

Effect of gender of the operator and application mode of adhesives on dentin hydraulic conductance in an in vitro model

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Work received on 22/06/2017. Approved for publication on 14/08/2017

ABSTRACT

Aim: To compare the hydraulic conductance of human dentin disks which have been treated with an adhesive by operators of different genders and by active or passive modes of application. 60 third molars of healthy adults were included in resin blocks. These were cut to obtain 60 dentin disks of 1mm +/- 0.1 mm thick. Group 1 and 2, the adhesive was applied by 15 male operators passively (group 1) and vigorously (group 2). Groups 3 and 4, the adhesive was applied by 15 female operators passively (group 3) and vigorously (group 4). The flow rate was measured with a diffusion chamber and the hydraulic conductance of the disks was determined. **Results:** Mean for hydraulic conductance of each group was: 1 (0.01752), 2 (0.00355), 3 (0.01215), 4 (0.00877) in $\mu\text{l}/\text{min}\times\text{cm}^2$. There was statistically significant difference in the hydraulic conductance between experimental and control groups. There was no difference in dentin hydraulic conductance between genders. There was statistically significant difference in hydraulic conductance between the different modes of application. There was a moderate association between the pressure exerted when applying the adhesive and the values of dentin hydraulic conductance in vitro

KEY WORDS

Gender, Application modalities, Adhesion, Hydraulic conductance.

Rev. Clin. Periodoncia Implantol. Rehabil. Oral Vol. 11(1); 06-08, 2018.

INTRODUCTION

Dentin is crossed by tubules that extend from the pulp to the enamel-dentin or cement-dentin junction, forming a permeable mineralized tissue in which there is an active exchange of substances, including noxious agents⁽¹⁾. Dentin permeability is understood as a state or quality of being open to the passage, especially fluids, ions, bacteria, and minute particles. This may have an effect on pulp reactions, becoming dentin permeability an integral part of modern restorative dentistry where adhesive technology plays a central role⁽²⁾.

Exposure of dentinal tubules is inherent in the process of cavity preparation, working as channels that transmit mechanical, chemical and bacterial stimuli to the pulp. Phenomena as dentin hypersensitivity are related to dentin permeability. This is defined as an acute, sharp pain initiated in a dentin exposed area of the tooth in response to chemical, thermal, tactile or osmotic stimuli. This pain cannot be attributed to any other form of dental pathology⁽³⁾. As exposed by Brännström⁽⁴⁾ fluid movement inside dentinal tubules triggered by external stimuli (as cold, hot or pressure) reaches nerve receptors at the pulp-dentin complex generating pain (hydrodynamic theory of dentinal pain). Dentin permeability study has been focused primarily on the concept of hypersensitivity and new adhesive materials⁽⁵⁾.

The use of adhesive materials over dentin has the effect of significantly reduce the fluid movement through it⁽⁶⁾. Adhesive materials occlude dentinal tubules, mechanically blocking extrinsic stimuli, thus decreasing dentin hypersensitivity⁽⁷⁾.

To compare dentin permeability between different teeth, with different dentin areas, it is important to know their hydraulic conductance, defined as the fluid flow through dentin over the surface area through which the flow occurs⁽²⁾.

In vitro⁽⁸⁾ and *in vivo*⁽⁹⁾ studies have reported that bond strength between dental adhesives and dentin can be improved by applying them vigorously. It has been suggested that active application may increase the smear layer dissolution, improving micromechanical interlocking and chemical interaction with the dentin. Probably this is because the acidic resin monomers of the adhesive are deposited in the basal part of the etched dentin. This produces a more aggressive demineralization,

facilitating monomer diffusion, promoting better interaction between dentin and the smear layer⁽¹⁰⁾. Also, recent findings have shown that the active application improves the adhesive mechanical properties^(8, 11) since it can improve the degree of conversion of the adhesive^(12, 13).

In the animal kingdom, adult males and females often differ in size⁽¹³⁾. Mammal males are often larger than females, being this sexual dimorphism evident for human body composition from fetal life^(13, 14). Human sexual size dimorphism is commonly measured as the male to female stature (height) ratio, being approximately 1.0713. Adult males often have greater arm muscle mass, larger and stronger bones, and reduced limb fat compared to females⁽¹⁴⁾. These differences are also expressed as differences in strength and muscle fiber characteristics between both genders, with male showing greater upper body strength⁽¹⁵⁾.

These differences in muscle mass could have an effect on dental adhesive application. Therefore, the aim of this in vitro study was to determine whether the gender and/or the modalities (passive or active) of the operator performing the adhesive dental application affects dentin permeability.

The hypothesis is that there are differences in dentin hydraulic conductance when dental adhesive is applied by male or female operators and according to the application modality.

MATERIALS AND METHODS

60 human caries free third molars (upper and lower) of healthy adult patients were included. Patients donated their teeth after reading and signing an informed consent form approved by the Ethics Committee of Universidad de Chile Faculty of Dentistry (PRO-ODO 12-004).

Teeth were cleaned with Gracey curettes (HuFriedy® USA) to remove traces of the periodontal ligament and disinfected for 24 hours in 0.1% thymol solution. Where embedded in 25mm diameter epoxy resin cylinders (Bosh®) that was allowed to polymerize for 48hrs as indicated by the manufacturer. Dentine discs of 1mm ($\pm 0.1\text{mm}$) were obtained cutting the cylinders perpendicular to their long axis (Buehler Isomet Ler Lakebuff IL, USA 1000) at 700 rpm, 500grs pressure and under copious irrigation. Occlusal and pulpal surfaces of each disc

were regularized with abrasive paper (Silicon Paper No. 600, SIA, Switzerland) under water to standardize thickness and obtain a smooth surface. Finally, both surfaces of each disc were acid etched with 37% phosphoric acid (3M™ ESPE® Scotchbond® Etchant Gel) for 15 seconds to remove the smear layer and washed with water for 30 seconds.

Discs were randomly assigned to:

Group 1 (n=15) (male operator-passive application – G control): one coat of dental adhesive (Single Bond Universal®, 3M ESPE) was gently rubbed for 20 seconds over one disc surface by a male operator. Adhesive was gently dried for 5 sec at a 10cm distance. A second layer was applied in the same way. The adhesive was light-cured for 20 seconds using a 3M ESPE Elipar 2500 lamp (900mW/cm²)

Group 2 (n=15) (male operator-active application): one coat of dental adhesive (Single Bond Universal®) was vigorously rubbed for 20 seconds over one disc surface by a male operator. Adhesive was gently blown for 5 sec at a 10cm distance, and a second layer was applied and light cured.

Group 3 (n=15) (female operator-passive application – G Control): a female operator performed the same procedure as in group 1.

Group 4 (n=15) (female operator-active application): a female operator performed the same procedure as in group 2.

Dental adhesive application was done by 15 male operators (groups 1 and 2) and 15 female operators (groups 3 and 4). Each operator applied adhesive passively over one disc and actively (vigorously) over another. All applications were done over a digital scale (Radweg WT2000®) to register in grams (gr) the force of every operator over the disc.

Flow measurement of each disc was done with an experimental model similar to that proposed by Pashley et al⁽²¹⁾, with a water column of 200mm long coupled to a stopcock. This water reservoir was connected by a silicone tube to a horizontally positioned graduated capillary tube in which an air bubble was included. The capillary tube was distally connected by a silicone tube to the chamber where the dentine disc under study was placed and sealed by silicone rings⁽¹⁶⁾.

Flow rate was determined recording the initial position of the air bubble and its final position after a period of 20 minutes.

Dentine exposed area of each disc was calculated using ImageJ software⁽²²⁾ and hydraulic conductance (HC) determined by the formula⁽²³⁾:

$$HC = F / (P(SA))$$

Where:

F: flow rate (µl/min),

P: hydrostatic pressure over dentine (20cm in accordance to water column height)

SA: surface area of exposed dentine (cm²)

Data distribution was evaluated by Shapiro-Wilk test and dentine hydraulic conductance compared considering operator genre and adhesive application (passive or active) as the independent variables. Additionally, hydraulic conductance was correlated with application force of the adhesive system. All tests were performed in SPSS 15 for Windows software, considering a p-value <0.05

RESULTS

Table 1 shows applied force values for each group. The mean applied force value by male operators was 56.85 grs and by female operators was 51.27 grs. When considering the adhesive mode of application, the mean value when the adhesive was applied passively was 5.73 grs and 102.4 grs when actively applied.

Table 1. Force applied on groups (gr)

	Group 1	Group 2	Group 3	Group 4
Mean	5.167	108.533	6.287	96.267
Standard deviation	4.568	49.716	4.929	28.267
Minimum	1.000	50.000	1.000	60.000
Maximum	18.000	250.000	18.000	135.000

Table 2 shows hydraulic conductance values of each group. The mean hydraulic conductance value for males treated groups was 0.0105µl/min*cm² and for females treated groups was 0.105µl/min*cm². When considering the adhesive application, the mean value for passively treated groups was 0.0148µl/min*cm² and 0.0062µl/min*cm² for actively treated groups.

Table 2. Hydraulic conductance of groups (µl/min*cm²)

	Group 1	Group 2	Group 3	Group 4
Mean	0.01752	0.00355	0.01215	0.00877
Standard deviation	0.01468	0.00160	0.00763	0.01628
Minimum	0.00381	0.00171	0.00491	0.00197
Maximum	0.05198	0.00622	0.03125	0.06649

Mann-Whitney test was performed to compare the applied force according to the gender or adhesive application. No differences were seen on applied force between male and female groups (p=0,877). Differences were seen when compared active or passive adhesive application (p<0.05), with higher forces where an active application of the adhesive was done.

When hydraulic conductance was compared, no differences were seen between genres (p=0.626, Mann-Whitney test). Differences were seen between active and passive adhesive application (p<0.05), with higher values for passive applied groups.

Spearman Rho test showed a moderate statistically significant correlation between the applied force and the hydraulic conductance (Rho=-0.681, p<0.05).

DISCUSSION

In this paper was tested under a known model⁽¹⁶⁾ the difference in hydraulic conductance by gender operator and/or the mode of application of a dental adhesive. While there were no differences in conductance according to the gender of the operator, differences were seen when considering the application mode. Thus, the hypothesis of this paper is partially rejected.

Dental adhesive materials attempt to occlude dentinal tubules mechanically blocking extrinsic stimulus, and so decreasing sensitivity⁽³⁾. In our study all treated groups showed dentin permeability, expressed as hydraulic conductance, although all were adhesively treated. *In vitro* studies have shown that after the application of either etch-and-rinse or self-etch adhesive systems, permeability is not blocked, being dentin permeable to fluid movement^(17,18). Therefore, it is interesting to know whether any factor related to the application of the adhesives could affect sealing dentin, this reflected as hydraulic conductance reduction.

The use of acid etching before adhesive application increase dentine permeability⁽¹⁸⁾. Also, this increases the bond strength over enamel and dentin by increasing superficial energy and contact surface⁽¹²⁾. One way to reduce dentin permeability is by vigorously applying the adhesive over the tooth surface⁽¹⁹⁾. In our study differences were seen between active and passive adhesive application groups, with decreased conductance in the actively applied groups. The active adhesive application can improve the degree of conversion of the adhesive increasing a chemical interaction between acidic monomers and dentin hydroxyapatite⁽¹²⁾. This chemical interaction could provide a buffer effect to the acidic monomer while otherwise more initiator is consumed, comprising the efficiency of photopolymerization^(20, 21).

Vigorous adhesive application implies greater strength when rubbing it. It has been demonstrated differences between male and female humans, with stronger males since they have greater muscle mass⁽¹³⁻¹⁵⁾. In our study, no differences were seen between male or female operators. When passive adhesive application was completed, the force generated by both genres was quite small, with no differences between them. Such small forces could be achieved by every operator.

Greater forces were applied when active application was carried out. Same as in passive application, the force reached when actively applied was equal for male and female operators, as the applicator tip broke down when higher forces were applied. So even though there are obvious differences between the force exerted between men and women, material fatigue of applicators (Microbrush) did not allowed to capture gender differences, resulting in an irrelevant variable in daily clinical practice. Unlike, the active application mode results in a more reliable seal and has relevance in clinical practice.

A limitation of this work is the high standard deviation of the hydraulic conductance reported, attributed to high inter-variability of the mineralization of the dentin of the teeth disks used. Future studies might use a bigger sample to validate null hypothesis, however in this case it was possible to find significant differences in the variable mode of application, with good statistical power.

Nevertheless, moderate correlation was encountered between the applied force and hydraulic conductance.

CONCLUSION

Dentin hydraulic conductance is not influenced by operator's gender, but it is by the application mode (active or passive). There was a moderate correlation between the strength of application and the

hydraulic conductance. A mode of active application with this Universal adhesive system could increase the dentinal sealing and consequently reducing the hydraulic conductance, very relevant topic in clinical practice.

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