Effect of Different Osmotic Stimuli on Fluid Flow Before and After Self-etching Adhesive Application

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Purpose: To determine the in vitro dentinal fluid movement produced by various osmotic stimuli, and evaluate fluid movement across human dentin in response to the application of various osmotic stimuli before and after the application of self-etching adhesive (Clearfil S3 Bond).

Materials and Methods: The experiments were carried out on 10 extracted premolars. Each tooth was cut transversely below to the cementoenamel junction with a diamond disk and water coolant. Dentin was exposed at the tip of the buccal cusp by cutting a cavity and was etched with acid. The osmotic stimuli were solutions of saturated $CaCl_2$, sugar syrup, chocolate, and sweet Thai dessert, used as osmotic test solutions, randomly applied to dentin. The fluid flow through dentin obtained after 15 s of application of each osmotic stimulus was measured before and after bonding with Clearfil S^3 Bond single-dose.

Results: Before bonding procedures, $CaCl_2$ produced peak rates of fluid flow that were significantly higher ($p < 0.001$), when compared with normal saline, sugar syrup, chocolate, and sweet Thai dessert. During the applications of all osmotic stimuli, the amount of fluid movement across resin-bonded dentin was significantly lower than that without adhesives. There were no significant differences of fluid shifts across resin-bonded dentin obtained during the application of any osmotic stimuli.

Conclusion: It appears that different osmotic stimuli produced different rates of outward fluid flow through dentin. Clearfil S^3 Bond produced similar significant reductions of fluid movement in response to osmotic stimulation, irrespective of the chemical composition, or the osmotic pressure of stimuli.

Keywords: osmotic stimuli, fluid flow through dentin, self-etching adhesive, dentin permeability, human teeth.

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Clinically, osmotic stimuli such as a wide variety of sweet food, particularly chocolate and sugar syrup, are one of the main factors causing dentin hypersensitivity. Previous studies showed that the different stimuli, including osmotic, when applied to dentin caused pain by a hydrodynamic mechanism that involves displacement of the dentinal fluid and excites intradental nerve endings.[5,17,18,22]

Hypertonic solutions of sucrose or $CaCl_2$ have been used to test the sensitivity of dentin.[2] In animal experiments,[4] no consistent effect of osmotic stimulation with 4 mol/l dextrose has been shown, although some preparations reveal a small outward flow. However, in human teeth,[15] 4 mol/l dextrose produced more flow than was observed in the recently extracted cat teeth. The authors suggested that this difference may be due to cat dentin having a lower reflection coefficient for dextrose. In studies of pain-producing activity of food materials, Anderson and Ronning[3] showed the relationship between osmotic pressure and pain-producing activity when $CaCl_2$ solution and other solutes in the range of 200 to 2800 atm were applied to human dentin.[2] Therefore, these results demonstrated the possibility of using an osmotic stimulus to evoke pain from dentin in human teeth.[23,25]

Patent dentinal tubules are commonly present in the area of hypersensitive dentin.[31,32] When those tubules are blocked with occluding agents such as oxalate treatment, some temporary relief of pain is found.[16,19] Also, several studies revealed that dentin bonded with self-etching adhesive systems produced some degree of reduction in dentin permeability[12,13] and hypersensitivity.[10,27,28] However, the results of in vivo and in vitro studies indicated that all single-bottle adhesives were somewhat permeable to dentin fluid

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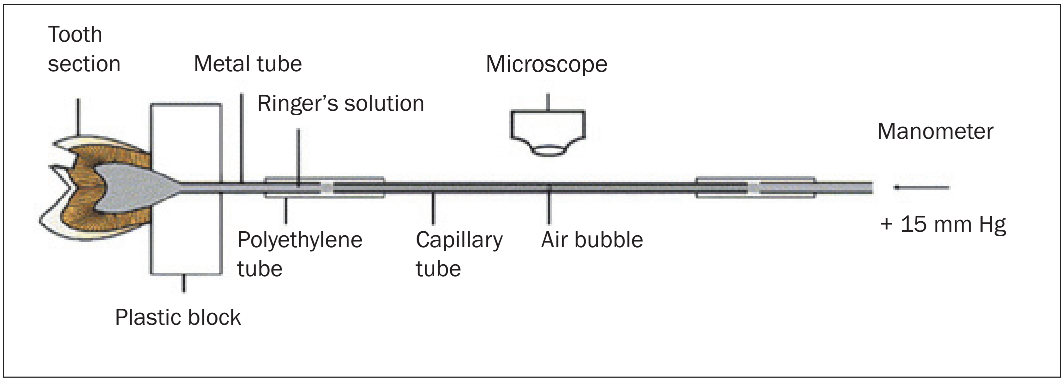

Fig 1 Diagram of the experimental setup for measuring the rate of fluid flow through dentin produced by an application of each osmotic stimulus. Regression analysis was used to test the correlations between fluid flow and osmolality of test solutions before and after bonding.

and small molecules after polymerization.[8,29] It is not known whether this fluid movement can be affected by the applications of various osmotic stimuli.[6,14] The aims of this study were to determine the dentinal fluid movement across human dentin in vitro in response to the application of various osmotic stimuli before and after the application of self-etching adhesive.

MATERIALS AND METHODS

The experiments were carried out on 10 recently extracted premolars. The teeth were extracted as part of orthodontic treatment. Each tooth was transversely cut 1-2 mm below to the cementoenamel junction with a diamond disk and water coolant. An endodontic file was used to remove coronal pulp tissue, during which water irrigation from a triple syringe was done to remove tissue debris.

A cylindrical preparation at the tip of the buccal cusp of a premolar was made by cutting a cavity (diameter 3 mm, depth 3 mm) with round and cylindrical high speed diamond burs No. 201 and No. 204 (Intensive, Viganello-Lugano, Switzerland) under air-water spray from an air-turbine handpiece. Dentin in the cut cavity was etched with 35% phosphoric acid for 30 s. An acrylic block penetrated by a stainless-steel tube (18G, outer diameter 1.27 mm, inner diameter 0.8 mm) had been bonded to the cut surface of the crown (Superglue Gel Extra; Locktite, Welwyn Garden City, UK). A glass capillary with an internal diameter of 300 µm was connected to this tube. Normal saline solution filled the pulp chamber, tube, and capillary. A manometer with a pressure of 15 mm Hg was applied through the capillary to maintain the pulp tissue pressure. A magnifying lamp was used to measure the movement of fluid between the pulp and dentin by recording the movement of a small air bubble that was introduced into the capillary. During the application of each chemical stimulus, the volume flow through the capillary was calculated from the distance moved by the bubble. The fluid flow through dentin produced by different stimuli was measured twice each under a magnifying lamp. The diagram of the experimental design is shown in Fig 1.

The solutions of saturated calcium chloride in distilled water at 25°C, concentrated flavored sugar syrup (Hale's blue boy, Bangkok, Thailaind), 1.25 g/ml chocolate (Van Houten, Switzerland), 1.26 g/ml sweet Thai dessert (Gold Egg Yolk Drops: S&P company, Thailand) were randomly used as osmotic stimuli. The control isotonic solution consisted of normal saline solution. Each osmotic stimulus was applied to the cavity in the same amount of 0.02 ml. After etching with 35% phosphoric acid to remove the smear layer from the cut cavity, the fluid flow was recorded by the same observer for 15 s after the application of each osmotic stimulus. A hydrostatic pressure of 300 mm Hg above atmospheric was applied through the pulp cavity at the end of each stimulation. This was done to remove any blockage of dentinal tubules and to restore the hydraulic conductance, so that the tubules were patent for next stimulation. This also insured that there was no residual hypertonic solution in the tubules.

The same procedures were repeated after the application of self-etching adhesive (Clearfil S^3 Bond single-dose, Kuraray, Tokyo, Japan) to the dentinal floor of the prepared cavity following the manufacturer's instruction, except that the normal saline solution was used to clean the cut cavity during the application of each osmotic stimulus.

The total osmolality of each test solution was determined by comparing the freezing point of pure water with the freezing point of the test solution by using an Osmomat auto (Gonotec, Berlin, Germany). Multiple 20-fold dilutions of saturated $CaCl_2$ solution were made, while the solutions of sugar syrup, chocolate, and Thai dessert were diluted 10-fold. After the osmotic activities of all dilutions were measured, the values were then multiplied by 20 and 10, respectively, to obtain the osmolalities of undiluted test solutions.

Statistical Analysis

The fluid flow rates obtained under different conditions are reported as means ± 1 standard deviation (SD).

One-way repeated-measures analysis of variance (one-way RM ANOVA) was used to determine the difference of mean fluid flow rates recorded before applying the bonding adhesive. Significant differences between the means were evaluated using the Holm-Sidak method for making multiple comparisons between them.

Since the fluid flow rates recorded after the application of dental adhesive were not normally distributed (Kol-

Table 1 Fluid flow rate ($nL/s/mm^2$) measured for 15 s immediately after the application of various osmotic stimuli with and without self-etching adhesive application

Stimuli	Without bonding£	With bonding°	%Change from without bonding§
Normal saline*	0.39 (0.14)^{A,1}	0.20 (0.13)^{a,2}	-48.27 (30.52)^{c}
sat. $CaCl_2$*	1.15 (0.37)^{B,1}	0.25 (0.15)^{a,2}	-79.33 (10.17)^{d}
Sugar syrup*	0.80 (0.22)^{C,1}	0.20 (0.15)^{a,2}	-75.65 (17.39)^{d}
Chocolate*	0.65 (0.22)^{C,D,1}	0.20 (0.14)^{a,2}	-72.46 (18.48)^{d}
Thai dessert*	0.55 (0.19)^{A,D,1}	0.20 (0.13)^{a,2}	-65.63 (17.69)^{c}

Values are mean (SD) in $nL/s/mm^2$ (n=10).

* For each osmotic stimulus, a significant difference ($p \leq 0.001$) between the "without bonding" subgroup and the "with bonding" subgroup is indicated by different numerical superscripts.

£ In the column "without bonding", subgroups with different uppercase letter superscripts indicate a significant difference ($p \leq 0.001$).

° In the column "with bonding", subgroups with different lowercase letter superscripts indicate a significant difference ($p \leq 0.001$).

§ In the column "% change from without bonding", subgroups with different lowercase letter superscripts indicate a significant difference ($p = 0.001$).

mogorov-Smirnov normality test), nonparametric statistical tests were used to compare the median fluid flow rates obtained under different conditions by using Friedman repeated-measures analysis of variance on ranks. Where this showed that there were significant differences between the medians, Dunn's method was used to make multiple comparisons between them. Correlations between the osmolalities of the different test solutions and the mean fluid flow through dentin were determined by using linear regression analyses. P values of less than 0.05 were considered significant.

RESULTS

Table 1 shows summarized data of mean fluid flow rates recorded under different conditions. With atmospheric pressure in the cavity and the pulpal pressure maintained at 15 mm Hg, $CaCl_2$ solution applied before using adhesive produced a significant increase in the mean value of fluid flow rates when compared with saline, sugar syrup, chocolate and sweet dessert ($p \leq 0.001$). Before the application of self-etching adhesive, outward fluid flow rates measured during application of sweet dessert and saline were significantly less than that of sugar syrup ($p \leq 0.001$). Chocolate produced a significantly higher rate of fluid flow than did saline. Sweet Thai dessert and chocolate seemed to produce similar fluid flow rates during the observed 15 s. After bonding application, all osmotic stimuli produced a significant reduction of fluid movement when compared with those without bonding ($p \leq 0.001$). The application of self-etching adhesive (Clearfil S3 Bond) to the cavity floor in vitro significantly decreased the rate of outward fluid movement from the pulp chamber toward the exposed dentin in response to application of all osmotic stimuli.

Compared with all the test solutions, saturated $CaCl_2$ solution produced the highest osmotic activity of 37.04 Osm/kg. The osmolalities of sugar syrup, Thai dessert and chocolate were 3.34, 4.30 and 4.63 Osm/kg, respectively, whereas that of isotonic saline was 0.29 Osm/kg. Correlations between the osmolalities of the test solutions and the mean rates of fluid flow through dentin before and after the applications of bonding are shown in Figs 2a and 2b, respectively. Fluid flow obtained before bonding was moderately correlated with the osmolality of test solutions ($R^2 = 0.788$, $p = 0.044$), while the strong correlations were observed between fluid flow after bonding and the osmolality of solutions ($R^2 = 0.99$, $p < 0.001$).

The fluid flow was always greater than zero when the osmolality of the solution was zero because they maintained a steady constant hydrostatic pressure of 15 mm Hg at all times. Thus, about 0.2 nL mm^{-2} s^{-1} were due to fluid filtration via the hydrostatic pressure. When the osmotic stimuli were applied, the outward fluid flow increased due to osmotic-induced fluid flow in addition to the convective flow.

DISCUSSION

This is one of the first studies to reveal dentinal fluid flow produced during the application of a wide range of osmotic stimuli to etched dentin,[24] and after self-etching adhesive application.[14] The experiments have demonstrated that saturated $CaCl_2$ produced higher rates of outward flow of fluid through dentin, compared with those of sugar syrup, 1.25 g/ml chocolate, 1.26 g/ml Thai dessert, and saline. It is likely that some of the fluid flow rate produced during saline application in our study might be partly due to the outward pulpal pressure we applied. Clinically, all the osmotic stimuli used in our study are capable of caus-

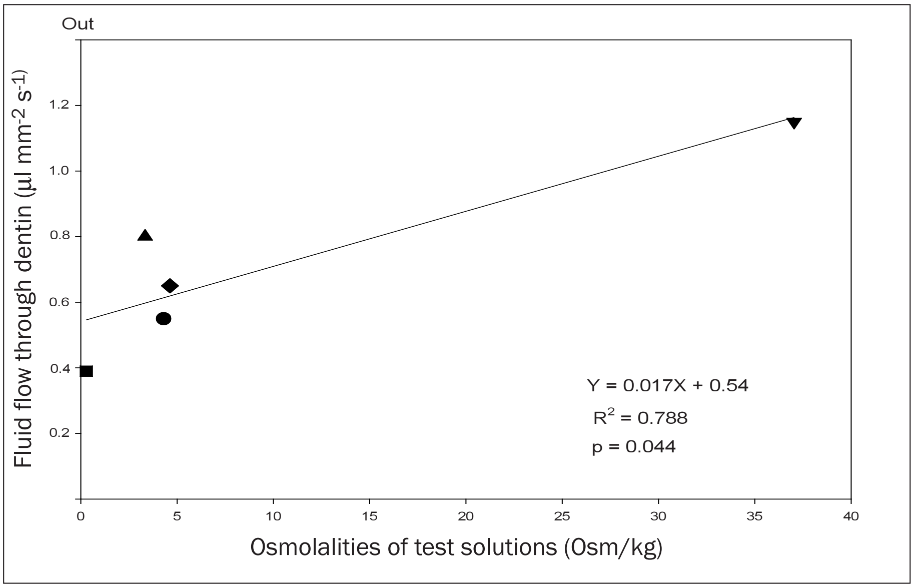

Fig 2a Correlations between the osmolalities of the test solutions and the mean fluid flow through dentin before the application of Clearfil S^3 Bond. The black symbols represent the mean fluid flow rate obtained before the application of Clearfil S^3 Bond. The test solutions were as follows: normal saline (■); sugar syrup (▲); Thai dessert (●); chocolate (◆); and saturated $CaCl_2$ solution (▼).

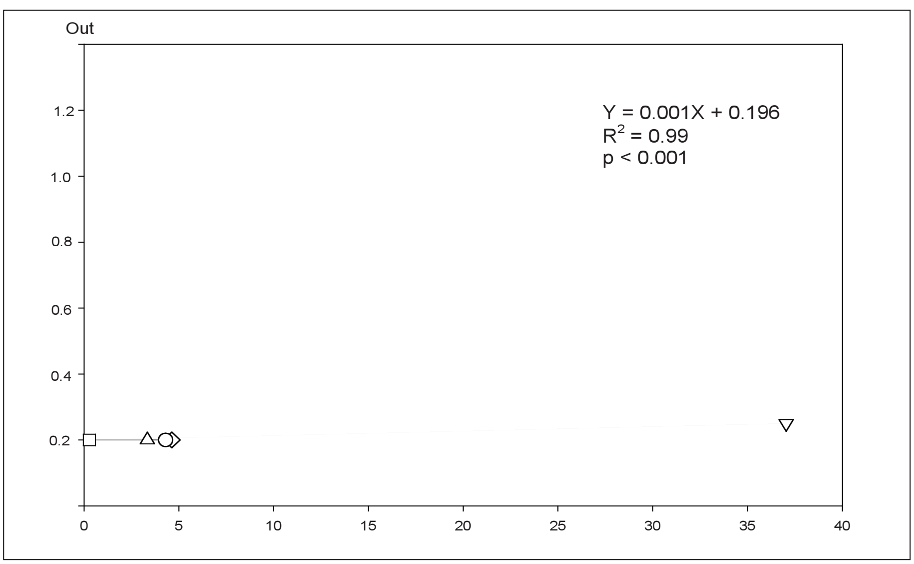

Fig 2b Correlations between the osmolalities of the test solutions and the mean fluid flow through dentin after the application of Clearfil S^3 Bond. The white symbols represent the mean fluid flow rate obtained after the application of Clearfil S^3 Bond. The test solutions were as follows: normal saline (❑); sugar syrup (Δ); Thai dessert (O); chocolate (◊); and saturated $CaCl_2$ solution (∇).

ing pain in humans, and almost all of them increased the rates of outward fluid flow through dentin when compared with that of saline.

Both electrophysiological recordings on experimental animals and human studies indicated the ability of hypertonic solutions to evoke responses from intradental nerves and induce fluid flow from dentin.[20,21] Anderson and Matthews[2] demonstrated the relationship between osmotic pressure of a solution and its ability to cause pain when applied to dentin in human subjects. In that study, they investigated the effects of varieties of either the chemical composition of the solute, or the degree of osmotic pressure on the pain sensation. However, no attempt was made to determine the relationship between the flow produced by various osmotic stimuli and the pain intensity. At present, the mechanism by which the movement of the tubule contents generates the nerve impulses is still unclear. The simple explanation may be that the nerve endings are deformed by the movement of the contents of dentinal tubules.[4]

Recently, the rate of dentinal fluid flow and pain thresholds during the application of positive and negative hydrostatic pressure stimuli to etched dentin in humans were determined.[7] In that study, the authors showed that the mean flow rate at the threshold for pain in an individual tooth was 3.29 ± 2.36 nL/(s mm^2) outwards for negative pressures and 5.75 ± 3.62 nL/(s mm^2) inwards for positive pressures.

The authors pointed out that the sensory transduction mechanism for pain in human teeth is more sensitive to outward than inward flow through dentinal tubules.[7] Using a similar procedure to record fluid flow in the current study, the maximum mean flow rate obtained during the application of the saturated $CaCl_2$ solution at 25°C was 1.15 nL/(s mm^2). With the methods employed in the present experiments, it was not possible to investigate the dental pain threshold caused by osmotic stimulation in vivo. However, it was found from previous studies in humans that the osmotic pressure of saturated $CaCl_2$ solution at 35°C was 2800 atm, and this preparation was proven to be the most potent osmotic stimulus to evoke pain.[3,15] Although we did not measure the osmotic pressure of test solutions, the measured values of the osmotic pressure produced by saturated $CaCl_2$ solution was stable from 25°C to 100°C.[26] Thus, fluid flow generated by saturated $CaCl_2$ solution before using the self-etching adhesive was also able to cause pain in humans. As the outward flow rate obtained in the current study was less than the flow rate at threshold during negative pressure stimulation in a previous study,[21] the transduction mechanism by which osmotic stimuli evoke pain in human teeth may be different from that of hydrostatic negative pressure stimulation. A possibility that has not yet been investigated is that osmotic stimuli may also cause the changes in both the ionic concentration and composition of extracellular fluid around the intradental nerve termini, so that this could either activate or sensitize slow-conducting A-δ or C fiber afferents.[1] Further studies are planned to investigate the relationship between the pain intensities during the application of osmotic stimuli before and after smear layer removal in humans. This will give us more explanation of the possible sensory transduction mechanism caused by osmotic stimulation in human teeth.

The osmotic stimuli used in our study contained a wide range of ingredients and osmolalities (Fig 2). Other than sugar syrup and $CaCl_2$ solutions, the main ingredients of sweet Thai dessert (Gold egg yolk drops) are egg yolks and a mixture of wheat and rice flours, whereas chocolate contains milk and cocoa butter. Figure 2 shows that the increase in the osmolality tended to increase the rate of fluid flow through etched dentin. Although the osmotic activity of sugar syrup was less than that of Thai dessert and chocolate, the fluid flow induced by sugar syrup was significantly greater than that of Thai dessert. This is because of differences in the degree to which the osmotic gradient is lowered by the permeability of test solutes, or the reflection coefficient values of the test solutions to acid-etched dentin.[6]

After the application of self-etching adhesive (Clearfil S[3] Bond) to cut dentin surfaces, a significant reduction of fluid flow rates recorded during osmotic stimulations might be attributed to the sealing ability of adhesive. Carrilho et al[6] suggested that both water and some small solutes might permeate through the hybrid, and adhesive layers in a varying degree, determined also by the reflection coefficients of various molecules for various resins. The presence of similar reductions of fluid flow rates produced across resin-bonded dentin during the application of hypertonic solution in the current study suggested that this adhesive did not seem to be permeable to any solutes.

By using our test system with the presence of a simulated pulpal pressure of 15 mm Hg,[9,30] Clearfil S[3] Bond was able to provide a great reduction in dentin permeability during the osmotic stimulation, regardless of the chemical compositions of the test solutes. This may be because resin tags occluded the tubules, as also confirmed by previous studies.[11,12] In a future study, it would be interesting to compare this sealing ability with other self-etching adhesives, including etch-and-rinse bonding systems.

CONCLUSION

In conclusion, it appears that different osmotic stimuli produced different rates of outward fluid flow through dentin. Clearfil Bond significantly reduced fluid movement in response to osmotic stimulation, irrespective of the chemical composition or the osmotic pressure of stimuli.

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Clinical relevance: The results of this study demonstrated the possibility that the hypertonic solutions may be initially used to test the occluding ability of agents in the treatment of hypersensitive dentin.