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Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions

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ABSTRACT

Objectives: To investigate the remineralising effect and bacterial growth inhibition of 38% silver diamine fluoride (SDF) solution and 5% sodium fluoride (NaF) varnish on artificial dentine caries lesions.

Methods: Demineralised dentine blocks were treated with SDF + NaF (Group 1), SDF (Group 2), NaF (Group 3) and water (Group 4) and subjected to a *Streptococcus mutans* biofilm challenge. Lesion depth, precipitates' characteristics and matrix (collagen)-to-mineral ratio were evaluated by micro-computer tomography (micro-CT), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR), respectively. The biofilm kinetics, viability and topography were assessed by counts of colony forming units (CFUs), confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), respectively. Data were analysed by two-way ANOVA test.

Results: The lesion depths of Groups 1–4 were 170 ± 28 μm, 160 ± 32 μm, 353 ± 38 μm and 449 ± 24 μm, respectively. The addition of NaF to SDF did not show better remineralisation than SDF ($p = 0.491$). Metallic silver and silver chloride were found in Groups 1 and 2. The amide I-to-hydrogen phosphate ratios of the four groups were 0.14 ± 0.02, 0.14 ± 0.01, 0.29 ± 0.05 and 0.49 ± 0.16, respectively, and the addition of NaF to SDF did not offer better protection against collagen exposure than SDF ($p = 0.986$). The Log_{10} CFUs of Groups 1–4 were 5.75 ± 0.56, 4.49 ± 0.57, 6.55 ± 0.39 and 6.40 ± 0.38, respectively. The presence of NaF reduced the antibacterial effect of SDF ($p < 0.001$). The SEM and CLSM images supported the findings.

Conclusion: Application of SDF with or without NaF reduced the demineralisation of dentine caries, but SDF exerted stronger inhibition of biofilm growth than SDF with NaF.

Clinical significance: NaF varnish affects the antibacterialeffects of SDF, the adjunctive application of SDF solution and NaF varnish is not recommended to arrest dentine caries in clinic.

1. Introduction

Sodium fluoride (NaF) varnish is one of the most concentrated fluoride products available commercially. It is relatively new in the United States, although it has been widely used in Europe for more than 50 years. Although NaF varnishes have different compositions and delivery systems, most of them contain 5% NaF (22,600 ppm fluoride) in a natural colophony base, which allows the varnish to adhere to tooth surfaces in the presence of saliva [1]. Because of its adherent nature, NaF varnish can stay in contact with the tooth surface for several hours. NaF varnish is one of the most common topical fluorides for prevention of dental caries. Apart from caries prevention, dentists also use NaF varnish to arrest dental caries [2]. A recent systematic review concluded that 5% NaF varnish (22,600 ppm fluoride) can arrest enamel caries [3]. However, whether NaF varnish can effectively arrest dentine caries was inconclusive [4].

Unlike caries on enamel, which is basically highly mineralised tissue, dentine caries is more complex. The complexity of dentine caries can be attributed to its structural composition; it contains approximately 50% organic materials and water by volume [5]. Compared to enamel, dentine in general has smaller hydroxyapatite crystallites, higher carbonate and magnesium content and a more porous structure [6]. Thus, dentine is more vulnerable than enamel to dental caries. Caries in dentine is a biochemical process starting with the dissolution of dentine's mineral content by acid [7]. When the minerals are lost, organic matrix—which is basically collagen—is exposed. It is subsequently degraded by bacteria- and host-derived enzymes [7].

Some dentists have used silver diamine fluoride (SDF) to arrest cavitated dentine caries [8,9]. The most commonly used concentration of SDF is 38% (44,800 ppm F) [10]. Mechanistic studies have found that

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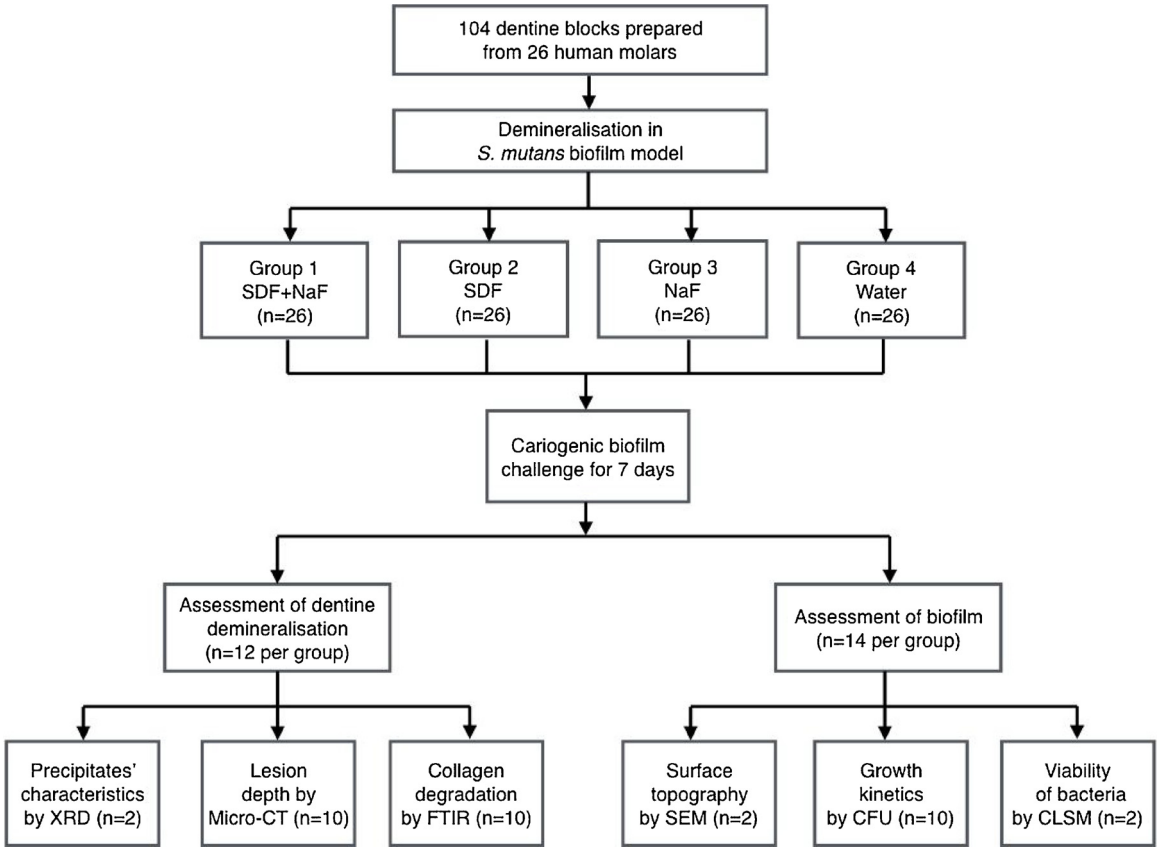

Fig. 1. Flow chart of the study.

SDF can remineralise carious dentine [11,12]. A highly remineralised layer with high calcium and phosphate content was found on arrested dentine caries lesions after SDF treatment [13]. SDF also inhibited the degradation of dentine organic matrix, which mainly consisted of Type I collagen [14,15]. Although SDF has been used in some countries in Asia and South America for many years, the US Food and Drug Administration only approved its use for the management of dental hypersensitivity in 2014. More evidence is required for the use of SDF in caries management, and a use protocol for SDF based on scientific evidence is essential. Although the School of Dentistry of the University of San Francisco has proposed a protocol for SDF use [16], the protocol was developed basically through specialists' experience. Because SDF is a clear liquid solution and its contact time with the caries lesion is limited due to its high fluidity, some clinicians have applied 38% SDF solution followed by 5% NaF varnish. The NaF varnish is believed to not only protect the SDF from being washed away by the saliva but also provide additional fluoride for an extended period of time. However, whether the adjunctive application of SDF and NaF (SDF + NaF) has a superior caries-arresting effect on dentine caries lesions has not been studied. Thus, this study investigates the antibacterial and remineralising effects of 38% SDF solution followed by 5% NaF varnish on dentine caries.

2. Materials and methods

2.1. Sample preparation

Twenty-six dentine slices were prepared from human third molars with the patients' consent. Ethics approval was obtained from the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (IRB number UW 12–221). The dentine slices were polished, and each slice was cut into four blocks ($3 \times 3 \times 2$ mm^3). A total of 104 dentine blocks were prepared. Half of the surfaces of 48 blocks were covered by acid-resistant nail varnish (Clarins, Paris, France) as internal controls to study mineral content (dentine demineralisation). The remaining 56 blocks were used for biofilm assessment. The dentine blocks were sterilised by autoclave. *Streptococcus mutans* (*S. mutans*) American Type Culture Collection (ATCC) 35668 was anaerobically cultivated on blood agar plates at 37 °C for 2 days. A single colony was picked and cultivated in brain heart infusion (BHI) at 37 °C in anaerobic conditions. Subsequently, bacterial cell pellets were collected and re-suspended in BHI with 5% sucrose. The concentration of the bacteria suspension was adjusted to McFarland 2 (6×10^8 cells/mL). Then, 1 mL bacteria culture was incubated with each dentine block in a well of a 24-well plate. The plates were incubated anaerobically at 37 °C for three days to create carious lesions that were approximately 80 μm in depth on the exposed surfaces of the dentine blocks [15]. The biofilm on the dentine surface was then removed by ultrasonication.

2.2. Experimental treatment

Four dentine blocks prepared from the same slice were randomly allocated into four experimental groups. The blocks in Group 1 received a topical application of 38% silver diamine fluoride (SDF) (Saforide; Toyo Seiyaku Kasei Co., Ltd., Osaka, Japan) followed by a 5% sodium fluoride (NaF) varnish (Duraphat; Colgate-Palmolive Co., New York City, NY, USA). Group 2 received a topical application of SDF. Group 3 received a topical application of NaF. The dentine blocks in Group 4 received sterile deionised water. A microbrush (Micro Applicator—Regular; Premium Plus International Ltd., Hong Kong, China) was used to apply SDF solution and NaF varnish to the dentine surface. The SDF solution and/or fluoride varnish were left on the dentine surface for 60 min before they were subjected to the *S. mutans*