INFLUENCE OF TYPE OF BUR AND ACID ETCHING ON DENTIN HYDRAULIC CONDUCTANCE

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ABSTRACT

The aim of this study was to compare ex vivo filtration rate (hydraulic conductance) in human dentin discs mechanically treated with diamond and carbide burs of different grain size with or without acid etching. Method: 60 healthy third molars, recently extracted from patients aged 18-30 years, were cleaned, disinfected (0.1% thymol) and embedded in epoxy resin blocks. Dentin discs were obtained by cutting the occlusal surface with cylindrical rotary instruments, forming nine groups containing 12 specimens each: 1: fine grain (FG); 2: medium grain (MG); 3: coarse grain (CG); 4: carbide (C) burs; 5: FG with acid etching (AE); 6: MG with AE; 7: CG with AE; 8: C with AE; 9: only AE. Hydraulic conductance was determined in the experimental model under constant pressure of 200mm H_2O.

No difference in hydraulic conductance was observed among dentin discs treated with different types of burs (p=0.5). Differences were found in the hydraulic conductance of etched and non-etched dentin discs (p < 0.001).

The type of mechanical bur treatment does not affect dentin hydraulic conductance. Acid etching significantly increases dentin hydraulic conductance.

Keywords: *Dentin -Acid Etching, dental- Instruments, dental*

INFLUENCIA DEL GRANO DE PIEDRAS DE DIAMANTE Y FRESAS DE CARBIDE EN LA CONDUCTANCIA HIDRAÚLICA DENTINARIA

RESUMEN

El objetivo de este estudio fue comparar la tasa de filtración ex vivo (conductancia hidráulica) en discos de dentina humana tratados mecánicamente con fresas de diamante de diferente granulometría y carbide con o sin grabado ácido. Método: 60 terceros molares sanos recientemente extraídos de pacientes entre 18-30 años, fueron limpiados, desinfectados (0.1% timol) e incluidos en bloques de resina epóxica. Los discos de dentina se obtuvieron mediante la reducción de la superficie oclusal con instrumentos rotativos cilíndricos, formando los siguientes nueve grupos de 12 muestras c/u: 1: grano fino (FG); 2: grano medio (MG); 3: grano grueso (CG); 4: fresas de carburo-tungsteno (C); 5: FG con grabado ácido (AO); 6: GM con AO; 7: GG con AO; 8: C con AO; 9: sólo grabado ácido. Se determinó la conductancia hidráulica en el modelo experimental bajo presión constante de 200 mm de altura de H_2O. No se observaron diferencias entre la conductancia hidráulica entre los diferentes tipos de fresas (p = 0,5).Se encontraron diferencias en la conductancia hidráulica de discos de dentina con y sin grabado ácido (p < 0,001).

El tipo de fresa no afecta la conductacia hidráulica dentinaria. El grabado ácido aumenta significativamente la conductacia hidráulica dentinaria

Palabras clave: *Dentina - Grabado Ácido Dental, Intrumentos dentales*

INTRODUCTION

Dentin is a mineralized tissue, composed in volume of 50% inorganic matrix, 30% organic matrix and 20% water[1]. It is crossed from the pulp chamber to the dentin-enamel junction by dentinal tubules. The presence of these tubules makes it a permeable tissue, allowing the passage of microorganisms, bacterial toxins or chemical products towards the pulp[2]. Another consequence of the movement of fluid through the dentin is dentin hypersensitivity[3]. Tubules are partially obstructed by dentinal fluid[4].

The composition of dentinal fluid is similar to plasma and it constitutes almost 22% of the total volume of dentin[5]. In normal function it is controlled by odontoblasts. However, disturbances such as dental caries, attrition or restorative procedures can lead to a change in its composition, because it probably transudes from pulp capillaries. Dentinal fluid transudation from exposed dentin *in vivo* contains large amounts of albumin[6].

Dentinal fluid pressure has not been established for humans or even animals under physiological conditions[6].

Dentinal fluid flow is subject to the law of Poiseuille-Hagen[7], accepting that the dentinal fluid, flowing through a complicated network of capillary tubules, has laminar performance. In the last thirty years, research protocols have been developed to study dentin conductance with the aim of measuring the passage of fluid through a dentin disc under a hydrostatic pressure gradient from the pulp[8-13]. The experimental model used in this study attempted to simulate the clinical conditions such that the data could be of interest to researchers and clinicians, although laboratory studies cannot be directly extrapolated to *in vivo* conditions.
The effectiveness of dentin as a barrier to diffusion of oral fluids and restorative materials to the pulp is dependent upon the methods of cutting and treating the exposed dentin during cavity preparation. Cutting with dental burs creates a smear layer on the surface of the dentin that occludes the dentin tubules[14-16]. In restorative dentistry, dental burs are still used for cavity drilling procedures on dentin, despite new developments. It is therefore important to know what effect this has on dentin permeability. There is no study comparing the effect of different types of burs on dentin permeability.
Acid etching dissolves debris and removes the smear layer, increasing permeability. Acid etching of dentin is a widely used technique[17], so it is important to measure its effect on increasing dentin hydraulic conductance, in addition to that of the different types of burs. The hydraulic conductance test has been widely used in studies carried out with different variables. Based on the diffusion mechanism, higher permeability means greater passage of molecules into the pulp. This simulation of a restorative procedure in a deep dentin cavity may result in relevant information which may contribute to understanding the generation of post-operative sensitivity.
The clinical relevance of this study is concerned with understanding permeability changes in dentin in an *ex vivo* simulation of cutting by means of different types of dental drills and the use of acid etching.
The purpose of this study was to determine the influence of different grains of diamond burs and carbide burs on trans-dentinal conductance, with or without acid etching of dentin surface.
The hypothesis of this study was that acid etching affects hydraulic conductance and the type of bur does not affect hydraulic conductance in an *ex vivo* model of dentin discs.

MATERIALS AND METHODS

This *ex vivo* experimental study was approved by the research and ethics committee of the School of Dentistry at the University of Chile (PRI ODO 12-007). All patients who donated their molars signed informed consent.
Teeth were collected and used in accordance with the applicable Chilean legislation and regulations pre-dating 2000 (DFL 725, Health Code Law 836). Sixty healthy extracted human third molars, without occlusal contact, with coronal diameter less than 12mm, from patients from 18 to 30 years old were included. Sample size was determined with a confidence level of p=0.05, a statistical power of 0.8 and an effect size of 0.35, using G*Power v3.1 software[18].
All molars were disinfected for 24 hours in 0.1% thymol solution (Sigma-Aldrich Gillingham, Dorset, UK), and stored in Hanks Balanced Salt Solution (HBSS, Sigma-Aldrich) for 2 weeks at room temperature.

Tooth preparation

Enamel of all specimens was acid etched for 30 seconds with 35% phosphoric acid (Coltène/Whaledent AG, Feldwiesenstrasse20, 9450 Altstätten, Hauptsitz, Switzerland), and washed for 60 seconds. After that, they and were embedded in epoxy resin blocks of cylindrical shape 13 mm in diameter. Teeth were coded and randomly distributed using PASS software (NCSS PASS 2008, v08.0.15) into different treatment groups. The occlusal surfaces of 48 teeth were mechanically prepared with cylindrical (0.12/diameter) diamond or carbide burs (according to treatment group) under abundant irrigation, using a high-speed dental handpiece (NSK air buddy, PA-S B2, JAPAN) mounted on a vertical holder (Dremel 220-01 Work Station, Racine, Wisconsin, USA) at a pressure of 91.5 g for 5 minutes by a calibrated operator[16] (CB).

Treatment Groups

Forty-eight teeth were randomly divided into 4 groups of 12 (Fig.1), and the occlusal faces of the teeth received the following mechanical treatment:
Group 1: teeth prepared with fine grain diamond burs (842F Meisenger ISO-No. 806 314 158 514).
Group 2: teeth prepared with medium grain diamond burs (842 Meisenger ISO-No. 806 314 158 524).

Group 3: teeth prepared with coarse diamond burs (842G Meisenger ISO-No. 806 314 158 544).
Group 4: teeth prepared with carbide burs (Meisenger HM 21L ISO-No. 500 316 110 006).

Disc preparation
Thereafter, dentin discs 1 mm (+/- 0.1 mm) thick were obtained by cutting the pulp side of the disc using an Isomet 1000 (Buehler, 41 Waukegan Road, Lake Bluff, Illinois 60044, USA) cutting machine at 750 rpm, at a pressure of 500grs, under abundant cooling water. All pulp sides of the discs were treated with 35% phosphoric acid (Colténe/Whaledent) for 15 seconds to remove the smear layer. Twelve teeth were cut on both sides to prepare the discs in group 9.

Experimental model
The experimental model used to measure water flow was a modification of that proposed by Pashley et al. A reservoir with a vertical water column of 200mm was connected to a stopcock. This was attached to a horizontal graph capillary tube by a silicone hose that allows the connection of a syringe to introduce an air bubble into the aqueous medium as a guide for visualization and measurement. The capillary tube was joined distally to a chamber where dentin discs were set. Flow rate through dentin discs was recorded by measuring the movement of the air bubble inside the capillary tube for a period of 20 minutes by a calibrated operator (PV)[19].

Dentin flow measurement
First, water flow through discs was measured in all discs. Three 20-minute measurements were recorded for each disc, and the average used as the final assessment (GX).
After that, all discs were acid etched on their occlusal surface using 35% phosphoric acid (Coltene-Whaledent) for 15 seconds and washed with abundant water for 60 seconds, forming the following groups (Fig.1):
Group 5: discs from group 1 treated on the occlusal surface with 35% phosphoric acid.
Group 6: discs from group 2 treated on the occlusal surface with 35% phosphoric acid.
Group 7: discs from group 3 treated on the occlusal surface with 35% phosphoric acid.
Group 8: discs from group 4 treated on the occlusal surface with 35% phosphoric acid.
Group 9: discs treated on the pulp and occlusal surfaces with 35% phosphoric acid.

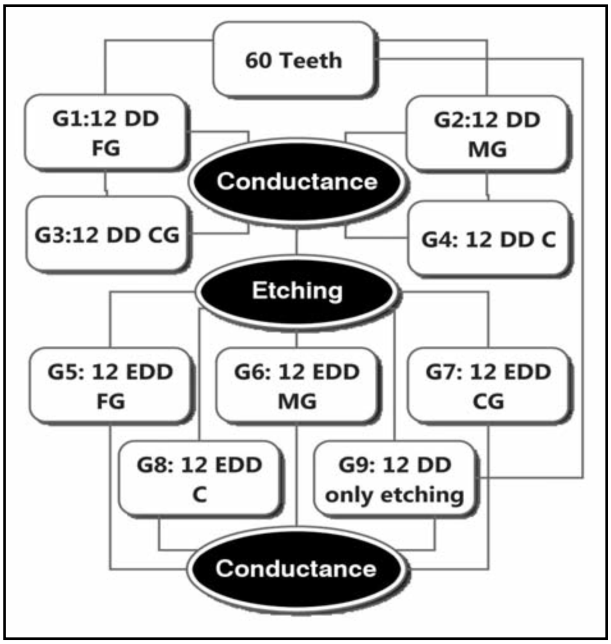

Fig. 1: Flow chart of groups and their treatments and times. Dentin discs(DD),Etched Dentin discs(EDD), Fine Grain (FG), Medium Grain (MG), Coarse Grain (CG), Carbide (C).

Conductance measurement
Hydraulic conductance was calculated using the following formula:

Where:
Ch = Hydraulic conductance $\mu l \ast cm^{-2} \ast min^{-1} cm \ast H_2O^{-1}$
F = flow rate $\mu l /min^{-1}$.
A = area of exposed dentin cm^2
P = intra-pulp pressure that corresponds to the height of water column(20)cm H_2O^{-1}
t = time min
The area of exposed dentin on each disc was measured by Image J computer software[20] using pictures taken by macro digital photography of each disc (Nikon D90, Japan), with macro lens (AF-S Micro Nikon 105mm 1:2.8 G) and wireless flash (Nikon Speedlight SB-R200) at a standardized distance of 30 cm.
In order to ensure that the flow measuring model worked correctly, measurements were taken without discs and with epoxy resin discs on the chamber as positive and negative controls respectively, before and after the measurements.
The distribution of data was evaluated by the Shapiro-Wilk test. To compare hydraulic conductance, statistical analysis was performed using two way ANOVA test and Tukey's post hoc test to determine differences between groups (SPSS, v21).

RESULTS

There was no detriment to the number of discs during the study. Normal distribution of data for all groups was observed by the Shapiro-Wilk test.

All groups showed dentin hydraulic conductance (Table 1).

Figure 2 shows the graph of the conductance for groups and standard deviations and trends. Group 9, discs with acid etching only, shows a wide standard deviation (Fig. 2).

The acid etched discs showed statistically more dentin hydraulic conductance than non-etched groups ($p<0.001$) (Table 2).

A comparison of mechanically treated and not mechanically treat-

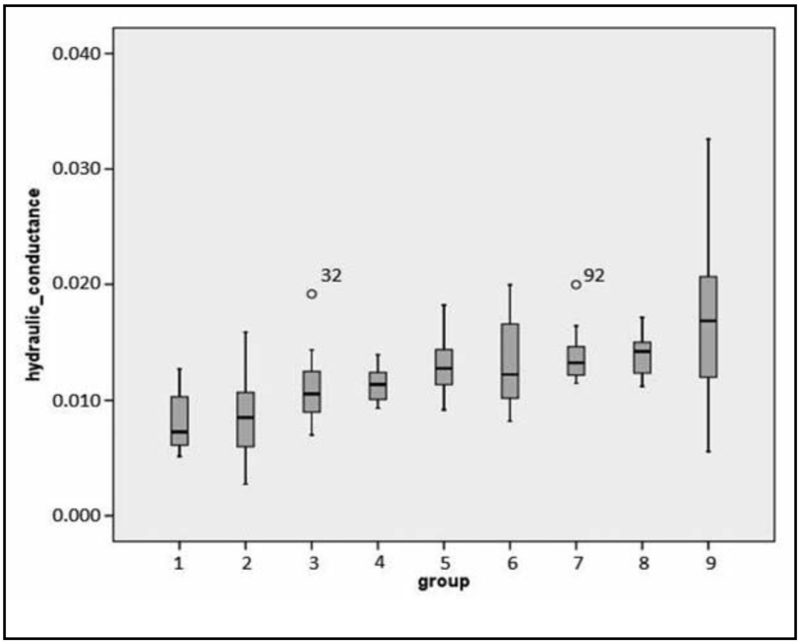

Fig. 2: Distribution of hydraulic conductance for group and standard deviation.

Table 1: Hydraulic conductance of dentin discs, average and standard deviation separated by groups, expressed in (µl $*cm^{-2}*min^{-1}cm*H_2O^{-1}$.

N Group	n	Treatment	Hydraulic Conductance	SD
1	12	Fine Grain	0.0082458	0.0026303
2	12	Medium Grain	0.0087017	0.0038548
3	12	Coarse Grain	0.01112	0.0033172
4	12	Carbide	0.0113342	0.0014259
5	12	Fine Grain + AE	0.0132758	0.0027212
6	12	Medium Grain + AE	0.0132783	0.0039067
7	12	Coarse Grain + AE	0.0138683	0.0024382
8	12	Carbide +AE	0.01398	0.0017781

Table 2: Tests of Between-Subjects Effects. Two way ANOVA analysis of dentin hydraulic conductance of all groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.001[a]	8	9.349E-005	6.642	.000
	.016	1	.016	1127.086	.000
etching	.000	1	.000	23.980	.000
treatment	.000	4	4.766E-005	3.386	.012
etching * treatment	2.727E-005	3	9.089E-006	.646	.587
Error	.001	99	1.408E-005		
Total	.019	108			
Corrected Total	.002	107			

Dependent Variable: hydraulic_conductance

[a] R Squared = .349 (Adjusted R Squared = .297)

ed groups showed a statistically significant difference in DHC (p=0.012) (Table 2).
The two-way ANOVA test showed that the combination of both variables (grain type and acid etching) did not have additive influence on hydraulic conductance, with no statistically significant difference ($p = 0.587$).
A comparison of differences between groups shows no difference among treated groups ($p > 0.41$). A comparison of the groups treated with burs to the group without treatment (no bur) shows a significant difference ($p<0.05$) (Table 3).

Table 3: Tukey's post hoc test. Differences in the individual groups.

	Medium	Coarse	Carbide	No bur
fine	1	0.5	0.41	0.00
medium		0.64	0.54	0.00
coarse			1	0.01
carbide				0.01

DISCUSSION

Restorative procedures usually involve tooth dentin, therefore phenomena affecting permeability are of great relevance to dentistry and they must be considered because of their many implications such as dental sensitivity, micro leakage of restorations or postoperative pulp damage[21-26]. All the methods of cutting dentin were effective in changing the permeability of dentin to water, and the differences among the bur groups were relatively small. Conductance of dentin discs treated with diamond or carbide burs was similar.
The design of this study differs primarily in the treatment performed on dentin discs and the hydrostatic pressure used (20 cm H_2O at 20°C). This technique may be compared with the one proposed by Sekimoto in 1999, which used the same model but with a pressure of 15 psi N_2 on an 0.2% phosphate buffered saline solution, equivalent to 1054.8 cm H_2O at 4°C. Ultimately, the pressure used becomes irrelevant, because the mathematical formula of the hydraulic conductance standardizes the results[15].
One problem of the model is that it is limited in sensitivity by the micropipette used, with a scale range of 0.001 ml. Thus, the observational accuracy has a range of error given by the observer. Another point to consider is the difficulty in obtaining the most homogeneous dentin possible because dentin differs at different levels within the same tooth, so the selection of the teeth used in the studies was rigorous. Nevertheless, achieving an accurate cut is an inherent difficulty and dentin samples are usually obtained from average depth[27].
Diamond and carbide burs are widely used on rotary instruments for restorative procedures. They each cut the dentin surface in a different way, so it is important to know their effects on dental tissues in order to select the most appropriate parameters, because since dentin tissue is so closely related to the tooth pulp, its permeability is of vital importance for any treatment decisions that the dentist may take[28].
In our study, by observing the surfaces of mechanically treated dentin discs, it was possible to note differences in the topography of macroscopic surfaces, in which a coarser grained diamond bur produces higher roughness, compared to carbide burs, which produce low roughness. This can be compared to the results obtained by Sekimoto *et al.* (1999), who compared the effects on dentin permeability and morphology produced by carbide and diamond burs by observing treated surfaces by scanning electron microscopy, and found slight differences between the dentin treated with carbide burs and those treated with diamond burs, which presented marked grooves along the surface after removing the smear layer with 6% citric acid for two minutes[29]. They observed that the orifices of the dentinal tubules of dentin treated with carbide burs were clear, unlike those treated with diamond burs, which were hardly distinguishable; thereby we can deduce that the smear layer produced by diamond causes greater occlusion of the tubules, so that permeability should be lower[15].
Our results show a tendency to greater hydraulic conductance, and thus greater dentine permeability, with coarser-grain diamond burs. They also show higher conductance with carbide burs than with diamond burs.
Scientific evidence clearly shows that dentinal tubules are responsible for dentin permeability[30]. Larger tubule diameters make dentin more permeable, while tubule obstruction and smear layer make it less permeable. The use of rotary instruments on teeth produces a smear layer and differences in permeability may occur due to differences in the smear layer formed. This leads to the assumption that more coarse-grained diamond burs would produce larger particles in the smear layer than would finer-grained burs, which would produce smaller parti-

cles, thereby causing greater blockage of dentinal tubules and hence, lower permeability. In addition, with acid etching of the surfaces there was an increase in hydraulic conductance and the difference between the groups decreased[15].

The ANOVA test showed that acid etching with 35% phosphoric acid for 15 seconds produces statistically significant differences between etched and non-etched groups, which is consistent with existing studies on the subject, where acid etching of the surface produces a significant increase in dentin permeability due to the removal of the smear layer occluding dentinal tubules[17]. This study agrees with Boyer and Svare (1981), who compared the hydraulic conductance of dentin discs cut with diamond wheels, polished with diamond burs and fissure carbide burs, with similar results and no statistically significant difference, which would support our results. It should be considered that the methods of treating the dentin were different; Boyer cut the dentin and then carved the surface while in our study the dentin of the occlusal surface was cut directly by the rotary cutting instruments [29].

The clinical relevance is that higher permeability in dentin results in greater sensitivity due to fluid movement. The relationship between *ex vivo* measurements of permeability and the clinical problems of sensitivity, pulp lesions and recurrent decay has not yet been established.

Permeability caused by diamond or carbide burs on dentin is a small yet important topic related to diffusion in dentin, regarding which there is no specific line of isolated research studies. Thus, analysing whether the grain size of diamond burs caused any differences in hydraulic conductance was a new initiative. The differences were not statistically significant; therefore permeability would not be a factor to consider when selecting which instrument to use for working on dentin.

CONCLUSION

- The type of bur does not affect hydraulic conductance in this *ex vivo* model of dentin discs.
- Acid etching affects the hydraulic conductance in dentin discs in this model.
- Further studies are needed to clarify the trends, perhaps by increasing the sample number.
- The diffusion chamber used in our work, derived from the Pashley model is a simple, reliable tool for studying diffusion in dentin.

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