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Research Paper

Optimizing concentration of titanium tetrafluoride solution for human dentine remineralization

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ABSTRACT

Objective: The aim of the present study was to select the optimal concentration of TiF_4 solution to facilitate the remineralization of early dentine caries lesions.
Design: Sixty human dentine specimens were cut and randomly divided into 6 groups (1%, 2%, 3%, 4% TiF_4 groups, 2.712% NaF group and distilled deionized water (DDW) control group). Artificial dentine caries-like lesions were created. After being subjected to fluoride treatment and immersed in remineralizing solution for 2 weeks, the specimens were observed by microCT, scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS). Data were analysed using linear regression analysis ($P < 0.05$).
Results: The lesion depths of the specimens treated by 2% TiF_4 solution were statistically less than those of the other groups. Further, the greyscale values of these lesion areas were greater. The 3% and 4% TiF_4 solutions caused further lesion demineralization. The 2.712% NaF solution seemed to be detrimental to remineralization during the experimental time, as the subsurface area remained hypomineralized with a thick precipitation layer on the surface.
Conclusions: The 2% TiF_4 solution demonstrated better remineralizing potency than did the other treatments.

1. Introduction

Root caries has become a prevailing dental problem in the aged population mainly attributed to gingival recession and exposure of the dentine and cementum to acid attack by cariogenic bacteria (Bignozzi et al., 2014). It has been reported that the prevalence of root caries ranges from 30% to 60% (Gluzman, Katz, Frey, & McGowan, 2013). The physicochemical process of an early caries lesion is dynamic and reversible, involving continuous mineral demineralization and remineralization (Cury & Tenuta, 2009). Due to the natural hypomineralization state of dentine and cementum, root caries progresses more rapidly than enamel caries does. Thus, interventions to prevent its initiation or to inactivate root caries lesions should be implemented aggressively at the earliest stage of the disease (Wierichs & Meyer-Lueckel, 2015).

For decades, fluoride has been used as an effective anti-caries agent. The action of fluoride is generally attributed to its effects on the demineralization and remineralization kinetics of dental hard tissues and to its disputed influence on cariogenic bacteria (Buzalaf, Pessan, Honorio, & ten Cate, 2011; Wiegand, Magalhães, & Attin, 2010; ten Cate, 1999, 2015). Recently, M. Kato et al. revealed that the effect of fluoride on prevention of dentine caries could be due to decreasing the activities of human matrix metalloproteinases (MMPs) and consequently inhibiting the breakdown of the demineralized organic matrix (Kato et al., 2014). Fluorides can be classified into traditional fluorides and polyvalent metal ion fluorides. The traditional fluorides usually refer to NaF, AmF, and others, which have been broadly used in caries prevention for many years; the polyvalent metal ion fluorides mainly include TiF_4, SnF_2, ZrF_4 and HfF_4. It is believed that fluoride with polyvalent metal counter-ions may have greater prospects for root caries remineralization (Comar, Souza, Grizzo, Buzalaf, & Magalhães, 2014; Comar et al., 2012; Lussi & Carvalho, 2015).

Most of the studies on TiF_4 that have reported its effectiveness on

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caries prevention or anti-erosion described only its anti-demineralization or mineral solubility reduction effects on dental hard tissues and seldom referred to its effect on the remineralization kinetics on dental hard tissues, especially on dentine (Alves, Souza, & Lima, 2005; Huysmans, Young, & Ganss, 2014; Magalhães, Levy, Rios, & Buzalaf, 2010; Vieira, Ruben, Bronkhorst, & Huysmans, 2011; Wiegand et al., 2010). Furthermore, the concentration of TiF_4 used in the reported studies ranged from 1% to 4%, leading us to the following question: which concentration is optimal when TiF_4 is used in practice? This study aimed to evaluate the effects of different concentrations of TiF_4 solutions on the remineralization of early dentine caries lesions compared with NaF. The null hypothesis was that the effects of various concentrations of TiF_4 solutions on dentine remineralization were similar.

2. Materials and methods

2.1. Dentine specimen preparation

Extracted human third molars were collected with patients' informed consent from the Stomatology Hospital of Xi'an Jiaotong University, which was under a protocol approved by the Ethics Committee for Human Studies of the Stomatology Hospital of Xi'an Jiaotong University. The teeth with caries, dental fluorosis, cracks and other structural abnormalities were excluded. Sound teeth were cleaned and stored in distilled deionized water at 4 °C before use. The teeth were mounted in a SYJ-160 low-speed diamond saw and cut into 3 mm × 2 mm × 1.6 mm-sized dentine slabs, embedded in acrylic resin and polished with serial water-cooled silicon carbide papers of 600-, 1200-, 2500- and 4000-grit. Approximately 100 μm of dental hard tissue was removed in the polishing process. On each specimen, two-thirds of the polished surface (2 mm x 2 mm) was left uncovered to create an open window area, while the remaining surface was covered with acid-resistant nail varnish. The 60 dentine specimens were randomly divided into 6 treatment groups (1%, 2%, 3%, 4% TiF_4 groups, 2.712% NaF group and DDW group). The molar concentration of fluoride in the 2.712% NaF solution was equal to that in the 2% TiF_4 solution.

2.2. Specimen treatment

All specimens were immersed in 50 mmol/L acetate buffer solution (2 ml per specimen) containing 2.2 mmol/L $CaCl_2$ and 2.2 mmol/L KH_2PO_4 at pH 5.0, 37 °C for 5 days to create artificial dentine caries-like lesions (Moron et al., 2013; ten Cate & Duijsters, 1982). The middle one-third of the surface on each specimen was covered with acid-resistant nail varnish, leaving a 1 mm x 2 mm area uncovered (Fig. 1).

An aliquot of 8 μl of each respective fluoride solution (1% TiF_4, pH 1.51; 2% TiF_4, pH 1.28; 3% TiF_4, pH 1.15; 4% TiF_4, pH 1.03; and

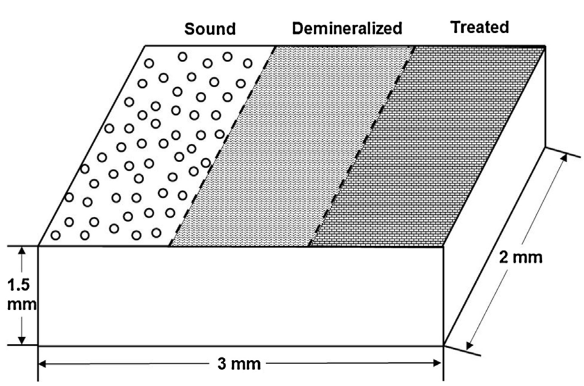

Fig. 1. Schematic drawing of the specimen surface. The surface was composed of sound area, demineralized area and treated area.

2.712% NaF, pH 7.18) or distilled deionized water as the control was pipetted on each specimen surface and left undisturbed for 60 s. After treatment, specimens were rinsed with distilled deionized water for 10 s and then immersed in the remineralizing solution (10 ml per sample) at 37 °C with no stirring. The remineralizing solution contained 1.5 mmol/L $CaCl_2$, 0.9 mmol/L KH_2PO_4, 130 mmol/L KCl and 20 mmol/L HEPES buffer, adjusted to pH 7.0 with KOH (Islam et al., 2012). The fluoride treatment was repeated twice a week for 2 weeks, while the remineralizing solution was changed every day.

After 2 weeks, the nail varnish was removed with acetone, and the specimens were fixed in 2.5% glutaraldehyde for 4 h at room temperature, followed by washing once with 0.1 mol/L PBS and twice with distilled deionized water in an ultrasonic cleaner for 30 s. The specimens were dehydrated in ascending grades of ethanol (50% for 10 min, 75% for 10 min, 95% for 10 min, and 100% for 1 h) (Lin, Douglas, & Erlandsen, 1993).

2.3. MicroCT analysis

Each dehydrated specimen was adhered to a resin rod with a known density of 1.40 g/cm^3 for standardization, mounted in the scanner, and scanned non-destructively using a Y.Cheetah microCT scanner (YXLON, Germany) with a spatial resolution of 5.6 μm at 80 kV and 35 μA. Software calibration was performed during the reconstruction phase using VGStudio MAX 2.2 software (YXLON, Germany) to reduce beam-hardening artefacts. After reconstruction with registration in a scene coordinate system, two coronal profiles for each sample were obtained. The lesion depths of demineralized dentine area and treated dentine area in each profile were measured, which included the crater depth (CD) and the advancing depth (AD) (Fig. 2).

It is believed that there is a positive linear relationship between greyscale values and mineral density (Liu, Hsu, Teo, & Teoh, 2013; Schwass, Swain, Purton, & Leichter, 2009). Thus, the greyscale values reflect the potency of fluorides in dentine remineralization. The greyscale values were analysed within a standardized region of interest (ROI) 250 × 400 $μm^2$ (Fig. 2). In each profile, the mean greyscale values of sound dentine, demineralized dentine, treated dentine and the resin rod were recorded. Greyscale values from all groups were standardized to the resin rod greyscale values.

2.4. Scanning electron microscopy (SEM) analysis

After MicroCT analysis, three specimens of each group were mounted on an aluminum stub, sputter-coated with platinum for 90 s and examined via scanning electron microscopy (SEM) (S-4800, Hitachi, Japan).

2.5. X-ray photoelectron spectroscopy (XPS) analysis

Another three specimens of 2% TiF_4, 2.712% NaF and DDW control groups were subjected to XPS analysis. Only three groups were analysed using XPS because XPS is generally qualitative. XPS spectra were acquired using an AXIS Ultra DLD electron spectrometer (Kratos, UK), employing Al K_α (1486.6 eV) monochrome X-rays at 180 W. For each specimen, a survey spectrum (0–1200 eV) was collected with a pass energy of 160 eV, and high-resolution spectra of C 1s, O 1s, F 1s, P 2p, Ca 2p and Ti 2p regions were collected with a pass energy of 40 eV. The collected data were analysed using CasaXPS Software (Casa Software Ltd, United Kingdom).

2.6. Statistical analysis

The lesion depth and greyscale values were analysed statistically using linear regression at the 5% level of significance (SPSS 14.0; SPSS Inc., USA). A set of dummy variables was constructed for the different groups. Both the dummy variables for the different groups and the