THE COMBINED EFFECT OF XYLITOL AND FLUORIDE IN VARNISH ON BOVINE TEETH SURFACE MICROHARDNESS

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Abstract. The aim of this study was to evaluate the combined effect of xylitol and fluoride in varnish on bovine tooth surface microhardness. Thirty caries-free bovine teeth were sectioned and embedded in self-cured acrylic resin and the buccal enamel surfaces were ground flat. Each tooth was then placed in demineralization solution for 40 hours. After demineralization, the teeth were randomly divided into 3 equal groups: group 1 was the control group (no treatment); group 2 was treated with fluoride varnish (Duraphat®); group 3 was treated with a xylitol and fluoride varnish (Flor-Opal®). All the specimens were then subjected to pH-cycling for 7 days, consisting of demineralization for 6 hours and remineralization for 18 hours repeated daily for five days followed by remineralization for 2 days. Surface microhardness was checked in each tooth at baseline, after demineralization and after pH-cycling. The results were recorded and the data were analyzed with the one-way ANOVA and Tukey tests. A p-value < 0.05 was considered significant. The mean surface microhardness values of the teeth treated with the fluoride varnish and the fluoride with xylitol varnish were not significantly different from each other and showed significantly better remineralization than control group. The fluoride and xylitol varnish combination was beneficial for preventing enamel demineralization but no better than in fluoride varnish alone *in vitro*. Adding xylitol to fluoride tooth varnish does not appear to give any significant benefit *in vitro*.

Keywords: fluoride, xylitol, microhardness, remineralization

INTRODUCTION

Fluoride is the most effective agent in preventing of dental caries (Sh *et al*, 2013). Topical fluorides have been available for several decades and have been proven to prevent caries (Miller *et al*, 2012). Topical fluoride is available in several forms, including fluoride-containing dentifrices, topical fluoride gels and foams, rinses and varnishes. Fluoride varnishes are the agents most commonly used (Chu *et al*, 2010). Fluoride varnish is indicated for prevention of caries because it decreases enamel solubility in acids *in vitro* (Delbem *et al*, 2006), reduces the incidence of dental caries and remineralizes white spot lesions *in vivo* (Weintraub *et al*, 2006) since it adheres to enamel. Calcium fluoride formed after application acts as a long-

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term reservoir for fluoride (Hazelrigg *et al*, 2003).

Fluoride varnish was originally introduced by Schmidt in 1964 under the trade name Duraphat® (Strohmenger and Brambilla, 2001). Fluoride varnish combined with xylitol (Flor-Opal®) is also available on the market.

Xylitol has become a widely used non-cariogenic food additive. Xylitol has been shown to be effective in preventing dental caries by inhibiting the growth and metabolism of mutans streptococci (Hayes, 2001). It is also thought to enhance the remineralization of caries lesions (Mäkinen *et al*, 1995). Although the mechanism of action is unknown, xylitol is belived to be associated with calcium in aqueous solution (Mäkinen and Söderlings, 1984) and it inhibits the dissolution of calcium and phosphate ions from enamel (Arends *et al*, 1990). However, the benefit of xylitol in combination with fluoride in topical oral products, such as varnish, is not clear with respect to the remineralization process.

The aim of this study was to evaluate the combined effect of xylitol and fluoride in varnish on surface microhardness of bovine teeth compared to fluoride alone.

MATERIALS AND METHODS

Specimen preparation

Thirty bovine teeth without wear or caries were used in this study. Specimens were cut from the labial surfaces of the teeth and embedded in self-cured acrylic resin to produce 3x4x3 mm specimens. The labial surfaces were wet ground using 400, 800, 1,000, 2,000 and 4,000 grit silicon carbide paper (Buerler, Lake Bluff, IL) using a rotating polishing machine (Grinder-Polisher, Metaserv 2000 Buehler, West Yorkshire, UK) to obtain a flat, smooth surface, then kept in deionized water at room temperature until used. The baseline surface microhardness of the sound enamel was measured on the labial surface by means of a Vickers indenter (FM-ARS 9000, Future-Tech, Kanagawa, Japan) with 100 grams of force for 15 seconds (Maupomé *et al*, 1999). Four areas on each specimen were measured to obtain a mean microhardness value.

Initial caries-like lesion formation

Caries-like lesions were prepared artificially using a method modified from Maia *et al* (2003). All specimens were immersed in vials containing 0.2 wt% of 450 kDa polyacrylic acid and 0.1 M lactic acid solution saturated with 50% hydroxyapatite and adjusted to a pH 5.0. These vials were subsequently loaded into an incubator for 40 hours at 37°C to establish initial caries-like lesions.

Remineralization procedure

The specimens were randomly divided into three groups of 10 teeth each: in group 1 (control) the teeth received no treatment, in group 2 the teeth were treated with 5% sodium fluoride (Duraphat®, A. Nattterman & Cie GmbH, Waltrop, Germany) and in group 3 the teeth were treated with 5% sodium fluoride combined with sweetened xylitol (Flor-Opal®, Ultradent Products, South Jordan, UT). The varnishes were applied using a microbrush. The specimens were then immersed in artificial saliva (Amaechi *et al*, 1999a,b) for 6 hours (Magalhães *et al*, 2008). After immersion for 6 hours, the varnish was carefully removed from the teeth using acetone and a scalpel blade, taking care to avoid damaging the enamel surface (Souza *et al*, 2010).

The pH-cycling process

The experimental process attempted to imitate the changes in pH in the oral

Table 1
Surface microhardness values at baseline, after caries-like lesion formation and after pH-cycling (N=30).

Group	Surface microhardness (mean VHN±SD)		
	Baseline	After caries-like lesion formation	After pH-cycling
Control	300.43 ± 12.28^{Aa}	50.62 ± 6.92^{Bb}	17.58 ± 4.76^{Cc}
Duraphat®	292.25 ± 19.56^{Aa}	48.36 ± 8.11^{Bb}	95.16 ± 7.79^{Dc}
Flor-Opal®	291.49 ± 13.08^{Aa}	48.82 ± 7.04^{Bb}	92.89 ± 6.25^{Dc}

Within columns, differences in upper-case superscript letters indicate significant differences among treatment groups (One-way ANOVA, $p<0.05$).
Within columns, differences in lower-case superscript letters indicate significant differences among conditions (One-way repeated measures ANOVA, $p<0.05$).
VHN, Vickers hardness number.

environment for 7 days (Vieira *et al*, 2005). All the specimens were subjected to a pH-cycling process. The teeth were placed in a demineralizing solution for 6 hours followed by a demineralizing solution for 18 hours. The demineralization solution was composed of 2.2 mM $CaCl_2$, 2.2 mM NaH_2Po_4, 0.05 M acetic acid with the pH adjusted to 4.0 using 1 M KOH. The remineralization solution was composed of 1.5 mM $CaCl_2$, 0.9 mM NaH_2Po_4, 0.15 M KCl with the pH adjusted to 7.0 using 1 M KOH (ten Cate,1982). This cycle was repeated daily for 5 days and then the specimens were placed in remineralizing solution for 2 days until analyzed. The specimens were then evaluated for surface microhardness again.

Statistical analysis

One-way repeated analysis of variance (ANOVA) and Tukey tests were used to compare the surface microhardness values among the 3 groups at baseline, after the initial caries-like lesion formation procedure and after the pH-cycling process. Significance was set at $p<0.05$.

RESULTS

The mean surface microhardness measurements at baseline, after initial caries-like lesion formation and after the pH-cycling process are shown in Table 1.

The groups had similar mean microhardness values at baseline and after initial caries-like lesion formation ($p>0.05$). After the pH-cycling process, the mean microhardness values in the specimens treated with the fluoride varnish and the fluoride combined with xylitol varnish were not significantly different from each other and increased significantly compared to the mean microhardness values after the demineralization process, but the microhardness values still decreased significantly compared to the microhardness values at baseline. At the end of the study the mean microhardness value in the control group was significantly lower than the mean microhardness values in the treated groups ($p = 0.000$).

DISCUSSION

The baseline surface microhardness value in this study for all groups was 295.75±15.79 VHN. This value is similar to that reported by Vongsawan *et al* (2010) (304.08±7.72 VHN) but lower than 343.87±14.14 VHN and 342.1±21.9 VHN reported by Rirattanapong *et al* in 2011 and 2012, respectively. This could be due to the different of types of teeth used.

In our study, after the caries-like lesion formation process, the mean surface microhardness value for all groups was 48.93±8.51 VHN. All the groups had a significant decrease in microhardness. This means the caries-like lesion formation process caused demineralization of the enamel. Our results are similar to those of Lippert *et al* (2012) who reported a microhardness value in human enamel of 48.0-48.9 VHN after demineralization but lower than Maia *et al* (2003) who reported a microhardness value in bovine enamel of 113.5-123.8 VHN. These differences may be due to different solutions and durations of demineralization.

Both treatment groups showed an increase in microhardness compared to the control group after the pH-cycling process. However, neither of the treatment products tested completely prevented the formation of lesions.

Fluoride ions in the solution are known to be protective against tooth demineralization caused by caries and erosions (ten Cate, 1997; Schlueter *et al*, 2007; Vieira *et al*, 2007). In this study, we used a high fluoride concentration (22,600 ppmF). Calcium fluoride acts as a fluoride reservoir on the tooth surface. When fluoride is applied to the enamel, remineralization occurs (ten Cate, 1997).

Xylitol may be involved in enamel remineralization and demineralization. The effect of xyltiol on remineralization was studied by Arends *et al* (1984), and Smits and Arends (1985). Amaechi *et al* (1998) found demineralization was decreased and remineralization was increased when xylitol was used with fluoride.

In our study, xylitol did not give any further benefit in remineralization than fluoride alone. Our results differ from those reported by Amaechi *et al* (1998) who found xylitol had an additive effect when combined with fluoride in inhibiting dental erosions (bovine incisors) *in vitro*. This difference may be due to differences in type of acid-induced tooth mineral loss, temperature and exposure time.

We used bovine teeth in this study. Yassen *et al* (2011) reported bovine teeth may be used as an alternative to human teeth for microhardness testing.

In conclusion, the fluoride and xylitol tooth varnish was beneficial for preventing enamel demineralization but no better than the fluoride varnish alone *in vitro*. Adding xylitol to fluoride tooth varnish does not appear to give any significant benefit *in vitro*.

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