, 3% organic matrix, and 12% water [Rošin-Grgić, 2013]. Regardless of the common chemical components, the structural differences between enamel and dentine will influence the development of caries and the reactivity of fluoride within these tissues [Buzalaf et al., 2011]. Enamel and dentine share the same mineral phase of hydroxyapatite crystallites, however, the size of these crystallites in dentine is much smaller than in enamel. As a consequence, the total combined surface area of these crystallites in dentine is larger than the surface area of enamel crystallites making dentine more prone to demineralization than enamel. The organic matrix of dentine is composed of 90% collagen that acts as a scaffold for mineral deposition during dentinogenesis. Besides collagen, the non-collagenous organic components, like proteoglycans, phosphoproteins, and acidic glycoproteins also play a role in the properties of the dentine organic matrix [Buzalaf et al., 2011]. In addition to the organic and mineral components of dentine, dentinal tubules that extend from the enamel-dentinal junction towards the pulp are another reason for the vulnerability of dentine as they make dentine tissue very permeable, creating diffusion pathways for bacterial acids [ten Cate et al., 1995].

Dentine caries starts when oral bacteria consume carbohydrates, releasing lactic acid and other acids into the biofilm, and decreasing the pH under the critical pH of dentine (pH 6.5). This process leads to mineral dissolution and, subsequently, denudation of the organic matrix. Mineral-depleted collagen is vulnerable to enzymatic degradation. The proteolytic enzymes are originated from bacteria [Hashimoto et al., 2011] and from the host (MMPs; matrix metalloproteinases) and present in sulcus fluid [Teng et al., 1992], saliva [van Strijp et al., 1994] and dentine (endogenous MMP) [Shimada et al., 2009].

The chemical and structural differences between enamel and dentine induce differences in the processes of de- and remineralization between these tissues [Buzalaf et al., 2011]: (1) the critical pH for dissolution of dentine is around 6.5 which is one log unit higher than for enamel, (2) due to the permeability and the amount of the organic matrix of dentine, dentin demineralizes faster and remineralizes slower than enamel when both tissues are exposed to the same experimental conditions [Arends et al., 1992; ten Cate et al., 1995], (3) to inhibit dentine lesion initiation and progressions or to enhance dentine remineralization, relatively high fluoride concentrations are needed [ten Cate, and Duijsters, 1983; ten Cate et al., 1998] (4) dentine benefits more than enamel from the increase in the frequency of fluoride exposure [Laheij et al., 2010] and from the combination of the different fluoride application methods [Vale et al., 2011].
Most of the studies on the fluoride efficacy have focused on enamel and caries progression in enamel. Due to the differences between enamel and dentine, experiments using enamel cannot be easily applied to dentine. Thus, more investigations on dentine and the fluoride interaction are needed.

Non restorative cavity control (NRCC)

In traditional restorative dentistry, clinicians tend to fully excavate the caries tissues, leaving only healthy dental tissues. This is to locate the restoration margins on healthy dental tissues which are less vulnerable to recurrent dental caries [Innes et al., 2016]. This approach was considered being the gold standard from a hundred and fifty years ago until recently [Black, 1908]. However, the philosophy of caries management has shifted from the restorative dental approach to a medical approach since dental caries is considered being a disease rather than a cavity [Gao et al., 2016]. Therefore, the advances in the cariology field are to treat the cause of the disease rather than the symptoms. For this reason, non-to minimally-invasive dental approaches have been developed to manage dental caries as a disease, and this could be achieved by involving the patient in the treatment plan [van Strijp and van Loveren, 2018]. Among these approaches, non-restorative cavity control (NRCC) is developed to treat cavitated dentine caries in primary dentition, and coronal smooth surfaces lesions or root caries in permanent dentition. NRCC can be described as a customized approach to treat the cause of cavitated dentine lesions [Nyvad and Fejerskov, 1986; van Strijp and van Loveren, 2018]. In young NRCC patients, their parents or caregivers are involved in the treatment strategy by maintaining good oral hygiene via plaque control and using fluoridated toothpaste, besides changing the dietary habits by reducing the frequency of cariogenic food intake. The communication between the clinician and the patient/parents or caregivers is an essential step in the NRCC approach to obtain the cooperation and compliance of the patient/parents or caregivers. The role of the clinician is also to make sure that the patient’s oral hygiene is maintained. For this, it may be necessary to expose the cavitated dentine lesions by removing the overhanging enamel and making the cavities accessible for dental home care. For proximal dental caries, slicing may also be helpful to expose the proximal dental cavities for home care brushing. Exposing dental cavities to the oral environment by slicing has to follow a technical protocol, this is essential to avoid food impaction and looseness of the interproximal contact. Topical fluoride treatments could also add extra protection to the exposed cavitated dentine lesions and could be of benefit to arrest or even remineralize the cavitated dentine lesions [Innes et al., 2016; van Strijp and van Loveren, 2018].
Scientific evidence of the NRCC approach showed the success of this approach in treating cavitated dentine lesions in both primary and permanent dentition [Nyvad and Fejerskov, 1986; Schwarz et al., 1996; Lo et al., 1998]. It has been reported that caries arrestment reached 28% after 3 years follow up when daily supervised tooth brushing with 1,000 ppm F toothpaste was applied in 3 - 6 years old Chinese children [Schwarz et al., 1996; Lo et al., 1998]. Another observational study reported a high success rate in caries arrestment reaching 90% after 1 year, when the NRCC was designed based on not only the daily supervised tooth brushing but also on slicing the interproximal cavities in the anterior primary teeth to facilitate brushing of the lesions, and on applying topical fluoride gel (2% NaF) to the interproximal cavities every 2 months [Peretz and Gluck, 2006]. Several studies have also compared the NRCC approach to different dental approaches from the minimally invasive to the traditional restorative approach. One study compared the survival percentage of cavitated primary molars in 6-7 years old children treated either with amalgam restoration (CRT), atraumatic restorative treatment (ART), or ultraconservative treatment (UCT) [Mijan et al., 2014]. The small cavities were treated with ART, while medium to large cavities were prepared to be accessible for dental home care (UCT). After 3.5 years, no significant difference in the survival rate among the three approaches was reported (CRT; 90.9%, ART; 90.4%, and UCT; 89.0%). Another clinical trial also compared the clinical efficacy of the NRCC approach (making cavities accessible for home care and applying fluoride varnish) to the traditional restorative approach (compomer), and Hall technique (dental caries is sealed under stainless steel crowns) in high caries risk children aged from 3-8 years old [Santamaría et al., 2018]. In the NRCC group, parents/caregivers were instructed about the tooth brushing technique, and 5% NaF varnish was applied every three months to the open cavities. After 2.5 years, the Hall technique showed a significantly higher survival rate (successful cases with no failures; 90%) compared to the traditional restorative approach (67%) and NRCC (70%). Both the traditional restorative approach and the NRCC approach revealed no significant differences in the survival rate. All these scientific evidence disclosed the efficacy of the NRCC approach in treating cavitated dentine lesions in the primary dentition.

In addition to the primary dentition, NRCC has also been used in the permanent dentition to treat cavitated dentine lesions in coronal smooth surfaces and root surfaces. An observational study used the NRCC approach in active root caries in the permanent dentition for 18 months follow up [Nyvad and Fejerskov, 1986]. In that study, patient brushed the root caries twice a day using fluoridated toothpaste, while 2% NaF was applied at the start and after eight weeks of the study. During the follow-up period of 18 months, it was observed that the yellow and soft texture of the active lesion was transformed into a dark and hard surface, indicating caries arrestment of the root cavities. Dental research has focused on elevating the fluoride concentrations of the toothpaste to control root caries. Four clinical trials have been conducted for 6-8 months on root caries in the permanent dentition.
dentition [Baysan et al., 2001; Ekstrand et al., 2008; Ekstrand et al., 2013; Srinivasan et al., 2014], concluding that root caries could be reduced to the half by replacing the traditional toothpaste (1,100, 1,350, 1,450 ppm F) with 5,000 ppm F toothpaste [Wierichs and Meyer-Lueckel, 2015].

The available evidence of the NRCC approach suggests that the professionally applied topical fluoride treatments could add protection to the cavitated dentine lesion, and support the clinical success of NRCC. Thus, to understand the clinical success of NRCC and the role of topical fluorides in caries arrestment, it is of importance to closely evaluate the chemical (de- and remineralization), histological (mineral content and distribution within the lesion), and microbiological aspects of dentine caries arrestment. This would help to explain the real efficacy of the topical fluoride treatments in arresting or even remineralizing dentine lesions.

### Topical fluoride treatments

One of the most effective preventive methods in the history of dentistry is the use of fluoride in dental care [Rošin-Grget, 2013]. This includes various types of fluoride application methods such as fluoridated water, fluoride tablets, fluoridated toothpastes, fluoride rinses, and professionally applied fluoride in the form of a gel, varnish, or solution. The interaction between fluoride and the tooth mineral could determine the condition of the teeth at different stages of tooth life, starting from the pre-eruptive stage to the post-eruptive stage of tooth development. In the pre-eruptive stage, fluoride was intended to be incorporated into the mineral during tooth development through systemic delivery methods (fluoridated water, fluoride tablets, and lozenges). It was believed that this incorporated fluoride was effective in reducing the solubility of the mineral [Rošin-Grget, 2013]. However, this belief had changed after several studies indicating that the pre-eruptive incorporated fluoride (firmly bounded fluoride) was less effective in inhibiting mineral dissolution than fluoride applied in the oral cavity [Fejerskov et al., 1981]. As a consequence, numerous studies have analyzed the method of topical fluoride treatments (post-eruptive) and their efficacies in de- and remineralization processes. Topically applied fluoride can inhibit demineralization and enhance remineralization through (1) deposition of fluoride to the partially demineralized crystals and formation of calcium fluoride (CaF₂) or CaF₂-like material, (2) Incorporation of fluoride into the crystallites and formation of firmly bound fluoride which is also known as fluoridated hydroxyapatite.

Fluoride concentrations ranging between 100-10,000 ppm F are required to produce calcium fluoride deposits on treated surfaces. These concentrations of fluoride are available
in topical forms of fluoride, such as professionally applied products, toothpastes, and mouth rinses [Rošin-Grget, 2013]. The insolubility of calcium fluoride deposits at neutral salivary pH is attributed to coverage by phosphate and/or protein. However, when plaque pH lowers, the phosphate and protein groups covering the calcium fluoride are released, and so the dissolution of calcium fluoride and fluoride release into the tooth environment will take place [Rølla, 1988; ten Cate, 1997; Rošin-Grget, 2013]. The released fluoride is subsequently incorporated by the reaction of reprecipitation into the hydroxyapatite to form the less soluble fluoridated hydroxyapatite.

In the 1940s, topical fluoride applications were introduced in dentistry to control dental caries [Knutson and Armstrong, 1946]. Most of these fluorides, such as gels and solutions, are aqueous agents, which are easily rinsed off after application. However, prolonged retention of fluoride on treated surfaces may increase effectivity for caries control. For this reason, the non-aqueous fluoride agents were developed to promote long retention [Chu and Lo, 2006; Fernández et al., 2014]. Among these agents, sodium fluoride varnish (5% NaF; 22,600 ppm F) was developed in 1964, and since then it was widely used in the dental office. As the most commonly used fluoride in the dental office, NaF varnish was the subject of investigations in the field of caries prevention. A systematic review of clinical trials revealed that the average preventive fraction of the NaF varnish in children reached 30% (0-69%) [Petersson et al., 2004]. The efficacy of NaF varnish is mainly due to its ability to retain and attach to the treated surface after the application for a long period. However, the mechanical forces by patients could disturb the retention of the NaF varnish to the surface. Therefore, other types of fluoride products that could overcome this problem are needed.

Metal fluoride products have been developed to increase the retention property of the fluoride to the tooth mineral and enforce the anti-demineralizing effect of the fluoride products in dental care. Among the metal fluoride products, titanium tetrafluoride (TiF$_4$) and stannous fluoride (SnF$_2$) are topical products used in caries prevention. The reason for combining fluoride with titanium (Ti) and stannous (Sn) ions is the ability of these metal ions to interact concurrently with fluoride and tooth mineral, and so enhance the fluoride retention to the treated surface [McCann, 1969; Grøn, 1977]. In addition, the acidity of the TiF$_4$ and SnF$_2$, can cause a slight demineralization of the treated surfaces and form hydrogen fluoride (HF) [Hjortsjö et al., 2009; Wiegand et al., 2010], which can transport the fluoride deeper in the dental tissues [Aasenden et al., 1968; Grøn, 1977]. Besides, the metal fluoride products create a surface coating layer on the treated surfaces, which can add an extra protective effect compared to the non-metal fluorides [Ellingsen, 1986; Skartveit et al., 1991]. Due to these special properties, metal fluoride products could be more effective than the non-metal fluoride products when it comes to remineralization of root caries. However, the drawbacks of these products could limit their clinical use. For instance, stannous ions in the
SnF$_2$ act as a mineral growth inhibitor in the co-presence of fluoride to some extent. This perhaps interferes with the remineralization process, even if it can provide acid resistance. For this reason, the role of the stannous ions in the caries process is complicated, and from a mechanistic aspect, it needs more investigations [Lippert, 2016]. Regardless of the several in vitro studies on the protective role of the TiF$_4$ in mineral dissolution, the clinical and in situ studies of this product are very limited [Lussi and Carvalho, 2015].

Recently, another metal fluoride product has gained the attention of clinical and laboratory studies [Horst, 2018]. As a metal fluoride product, silver diamine fluoride (SDF) has an extra property over other metal fluoride products. Besides the fluoride content of SDF, silver may also play a role in the protective mechanism of this product [Hu et al., 2018]. SDF has been developed in Japan in the sixties as a colorless solution composed of the active agent silver fluoride (AgF) and the stabilizing agent of ammonia (NH$_3^+$) [Yamaga et al., 1972; Liu et al., 2012]. The developmental idea of SDF solution originated from combining the two working mechanisms of the two separate products, silver nitrate (AgNO$_3$) and sodium fluoride (NaF) used in preventing and treating dental caries. The application of silver nitrate to the demineralized dental tissues enhanced the interaction between the silver ions and the free phosphate ions of the demineralized tissues. The application of NaF enhanced the interaction between the fluoride ions and the free calcium ions of the demineralized tissues. Likewise, the application of SDF enhances the interaction between the free calcium and phosphate ions with the fluoride and silver ions of the SDF, respectively. This interaction produces calcium fluoride (CaF$_2$) and silver phosphate (Ag$_3$PO$_4$) that both act as reservoirs for fluoride and phosphate, respectively, during the acid attack and so protect against dissolution [Yamaga et al., 1972]. In addition to the anti-demineralizing activity of SDF, the presence of silver in the product was claimed to have bactericidal activity against the cariogenic bacteria [Chu et al., 2012; Mei et al., 2013b]. However, recent studies did not verify changes in the plaque composition covering dentin surfaces treated with SDF using sequencing methods [Milgrom et al., 2018; Mitwalli et al., 2019]. Therefore, it is not clear whether the superior clinical effect of the SDF is attributed to the high fluoride concentration, the presence of silver or both.

Three different concentrations of SDF are available in the market for caries control (SDF; 12%, 30% and 38%) [Mei et al., 2013a]. The highest concentration of SDF (38%) is the most frequently studied concentration. Several clinical studies reported the efficacy of 30% and 38% SDF in dentine caries arrestment in primary dentition compared to other treatment methods [Contreras et al., 2017], while the one time application of 12% SDF showed no significant effect in dentine caries arrestment compared to one time application of 38% SDF [Fung et al., 2013]. Indeed, 38% SDF contains the highest fluoride concentration (44,800 ppm F) among all topical fluoride products, including the commonly used NaF varnish.
A recent umbrella review reported eleven systematic reviews; four reported the effect of SDF on root caries in geriatric populations and seven on the coronal dentine cavities in pediatric populations [Seifo et al., 2019]. In that review, a superior clinical effect of SDF was reported on arresting root or coronal dentine caries in comparison to different treatment methods (fluoride varnish, atraumatic restorative treatment, and placebo). For root caries, the prevented fraction (PF) of SDF for caries arrestment ranged from 25 to 71%. For coronal dentine caries treated by SDF, the PF for caries arrestment was ranging between 70 to 78%. It has been concluded that the larger beneficial effect of SDF in arresting coronal dentine and root caries in primary and permanent dentitions compared to the other treatment methods, was repeatedly endorsed by systematic reviews.

Various clinical and laboratory studies were conducted to compare the effect of NaF varnish to SDF solution [Chu et al., 2002; Duangthip et al., 2016; Duangthip et al., 2018; Wierichs et al., 2018]. A randomized clinical trial on Chinese children aged 3-4 years compared the effect of annual application of 30% SDF solution to the three times application of 5% NaF varnish on a weekly interval over a 30-month follow up [Duangthip et al., 2018]. In that study, it was found that the cavitated dentine lesions treated annually with SDF solution showed a significant better caries arrest rate (48%) compared to the lesions treated three times for three weeks (one application time/week) using NaF varnish (34%). Similar results were reported by another clinical study on Chinese children aged 3-5 years, which found a significant dentine caries arrestment of the upper primary anterior teeth treated with 38% SDF annually compared to the three-month interval of 5% NaF varnish over thirty months [Chu et al., 2002]. These findings are consistent with those of systematic reviews that support the effectiveness of SDF solution in arresting dentine caries [Seifo et al., 2019]. The beneficial effect of SDF was also reported by an in vitro study that showed significant higher remineralization of the dentine lesions treated once with SDF (3.5%F⁻) compared to one time application of NaF varnish (2.2%F⁻) and no treatment [Wierichs et al., 2018].

Most of the studies compared the effect of SDF and NaF on dentine lesions, while their effect on enamel lesions was rarely studied [Delbem et al., 2006]. Delbem et al. [2006] showed a significant reduction in the demineralization of enamel samples treated with NaF varnish compared to SDF and placebo samples, whereas no significant difference was reported between SDF and placebo. In contrast, SDF showed a higher caries arrestment/anti-demineralizing effect compared to NaF varnish when both treatments were applied to dentine lesions [Chu et al., 2002; Duangthip et al., 2016; Duangthip et al., 2018; Wierichs et al., 2018]. Although the reason of the superior effect of SDF in dentine lesions compared to NaF is not fully understood, it was hypothesized that SDF interacts better with dentine than NaF, and this is perhaps related to the dentine composition of mineral and collagen [Delbem et al., 2006; Sinha et al., 2011]. An in vitro study found a good interaction between
38% SDF to the organic and non-organic components of dentine tissue compared to 4% NaF that showed no effect on the organic component [Lou et al., 2011]. This may explain the high clinical effect of SDF when it is applied to cavitated dentine lesions compared to NaF and questioning the real efficacy of NaF varnish in treating cavitated dentine lesions compared to SDF.

In conclusion, it is clear that the evidence in the field of NRCC and topical fluoride treatments is yet scarce. In order to understand the effect of different topically applied fluoride products on the NRCC approach and how they could contribute to caries arrestment and remineralization of cavitated dentine lesions, an investigation in that field is highly recommended.

Despite the various studies conducted on the SDF efficacy in arresting or remineralizing dentine caries lesions, the evidence is still insufficient to draw conclusions about the superiority of SDF. Therefore, more investigations on the effect of SDF on dentine lesions are needed. It remains unclear whether the beneficial effect of SDF is mainly due to its high fluoride concentration or additionally because of the silver component that might participate in inhibiting demineralization or enhancing remineralization of dentine lesions. A good understanding of that topic is of paramount importance in order to understand the efficacy of the topical fluoride in NRCC and how it will influence the success of this approach.

**Outline of the thesis**

The overall aim of this thesis was to investigate the effect of the various topically applied fluoride products on the demineralized dentine lesions in vitro. This would help to draw a conclusion about the effect of the topical fluoride treatments in the NRCC approach and how they could arrest the cavitated dentine lesions or even enhance the remineralization of the dentine cavities. For this reason, the current thesis focuses on the chemical (de- and remineralization) and histological (mineral content and distribution within the lesion) aspects of the dentine lesions.

In Chapter 2 it was of interest to understand the remineralizing effect of different fluoride products (SDF, NaF, TiF₄, and SnF₂) and no treatment group (control) on dentine lesions under constant remineralization conditions using transversal microradiography (TMR). The mineral content and the mineral distribution profiles were examined after 14 days of remineralization. The surface morphology of the experimental groups was also examined using scanning electron microscopy (SEM). In Chapter 3, the same fluoride products were...
used in a more realistic condition to mimic the de- and remineralization processes. In this study, we investigated the effect of different fluoride products on dentine lesions during 15-day pH-cycling. Calcium loss and uptake in de- and remineralization cycles were analyzed daily, as well as the net calcium values were also calculated. The fluoride release was also measured during the pH-cycling. The microradiographic data of the above mentioned pH-cycling experiment are presented in Chapter 4. In this experiment, the mineral distribution profiles and content throughout the lesion depth were analyzed using transversal microradiography (TMR). A subsequent experiment of a perpendicular acid exposure for 12 h on the sides of the pH-cycled specimens was conducted in order to examine the anti-demineralizing effect of the selected products throughout the depth of the dentine lesions. Chapter 5 aimed to compare the effect of equal fluoride concentrations as either SDF or KF on dentine lesions. This was to examine whether the superior effect of the SDF is due to its fluoride and silver components, or because of the high fluoride concentration. Therefore, three different concentrations (4.1%, 1.025%, 0.26% F⁻) of either SDF or KF were applied once to the dentine lesion, and then the dentine specimens were pH-cycled for 15 days. The calcium loss and uptake in the de- and remineralization cycles were analyzed daily. The net calcium values of both cycles were calculated. The fluoride release into the de- and remineralization cycles on day 1, 2, 3, and 8 was also analyzed. The microradiographic data of this experiment is presented in Chapter 6 to investigate the pattern of mineral deposition and the amount of mineral content in the deeper layers of the dentine lesions after application of 4.1%, 1.025% or 0.26% F⁻ of either SDF or KF.
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