THE EFFECT OF DIFFERENT TYPES OF MILK ON THE HYDRAULIC CONDUCTANCE OF HUMAN DENTIN IN EXTRACTED TEETH

Kanittha Kijsamanmith, Rudee Surarit and Noppakun Vongsavan

Department of Oral Biology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Abstract. Tooth erosions exposing dentin can cause dentin hypersensitivity treated by reducing hydraulic conductance of the dentin. We aimed to determine the effect of cow, buffalo, soy, and high-calcium milk on the hydraulic conductance of dentin. We obtained 36 human extracted premolars and sectioned each one transversely, 2 mm below the cemento-enamel junction. We then exposed the dentin by cutting a 3 mm diameter, 3 mm deep cavity into the buccal cusp of each tooth. Twenty-four teeth were used for the hydraulic conductance measurements and the other 12 teeth were for examination of morphology of exposed dentin after treatment with each test agent using a scanning electron microscope (SEM). Each of the 24 teeth was subjected to all test materials by sequential random treatment with cow's milk, buffalo milk, soy milk and high-calcium milk. After each treatment, the treated dentin surface was re-etched with 37% phosphoric acid for 30 seconds to reopen dentinal tubule and then re-measured the baseline hydraulic conductance. In the final treatment, dentin was applied with 3% potassium tetraoxalate. The cow's milk, buffalo milk, soy milk, high-calcium milk and 3% potassium tetraoxalate reduced hydraulic conductance by 9.4%, 15.9%, 8.2%, 14.0% and 71.1%, respectively, from their baseline values ($p \leq 0.001$, paired *t*-test). The buffalo milk and high-calcium milk reduced hydraulic conductance significantly more than the cow's milk and soy milk ($p \leq 0.001$, one-way RM ANOVA). SEM showed potassium oxalate occluded tubules most effectively, followed by buffalo milk, high-calcium milk, cow's milk and soy milk. This suggests buffalo milk and high-calcium milk may be more effective at reducing dentin hypersensitivity.

Keywords: dentin hypersensitivity, human dentin, hydraulic conductance, milk

INTRODUCTION

Dentin hypersensitivity is an oral health problem with prevalence rates ranging between 3% and 98% (Splieth and Tachou, 2013). This wide range in prevalence may be explained by different selection criteria for study samples, and variations in diagnostic approaches and/or time-frames (Splieth and Tachou, 2013). About 25-30% of adults report dentin hypersensitivity affecting quality of life (Flynn *et al*, 1985; Amarasena *et al*, 2011). The prevalence of erosions exposing dentin and causing hypersensitivity appears

Correspondence: Dr Rudee Surarit, Department of Oral Biology, Faculty of Dentistry, Mahidol University, Yothi Street, Bangkok 10400, Thailand.
Tel: +66(0) 2200 7849; Fax: +66(0) 2200 7848
E-mail: rudee.sur@mahidol.ac.th

to have increased among younger adults (Bamise *et al*, 2010).

Dentin hypersensitivity is defined as a short, sharp pain arising from exposed dentin in response to stimuli (thermal, evaporative, tactile, osmotic or chemical) which cannot be explained by other dental defects or pathology (Addy, 1990; Holland *et al*, 1997). These stimuli can generate impulses in the intradental nerves via a common sensory transduction mechanism detecting fluid movement in the dentinal tubules (Andrew and Matthews, 2000). Exposed dentinal tubules act as hydraulic links between the site of stimulation and the nerve endings, which are located either in the pulpal ends of the tubules or in the underlying pulp (Vongsavan and Matthews, 2007). The hydrodynamic theory is the most accepted explanation of this phenomenon (Brännström, 1963; Pashley *et al*, 1996; Andrew and Matthews, 2000; Orchardson and Cadden, 2001; Vongsavan and Matthews, 2007).

If dentin sensitivity is due to hydrodynamic fluid movement, decreased dentin permeability should decrease dentin sensitivity (Pashley *et al*, 1984). A decrease in the functional radius of the dentinal tubule should reduce the fluid flow rate in the tubule, or reduce the hydraulic conductance of the dentin (*ie*, the ease with which fluid can flow across the dentin), thus reducing dentin sensitivity. Dentinal tubule-occluding agents have been used clinically to reduce hypersensitivity; for example Sensodyne toothpaste (active ingredient, strontium chloride), which reduces hydraulic conductance by 5.20% *in vitro* (Pashley *et al*, 1984).

Many desensitizing agents have been proposed, such as 8% arginine/calcium carbonate paste, 8% strontium acetate base paste, and milk protein casein GC Tooth Mousse (Recaldent, GC Corp, Japan) (Schiff *et al*, 2011; Sabir *et al*, 2015). Sabir *et al* (2015) found rinsing with milk is effective in reducing dentin hypersensitivity after periodontal procedures.

Milk contains protein and calcium, which may play a role in remineralization and occlusion of dentinal tubules (Sabir *et al*, 2015). Kijsamanmith *et al* (2018) reported cow's milk decreased hydraulic conductance of human dentin by 15%, which was not significantly different from a desensitizing dentifrice containing proargin (Colgate Sensitive Pro-relief™) and a toothpaste containing strontium acetate (Sensodine® Rapid Relief), but was significantly lower than 3% potassium tetraoxalate. Milk composition varies by species (Rafiq *et al*, 2016). Caseins and whey are the main milk proteins and are found in different ratios depending on the species (Rafiq *et al*, 2016). Soy milk typically contains water, protein, oil, simple carbohydrates (sugar) and mineral salts (Friedman and Brandon, 2001). Soy milk is often promoted as a healthy alternative to bovine milk, since it contains lecithin, isoflavones, and vitamin E, does not contain lactose and has less saturated fat than bovine milk (Friedman and Brandon, 2001; Matthews *et al*, 2011). Therefore, it is useful to determine the effect of cow's milk, buffalo milk, soy milk and high-calcium milk on the hydraulic conductance of human dentin, in order to determine which is more effective in treating dentin hypersensitivity.

MATERIALS AND METHODS

Tooth preparation

We conducted a study on 36 healthy premolars extracted for orthodontic treatments. All the teeth were fully erupted, free of caries, without restorations, and intact. After extraction, the teeth were kept in 0.9% normal saline solution with amoxicillin (500 mg/l) and used within 2 weeks. Twenty-four premolars were used to measure hydraulic conductance, while twelve were examined by scanning electron microscopy (SEM).

Each tooth was sectioned transversely 1-2 mm below the cemento-enamel junction to remove the roots, using a diamond disc (Komet type 917; Komet Dental Gebr Brasseler, Lemgo, Germany) under water coolant. The coronal pulp was removed, and a cavity 3 mm in diameter and 3 mm deep was prepared in the buccal cusp using a diamond burs (Intensive® No.204, Viganello-Lugano, Switzerland) with a high speed hand-piece under water spray. The exposed dentin was prevented from drying using normal saline solution.

Hydraulic conductance measurements

After preparing each tooth, 24 premolar crown specimens were used for the hydraulic conductance measurements. The crown segments were connected to the fluid movement model system following the procedure of Kijsamanmith *et al* (2018). Hydraulic conductance was measured by following the movement of an air bubble in a capillary (internal diameter 300 μm, Supracaps Cat No. 709007) (Fig 1). System pressure was controlled by attaching the tubing from the capillary to a manometer. Fluid flow measurements were recorded; the pulpal pressure was maintained at 100 mmHg (13.33 KPa). Three consecutive measurements were taken and hydraulic conductance (L_p) was calculated using the following equation:

L_p = (Flow rate / Pressure) / Area of dentin exposed.

Treatment regimens

After the tooth was prepared, the dentin surfaces were etched with 37% phosphoric acid (Ivoclar Vivadent AG, FL-9494 Schaan, Liechtenstein) for 30 seconds, to remove the smear layer created during the preparation process. Distilled water (negative control) was placed in the cavity after the smear layer had been removed for 5 minutes at a pulp pressure of 11 mmHg (1.46 KPa) above atmospheric pressure (Vongsavan and Matthews, 1992). The baseline value for hydraulic conductance through dentin was recorded at a pressure of 100 mmHg (Kijsamanmith *et al*, 2018). Each of the study 24 teeth was subjected to all test agents by sequential random treatment with cow's milk (Meiji, Saraburi, Thailand), buffalo milk (Murah Farm, Chachoengsao, Thailand),

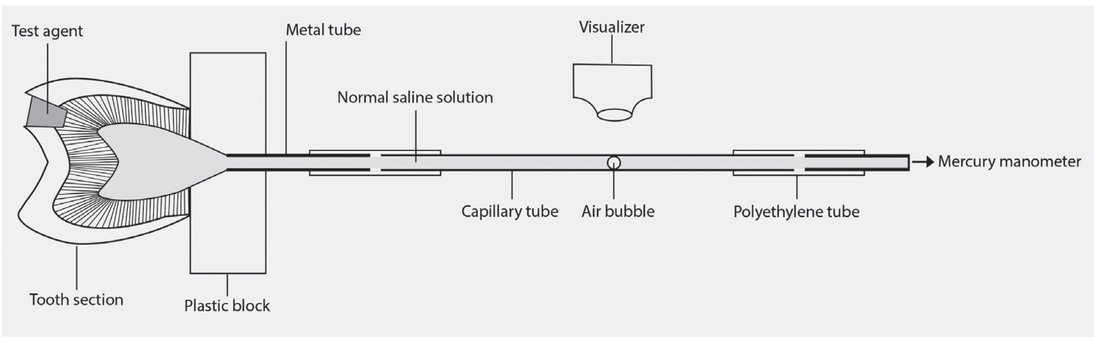

Fig 1 - Diagram of the experimental set-up for measuring fluid flow through dentin *in vitro* (not to scale).

soy milk (Ohayo®, Pathum Thani, Thailand), and high-calcium milk (Anlene™, Nakhon Sawan, Thailand). Of the 24 test specimens, each studied milk type had an equal chance of being used first in the order of treatment. For each treatment, the studied milk was applied to the studied cavity for 5 minutes and rinsed with distilled water for 15 seconds. During the application of each agent, the tubule pressure was maintained at 11 mmHg (1.46 KPa) to represent normal pulpal tissue fluid pressure (Vongsavan and Matthews, 1992). A fluid-filtration method was then used to measure the hydraulic conductance of the treated dentin with a positive pulpal pressure of 100 mmHg (13.33 KPa), as described above. After measuring hydraulic conductance of the first milk treatment, the cavity was re-etched with 37% phosphoric acid for 30 seconds and a pressure of 300 mmHg was applied to remove any remaining agent occluding the dentinal tubules. Baseline hydraulic conductance was again measured before treatment with the other studied milk. Exactly the same procedure was repeated on the same dentin for all other studied milk. Finally, the hydraulic conductance of dentin was confirmed before and after treatment with 3% potassium tetraoxalate (positive control).

After these measurements, each tooth was sectioned buccolingually through the cavity and the remaining dentin thickness, or the minimal length between the pulpal horn and the floor of the cavity, was measured using a digital veneer caliper to control the length of the dentinal tubules (Kijsamanmith *et al*, 2016).

Scanning electron microscopy

After cavity preparation and smear layer removal, twelve crown specimens were divided randomly into 6 groups (two specimens each group) and examined under SEM. In each tooth, only one treatment was conducted. In group 1, no treatment was conducted on the exposed acid-etched dentin surface. In groups 2, 3, 4, and 5, the cavity was filled for 5 minutes with cow's milk, buffalo milk, soy milk, and high-calcium milk, respectively. In group 6, the exposed acid-etched dentin was treated with 3% potassium tetraoxalate for 5 minutes. All the teeth were dried overnight at room temperature. The specimens were fixed to a stub with conductive adhesive tape (SPI Supplies, West Chester, PA) and sputter-coated under vacuum with gold-palladium (SPI-Module™: SPI Supplies Division of Structure Probe, West Chester, PA), and examined by SEM (JSM-5410 LV; JEOL, Tokyo, Japan).

The images were assessed for level of tubule occlusion by three different blinded reviewers. The visual assessment followed the protocol of Davies *et al* (2011) where grades 1, 2, 3, 4 and 5 were defined as 100%, 75%, 50%, 25% and 0 % of the tubules being occluded, respectively.

Statistical analysis

For each treatment, the mean [standard deviation (SD)] hydraulic conductance at baseline and after treatment was compared using the paired *t*-test. A p-value < 0.05 was considered statistically significant. Reductions in percentages of hydraulic conductance were recorded as mean and SD. Comparisons among the different milk types were made using the one-way repeated-measures analysis of variances (one-way RM ANOVA). The Tukey test was used to make comparisons among means.

Ethical considerations

The study protocol was approved by the Institutional Review Board, Faculty of Dentistry/Faculty of Pharmacy, Mahidol University (COE. No. MU-IRB 2011/029.2211).

RESULTS

After acid-etching with 37% phosphoric acid for 30 seconds, baseline hydraulic conductance was determined, to permit the maximum hydraulic conductance for each specimen. The mean (±SD) hydraulic conductance values before treatment with the cow's milk, buffalo milk, soy milk, high-calcium milk and 3% potassium tetraoxalate groups were 1.30 (±1.35) x 10^{-2} nL/s mm^2 mmHg, 1.34 (±1.41) x 10^{-2} nL/s mm^2 mmHg, 1.27 (±1.32) x 10^{-2} nL/s mm^2 mmHg, 1.22 (±1.25) x 10^{-2} nL/s mm^2 mmHg and 1.47 (±1.82) x 10^{-2} nL/s mm^2 mmHg, respectively. The mean hydraulic conductance before treatment in all groups were not significantly different (p=0.248, one-way RM ANOVA). The mean (±SD) hydraulic conductance values after treatment in the cow's milk, buffalo milk, soy milk, high-calcium milk and 3% potassium tetraoxalate groups were: 1.17 (±1.24) x 10^{-2} nL/s mm^2 mmHg ($p<0.001$, paired t-test), 1.15 (±1.22) x 10^{-2} nL/s mm^2 mmHg ($p<0.001$), 1.17 (±1.22) x 10^{-2} nL/s mm^2 mmHg ($p<0.001$), 1.06 (±1.09) x 10^{-2} nL/s mm^2 mmHg ($p<0.001$) and 0.30 (± 0.86) x 10^{-2} nL/s mm^2 mmHg ($p<0.001$), respectively (Fig 2).

All the studied milk groups significantly reduced the hydraulic conductance of human dentin in the extracted teeth. The percentage reductions in hydraulic conductance in the cow's milk, buffalo milk, soy milk, high-calcium milk and 3% potassium tetraoxalate groups were 9.4%, 15.9%, 8.2%, 14.0% and 71.1%, respectively.

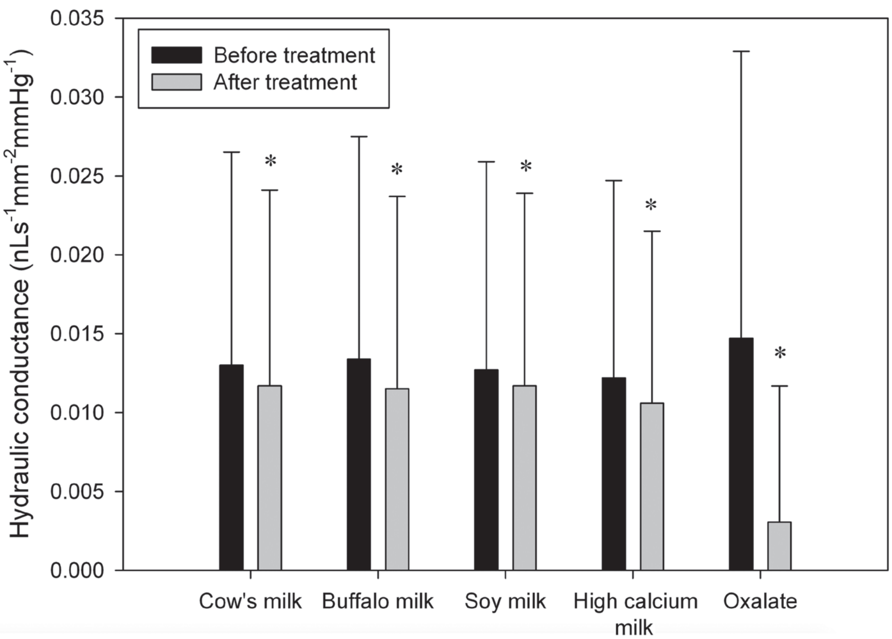

Fig 2 - Mean values for hydraulic conductance by studied agent. The line above each bar represents 1 standard deviation. *After treatment hydraulic conductance was significantly lower than before treatment ($p < 0.001$).

The buffalo milk and high-calcium milk reduced hydraulic conductance significantly more than cow's milk and soy milk groups ($p \leq 0.001$, one-way RM ANOVA, Tukey test) (Fig 3). The average remaining dentin thickness was 1.85±0.37 mm.

SEM showed all 4 studied milk types and 3% potassium tetraoxalate occluded dentinal tubules. The potassium tetraoxalate occluded tubules most effectively, followed by buffalo milk, high-calcium milk, cow's milk and soy milk (Fig 4). Group 1(untreated group) had a clean dentin surface with open dentinal tubule orifices, with each tubule having approximately the same diameter. The cow's milk and soy milk treated teeth were classified as having grade 3 occlusion. The buffalo milk and high-calcium milk were classified as having grade 2 occlusion. The 3% potassium tetraoxalate treated teeth were classified as having grade 1 occlusion. The untreated group was classified as having grade 5 occlusion.

DISCUSSION

Our study results show several milk products can reduce hydraulic conductance of human dentin *in vitro*. Both cow's milk and buffalo milk contained casein phosphopeptide-stabilized calcium phosphates, which exhibit remineralizing potential (Mcdougall, 1977; Featherstone and Zero, 1992; Cochrane *et al*, 2010). Casein protein and high-molecular-weight proteins may also precipitate calcium to occlude dentinal tubules, resulting in reduced hydraulic conductance in dentin (Kijsamanmith *et al*, 2018).

Cow's milk contains 30-35 grams of protein per liter, of which about 80% is arranged in casein micelles (Aimutis, 2004). Besides casein, milk also contains other

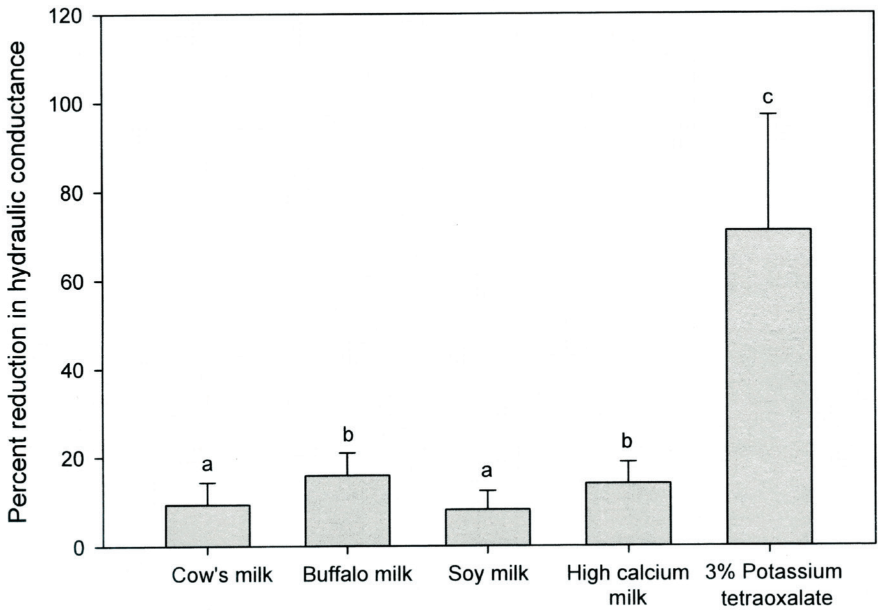

Fig 3-Percent reduction in hydraulic conductance by studied agent. Line represents 1 standard deviation. Same lower-case letter represents no significant difference.

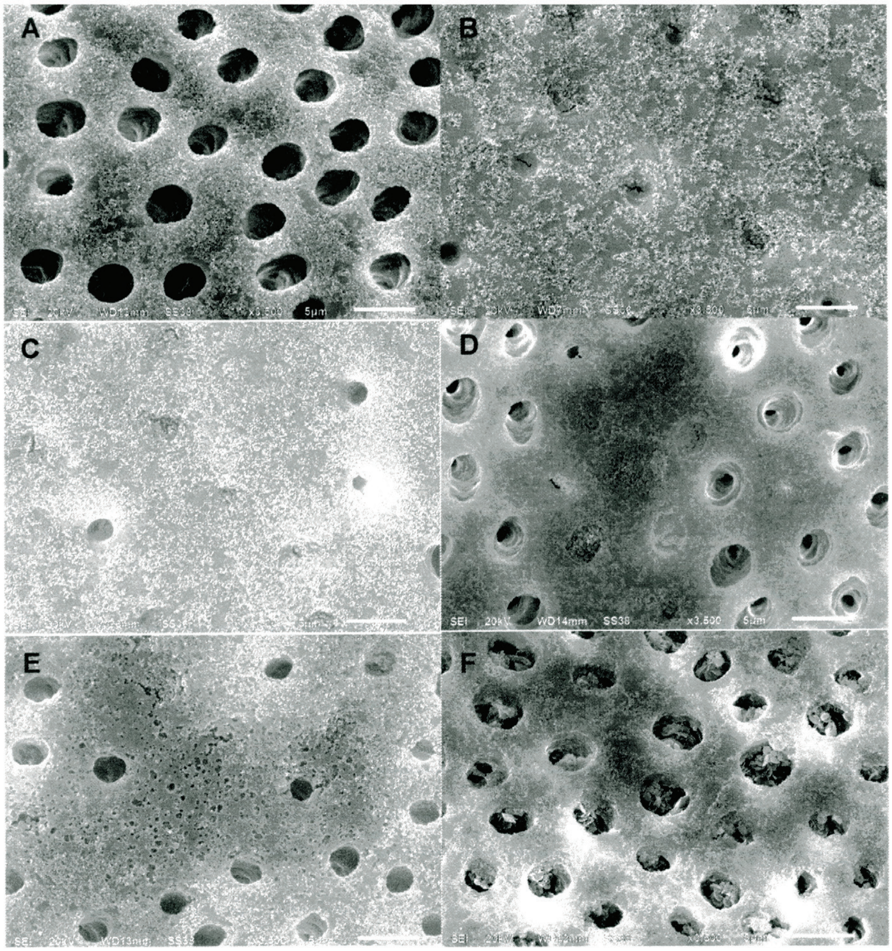

Fig 4 - Scanning electron micrographs (3,500 x magnification) of studied dentin treated by A) 37% phosphoric acid, B) buffalo milk, C) high-calcium milk, D) cow's milk, E) soy milk, F) 3% potassium tetraoxalate.

types of proteins, such as arginine-rich protein, which can help occlude dentinal tubules by working with calcium carbonate and phosphate to create a dentinal-tubule plug (Cummins, 2010). Buffalo milk is consumed in many countries, especially in South Asia, with India, China, and Pakistan being the largest consumers (Rafiq *et al*, 2016). Buffalo milk has been reported to contain higher protein, calcium and phosphate concentrations than cow's milk (Rafiq *et al*, 2016). In our study, buffalo milk and high-calcium milk were more effective than cow's milk and soy milk in reducing hydraulic conductance.

Soy milk contains soy protein, which is rich in sulfur-containing amino acids and resembles casein protein in animal milk (Friedman and Brandon, 2001). The negative charges in soy milk bind to calcium, occlude the dentinal tubules and reduce the hydraulic conductance of dentin (Friedman and Brandon, 2001). Although cow's milk contains more calcium than soy milk, no significant difference was seen in the percent reduction in hydraulic conductance between them in our study.

The greatest reduction in hydraulic conductance in our study was seen with potassium tetraoxalate, similar to previous studies (Pashley and Galloway, 1985; Pashley, 1986). Dentin treated with potassium tetraoxalate caused calcium oxalate crystals to partially occlude dentinal tubules. Calcium oxalate crystals block dentinal tubules, preventing fluid flow in the tubules, reducing hydraulic conductance (Greenhill and Pashley, 1981; Paes Leme *et al*, 2004).

In our study, cow's milk occluded dentinal tubules, reducing dentin hypersensitivity. Milk has also been found to promote remineralization of teeth (Featherstone and Zero, 1992; Aimutis, 2004). To summarize, cow's milk, buffalo milk, soy milk, high-calcium milk and potassium tetraoxalate all significantly reduced dentinal tubule hydraulic conductance in human teeth. However, buffalo milk and high-calcium milk caused a significantly greater reduction in hydraulic conductance than cow's milk and soy milk. The protein and calcium compositions of these milk products may have play a role in this reduction. Further studies are needed to evaluate this hypothesis.

Milk, especially buffalo and high-calcium milk should be considered as natural desensitizing agents for those with dentin hypersensitivity.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Suriyakul Wongsue, Dr Kongpob Dangchot, Dr Supawadee Putthapibal, and Dr Apakorn Boonyai for their extensive help with the experimental work. This study was supported by the Faculty of Dentistry, Mahidol University.

REFERENCES

Addy M. Etiology and clinical implications of dentine hypersensitivity. *Dent Clin North Am* 1990; 34: 503-14.

Aimutis WR. Bioactive properties of milk proteins with particular focus on anticariogenesis. *J Nutr* 2004; 134: 989s-95s.

Amarasena N, Spencer J, Ou Y, Brennan D. Dentine hypersensitivity in a private practice patient population in Australia. *J Oral Rehabil* 2011; 38: 52-60.

Andrew D, Matthews B. Displacement of the contents of dentinal tubules and sensory transduction in intradental nerves of the cat. *J Physiol* 2000; 529: 791-802.

Bamise CT, Kolawole KA, Oloyede EO, Esan TA. Tooth sensitivity experience among residential university students. *Int J Dent Hyg* 2010; 8:95-100.

Brännström M. A hydrodynamic mechanism in the transmission of pain-producing stimuli through the dentine. In: Anderson DJ, ed. Sensory mechanisms in dentine. Oxford: Pergamon, 1963: 73-9.

Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. *J Dent Res* 2010; 89: 1187-97.

Cummins D. Recent advances in dentin hypersensitivity: clinically proven treatments for instant and lasting sensitivity relief. *Am J Dent* 2010; 23: 3A-13A.

Davies M, Paice EM, Jones SB, Leary S, Curtis AR, West NX. Efficacy of desensitizing dentifrices to occlude dentinal tubules. *Eur J Oral Sci* 2011; 119: 497-503.

Featherstone JD, Zero DT. An *in situ* model for simultaneous assessment of inhibition of demineralization and enhancement of remineralization. *J Dent Res* 1992; 71(Special Issue): 804-10.

Flynn J, Galloway R, Orchardson R. The incidence of hypersensitive teeth in the west of Scotland. *J Dent* 1985; 13: 230-6.

Friedman M, Brandon DL. Nutritional and

health benefits of soy proteins. *J Agric Food Chem* 2001; 49: 1069-86.

Greenhill JD, Pashley DH. The effects of desensitizing agents on the hydraulic conductance of human dentin *in vitro*. *J Dent Res* 1981; 60: 686-98.

Holland GR, Narhi MN, Addy M, Gangarosa L, Orchardson R. Guidelines for the design and conduct of clinical trials on dentine hypersensitivity. *J Clin Periodontol* 1997; 24: 808-13.

Kijsamanmith K, Surarit R, Vongsavan N. Effect of tropical fruit juices on dentine permeability and erosive ability in removing the smear layer: an in vitro study. *J Dent Sci* 2016; 11: 130-5.

Kijsamanmith K, Surarit R, Vongsavan N. Comparison of milk and desensitizing dentifrices in reducing hydraulic conductance of human dentin *in vitro*. *Southeast Asian J Trop Med Public Health* 2018; 49: 345-54.

Matthews VL, Knutsen SF, Beeson WL, Fraser GE. Soy milk and dairy consumption are independently associated with ultrasound attenuation of the heel bone among postmenopausal women: The Adventist Health Study-2 (AHS-2). *Nutr Res* 2011; 31: 766-75.

Mcdougall WA. Effect of milk on enamel demineralization and remineralization *in vitro*. *Caries Res* 1977; 42: 171-84.

Orchardson R, Cadden SW. An update on the physiology of the dentine-pulp complex. *Dent Update* 2001; 28: 200-6.

Paes Leme A, Santos JC, Giannini M, Wada RS. Occlusion of dentin tubules by desensitizing agents. *Am J Dent* 2004; 17: 368-72.

Pashley DH. Dentin permeability, dentin sensitivity, and treatment through tubule occlusion. *J Endod* 1986; 12: 465-74.

Pashley DH, Galloway SE. The effects of oxalate treatment on the smear layer of ground surfaces of human dentin. *Arch Oral Biol* 1985; 55: 522-5.

Pashley DH, O' Meara JA, Kepler EE, Galloway SE, Thompson SM, Stewart FP. Effects of desensitizing dentifrices *in vitro*. *J Periodontol* 1984; 30: 731-7.

Pashley DH, Matthews WG, Zhang Y, Johnson M. Fluid shifts across human dentine *in vitro* in response to hydrodynamic stimuli. *Arch Oral Biol* 1996; 41: 1065-72.

Rafiq S, Huma N, Pasha I, Sameen A, Mukhtar O, Khan MI. Chemical composition, nitrogen fractions and amino acids profile of milk from different animal species. *Asian Australas J Anim Sci* 2016; 29: 1022-8.

Sabir M, Nazish S, Alam M. Milk as desensitizing agent for treatment of dentine hypersensitivity following periodontal treatment procedures. *J Clin Diagn Res* 2015; 9: ZC22–5.

Schiff T, Mateo LR, Delgado E, Cummins D, Zhang YP, Devizio W. Clinical efficacy in reducing dentin hypersensitivity of a dentifrice containing 8.0% arginine, calcium carbonate, and 1450 ppm fluoride compared to a dentifrice containing 8% strontium acetate and 1040 ppm fluoride under consumer usage conditions before and after switch-over. *J Clin Dent* 2011; 22: 128-38.

Splieth CH, Tachou A. Epidemiology of dentin hypersensitivity. *Clin Oral Invest* 2013; 17(Suppl 1): S3-8.

Vongsavan N, Matthews B. Fluid flow through cat dentine *in vivo*. *Arch Oral Biol* 1992; 37:175-85.

Vongsavan N, Matthews B. The relationship between the discharge of intradental nerves and the rate of fluid flow through dentine in the cat. *Arch Oral Biol* 2007; 52: 640-7.