

# Surface preparation and isotropic shear bond strength properties of superficial bovine dentin

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## ABSTRACT

The effect of different types of surface preparation with SiC abrasive paper on the shear bond strength (SBS) of superficial bovine dentin obtained from the incisal, middle and cervical thirds were evaluated. Dentin substrates were obtained with twenty specimens for each location-grit combination. Superficial dentin was exposed and prepared to 120-, 320-, or 600-grit SiC; the dentin surfaces were treated with Optibond Solo Plus (Kerr) and polymerized for 20 s. The specimens were placed in a jig, filled with resin composite Z100 (3M-ESPE), polymerized for 40 s according to manufacturer's instructions, and stored for 24 h at 37°C and 100% humidity. After 24 h, SBS was measured using a loading testing machine (Ultradent) and expressed in megapascals. A two-way ANOVA and Tukey test were used for data analysis. No statistically significant effect of the location ( $P = 0.254$ ) or interaction grit-location ( $P = 0.629$ ) were observed on SBS. Statistically significant effect of the grit on the SBS was detected ( $P < 0.001$ ) with 320-grit being statistically different from 600-grit ( $P = 0.011$ ) and 120-grit ( $P < 0.001$ ). No significant differences were observed between 600-grit and 120-grit ( $P = 0.413$ ). Regardless of the location, 320-grit consistently showed the lowest SBS indicating that different surface grit preparations have an effect on dentin SBS values.

**Keywords:** Resin Composite; Dentin Adhesion; Bond Strength; Isotropic; Surface Preparation; Failure Mode

## 1. INTRODUCTION

Adhesion to tooth structure, particularly to dentin, has been subject of extensive research in the last few dec-

ades [1-3]. The success of adhesive restorations is largely a function of factors relative to the bonding system, substrate and composite. Furthermore, *in-vitro* bond strength tests incorporate additional variables relative to the sample preparation, testing itself and storage conditions that are also known to have an effect in the complex nature and interactions taking place at the adhesive interfaces [4] and they all deserve consideration when evaluating strength of these interfaces. Bond strength tests have been extensively used and play a paramount role in the screening of dental adhesives since they are relatively easy to perform, do not require much armamentarium and are able to provide immediate information regarding the strength, adhesive or cohesive, of the bonded interface. While bond strength tests have allowed the development of improved adhesive systems and techniques, they are subject to a number of limitations and its results vary considerably depending on a number of structural and testing procedures [5]. A set of guidelines was developed by the International Organization for Standardization (ISO) to standardize bond strength testing of adhesive interfaces and thus facilitate reproducibility of the testing conditions and allow comparisons among studies. (ISO/TR 11405:1994) However, despite standardization efforts, variability in the testing conditions still exists particularly in aspects related to the substrate and testing itself compromising the validity of the comparisons among studies [6].

Variables inherent to the substrate, particularly dentin, are especially critical due to the large morphological variability known to exist between different teeth and in various regions of the same tooth [7]. Bond strength studies commonly use human third molars or bovine incisors as a substrate for bonding. Despite some anatomical and permeability differences [8], histochemical and morphological studies have shown that human and bovine dentin are essentially the same [9-12]. Studies have reported no differences in the concentration of

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tubules per  $\text{mm}^2$  between coronal human and bovine dentin [12]. However, compared to human molars, bovine incisors dentinal tubules are of larger size and show more porous intertubular dentin [13]. According to Nakamichi, only superficial dentin may be considered a suitable substitute to human dentin [10]. Irrespective of the type of substrate used, most studies fail to provide critical information regarding aspects such as the specific location of the bonding and the potential effect that this location may have on the bond strength results. The anisotropic bond strength behavior of human dentin has been established in previous studies [14,15]. However, limited information is available regarding the anisotropic bond strength properties of bovine dentin.

Surface preparation of the substrate has also been shown to have an effect in the bond strength of adhesive interfaces [16,17]. Studies have evaluated the effect of different types of surface preparation on dentin surface roughness [18,19]. Carbide and diamond rotary cutting instruments and silicon carbide abrasive paper create rough surfaces that are different in topography and smear layer thickness [20]. Studies evaluating the effect of surface preparation on bond strength normally use carbide or diamond burs simulating a clinical situation [21,22]. However, current ISO standards for bond strength testing recommend surface preparation with 600-grit silicon carbide (SiC) abrasive paper. This method produces a relatively smooth surface that does not reproduce the surface topography and smear layer thickness obtained with rotary cutting instruments [20]. Studies have evaluated the effect of different surface preparation methods on the surface roughness [20], wettability [17] and thickness of smear layer [23,24]. However, most of these studies fail to provide critical information regarding specific location of bonding within the crown. The authors are not aware of studies evaluating the shear bond strength of surfaces prepared with different progressively higher grits of silicon carbide (SiC) abrasive paper when bonded to different crown locations.

The objective of this study was to evaluate the effect of different types of surface preparation with silicon carbide (SiC) abrasive paper on the Shear Bond Strength (SBS) of superficial coronal dentin obtained from the incisal, middle and cervical thirds of bovine incisors. The null hypotheses were: 1) there would be no difference in SBS values obtained from the incisal, middle and cervical thirds; 2) there would be no difference in SBS values after surface preparation with the different grits of abrasive paper.

## 2. MATERIALS AND METHODS

### 2.1. Specimen Preparation

Thirty non-carious bovine mandibular central incisors

were used to obtain dentin substrates for bonding. The incisors were used within one month of extraction and stored in an aqueous disinfectant (0.5% chloramine T solution at  $4^\circ\text{C}$ ) until ready to be used. A slow-speed saw (Isomet, Buehler, Lake Bluff, IL, USA) with a diamond disk under cooling water was used to separate the crowns from the roots at the CEJ level. From each crown, six sections were obtained by making one longitudinal section along the middle of the crown and three transverse sections between the crown's incisal, middle and cervical thirds. This yielded a total of 180 specimens with a sample size of twenty ( $N = 20$ ) for each location-grit combination. The sectioned specimens were embedded in a chemically-polymerized methacrylate (Fastray, HJ Bosworth, Skokie, IL, USA) with the facial surface exposed. The exposed surface was ground flat on a model trimmer until superficial dentin was revealed. Each group of sixty specimens including samples obtained from the incisal, middle and cervical thirds were polished to a final coarseness of 120-, 320- or 600-grit with silicon carbide (SiC) abrasive paper (Buehler). The prepared specimens were stored in de-ionized water at  $4^\circ\text{C}$  until ready to be bonded.

### 2.2. Shear Bond Strength Testing and Statistical Analysis

Restoration of the different study groups was randomized to avoid bias relative to sequence of tooth restoration. One hour prior to bonding, the specimens were acclimatized to room temperature ( $23^\circ\text{C} \pm 2^\circ\text{C}$ ). Immediately before the start of the procedures, the specimen surfaces were slightly refinished with the same final coarseness of SiC paper used to prepare the surface initially in order to expose fresh dentin. Dentin specimens were etched with 35% phosphoric acid (Ultra-etch, Ultradent, South Jordan, UT, USA) for 15 seconds, rinsed and blot dried for moist bonding. An etch-and-rinse adhesive system (Optibond Solo Plus, Kerr, Orange, CA, USA) was applied and polymerized for 20 seconds following manufacturer's instructions with a light curing unit (Bluephase C8, Ivoclar-Vivadent, Amherst, NY, USA). A minimum power density of  $800 \text{ mW/cm}^2$  was ensured by periodically monitoring the unit's output with a radiometer (Demetron, Kerr). The specimens were placed on a specially fabricated bonding jig (Ultradent) with a cylindrical mold of 2.38 mm in diameter. The mold was filled with resin composite (Z100, 3M-ESPE, Saint Paul, MN, USA) in a single increment no greater than 2 mm and polymerized for 40 seconds. Immediately after bonding, specimens were stored in an incubator at  $37^\circ\text{C}$  and 100% humidity for 24 hours.

Shear bond strength was measured using a testing machine (Ultratester, Ultradent) at a test speed of 1 mm/min with a load cell of 1000 lbs (453.6 Kg). A notch-

ed crosshead designed to match the diameter of the bonded specimen was used to apply the testing load. Specimens were stabilized in a testing jig which was free to move to facilitate positioning under the load. The test base was then positioned so that the notched crosshead was placed against the specimen surface and the notch was fitted to the bonded specimen. The load required to debond the specimen was recorded and expressed in megapascals (MPa) by dividing the load by the surface area of the bonded specimen. Shear bond strength values were recorded at 24 hours and the mean SBS values per study group were calculated. A two-way analysis of variance (ANOVA) was used to evaluate the effect of the crown location and surface preparation on shear bond strength. Pairwise multiple comparison procedures (Tukey test) was used to identify these differences among the individual groups. A significance level of 0.05 was used for all tests.

### 2.3. Analysis of the Mode of Failure

Mode of failure was analyzed by field emission scanning electron microscope (FE-SEM) (Hitachi SU-70, Hitachi, Krefeld, Germany) observation of the dentin-adhesive interface in backscattered electron mode. The assessment was conducted by one trained examiner (CS). Both sides of the fractured interface of three representative specimens for each location-grit combination was observed and scored. The highest value, a value close to the mean and the lowest bond strength value were subjected to SEM analysis at a magnification of 50 $\times$ . The orientation of the dentinal tubules for the different crown thirds and the surface topography obtained after preparation with the different grits of abrasive paper was confirmed by SEM observation of additional dentin surfaces in secondary electron mode. The specimens were desiccated, and coated with a 20 nm layer of evaporated carbon to provide conductivity.

The fractured surfaces were classified into one of five

failure modes: 1) cohesive in dentin; 2) adhesive at the interface between dentin and adhesive resin; 3) cohesive in adhesive resin; 4) adhesive at the interface between the adhesive resin and composite; 5) cohesive in composite; 6) mixed failure across the different layers of the interface.

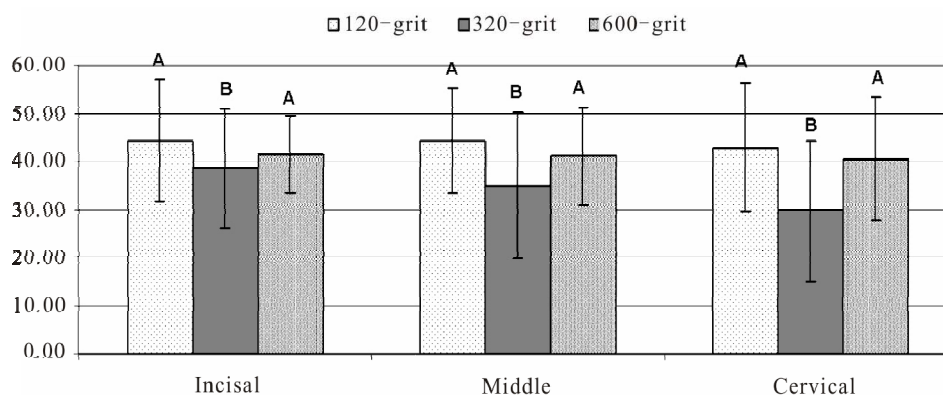
### 3. RESULTS

The mean SBS values obtained for the different location-grit combinations are shown in **Figure 1** and **Table 1**. **Table 2** summarizes the two-way ANOVA results. Statistically significant effect of the surface preparation on the SBS ( $P < 0.001$ ) was detected with 320-grit being statistically lower than 600-grit ( $P = 0.011$ ) and 120-grit ( $P < 0.001$ ). No significant differences were observed between 600-grit and 120-grit ( $P = 0.413$ ). No statistically significant effect of the location variable ( $P = 0.254$ ) or the interaction grit-location on the SBS was detected ( $P = 0.629$ ).

Failure modes for representative specimens, the highest, middle and lowest bond strength values, for each location-grit combination are depicted in **Table 3**. Overall, the highest and middle SBS values showed mixed-type failures irrespective of the surface preparation or location of the bonding. The only two exceptions were the highest SBS values within each of these groups which showed cohesive failures. The lowest SBS values displayed predominantly adhesive failures.

### 4. DISCUSSION

This study evaluated the shear bond strength of specimens bonded to different crown regions of superficial bovine incisor dentin after preparation with 120-grit, 320-grit or 600-grit SiC abrasive paper. Overall, high bond strength values were demonstrated irrespective of the crown location and surface preparation. The first null hypothesis was confirmed as no significant differences in



**Figure 1.** Mean shear bond strength values for the different location-grit combinations. Same letter indicates no significant differences between grits for each crown third.

**Table 1.** Mean shear bond strength and standard deviation values for the different location-grit combinations.

SBS MPa (SD)	120-grit	320-grit	600-grit
<b>Incisal third</b>	44.4 (12.7)	38.6 (12.3)	41.4 (7.9)
<b>Middle third</b>	44.4 (10.8)	35.0 (15.4)	41.0 (10.1)
<b>Cervical third</b>	43.0 (13.5)	30.0 (14.8)	40.5 (12.9)

bond strength values were shown for the different crown thirds. Despite the lack of significant differences, certain trends were observed indicating that orientation of the dentinal tubules may have had an effect on the bond strength results. Regardless of the surface preparation, the cervical third consistently showed the lowest SBS values and the middle and incisal thirds showed similar SBS values except for the groups prepared with 320-grit for which the incisal third displayed higher mean SBS value than that shown for the middle third. Similarly, a previous study evaluating shear bond strength to different crown regions demonstrated that, despite the absence of significant differences, a trend to higher values could be observed when bonding to the middle third of superficial bovine coronal dentin [25]. Hence, it appears that there could be an influence of the dentinal tubule orientation in the bond strength results that may only be detectable with a larger sample size. However, the sample size of twenty specimens per study group used in both of these studies doubles the sample size of ten commonly used in bond strength studies [15,26]. Based on these results, it may be safe to make the assumption that superficial bovine incisor dentin displays isotropic SBS properties and that provided that the same dentin depth is used, bonding can be performed to the different crown regions without a significant effect on the bond strength. The literature shows conflicting evidence as to the effect of dentinal tubule orientation in bond strength with studies showing a positive correlation between tubular orientation and bond strength results [27] and studies showing no correlation [28]. Histochemical and morphological studies have shown that human and bovine dentin are essentially the same [9-11]. Hence, the reported isotropic SBS behavior of bovine incisor dentin offers the added advantage of providing a much larger surface area for bonding compared to the use of human molars. Dentinal tubules are arranged in a radial pattern from the pulp chamber to the dentino-enamel junction (DEJ) and follow an "S" or zigzag course. Despite the zigzag course, dentinal tubules are oriented approximately at 90° to the DEJ in superficial dentin irrespective of the crown third suggesting that different crown regions may exhibit similar mechanical properties [29].

The second null hypothesis was rejected as differences in SBS were observed when the surface was prepared with different grits of SiC abrasive paper. Higher bond strength results were obtained for 600- and 120-grit

**Table 2.** Two-way ANOVA results (DF: Degrees of freedom, SS: Sum of squares, MS: Mean Squares).

Source of Variation	DF	SS	MS	F	P
Location	2	429.149	214.574	1.381	0.254
Grit	2	2815.321	1407.661	9.062	<0.001
Location × Grit	4	403.047	100.762	0.649	0.629
Residual	171	26561.583	155.331		
Total	179	30209.100	168.76		

abrasive paper with both grits showing values statistically higher than those obtained with the 320-grit group. Both viscosity of the adhesive and specimen's surface topography have been reported to be the main factors responsible for the nature and intimacy of the interactions taking place between the adhered and the adhesive [30]. Optibond Solo Plus is a single-component dental adhesive filled 15% by weight with 0.4 µm barium aluminoborosilicate glass and silicon dioxide. The filler reinforces the hybrid zone but at the same time, makes the adhesive more viscous perhaps hindering its ability to flow into areas of compromised access. The success of adhesive interfaces with etch-and-rinse systems depends largely on the ability of the resin monomers to penetrate to the same depth to which demineralization has occurred creating a strong hybrid layer [31]. Studies have shown that greater surface roughness promotes wettability by producing greater surface area for bonding, and as a consequence, a stronger micro-mechanical bond can be obtained [19]. The results of our study challenge this assumption. Although the highest SBS values were observed when µ surfaces were prepared with 120-grit, the second highest SBS results were obtained for the groups prepared with 600-grit rather than 320-grit. Interestingly, no significant differences were observed between SBS values obtained with 120-grit and 600-grit groups indicating no clinical difference between the two very different surface roughnesses.

Variations of micropore size are known to yield different surface topographies. According to the ISO standards for coated abrasives, the average grit size for the SiC abrasive papers are approximately: 25.8 µm for 600-grit, 46.2 µm for 320-grit, and 125 µm for 120-grit abrasive paper. (ISO 6344-1:1998) **Figure 2** depicts scanning electron microscope images of dentin prepared with the three different grits of abrasive paper. The difference in surface topography obtained with each grit size relative to the smaller size of a dentinal tubule, which ranges between 1 - 2.5 µm, becomes evident in these images. A highly undulating surface topography was observed for the 120-grit abrasive paper. The pore size of 120-grit abrasive paper was approximately three times larger than that of 320-grit paper and five times larger than that of 600-grit paper. Presumably this sur-



**Table 3.** Mode of failure for representative SBS values for the incisal, middle and cervical crown thirds.

SBS Value	Grit	Crown third	SBS (MPa)	Mode of Failure
Highest	120	Incisal	56.2	Mixed
Highest	120	Middle	59.1	Cohesive (dentin)
Highest	120	Cervical	58.5	Mixed
Highest	320	Incisal	58.6	Mixed
Highest	320	Middle	54.6	Mixed
Highest	320	Cervical	55.2	Mixed
Highest	600	Incisal	55.5	Mixed
Highest	600	Middle	56.3	Mixed
Highest	600	Cervical	56.8	Mixed
Middle	120	Incisal	44.7	Mixed
Middle	120	Middle	45.9	Cohesive (dentin)
Middle	120	Cervical	42.2	Mixed
Middle	320	Incisal	41.1	Mixed
Middle	320	Middle	34.5	Mixed
Middle	320	Cervical	30.5	Mixed
Middle	600	Incisal	41.2	Mixed
Middle	600	Middle	41.2	Mixed
Middle	600	Cervical	42.8	Mixed
Lowest	120	Incisal	27.7	Mixed
Lowest	120	Middle	19.1	Adhesive (DBA/composite)
Lowest	120	Cervical	7.7	Adhesive (DBA/dentin)
Lowest	320	Incisal	16.1	Adhesive (DBA/dentin)
Lowest	320	Middle	12.8	Adhesive (DBA/dentin)
Lowest	320	Cervical	11.8	Adhesive (DBA/dentin)
Lowest	600	Incisal	26.7	Adhesive (DBA/dentin)
Lowest	600	Middle	17.5	Adhesive (DBA/composite)
Lowest	600	Cervical	10.7	Adhesive (DBA/composite)

DBA: Dentin Bonding Agent.

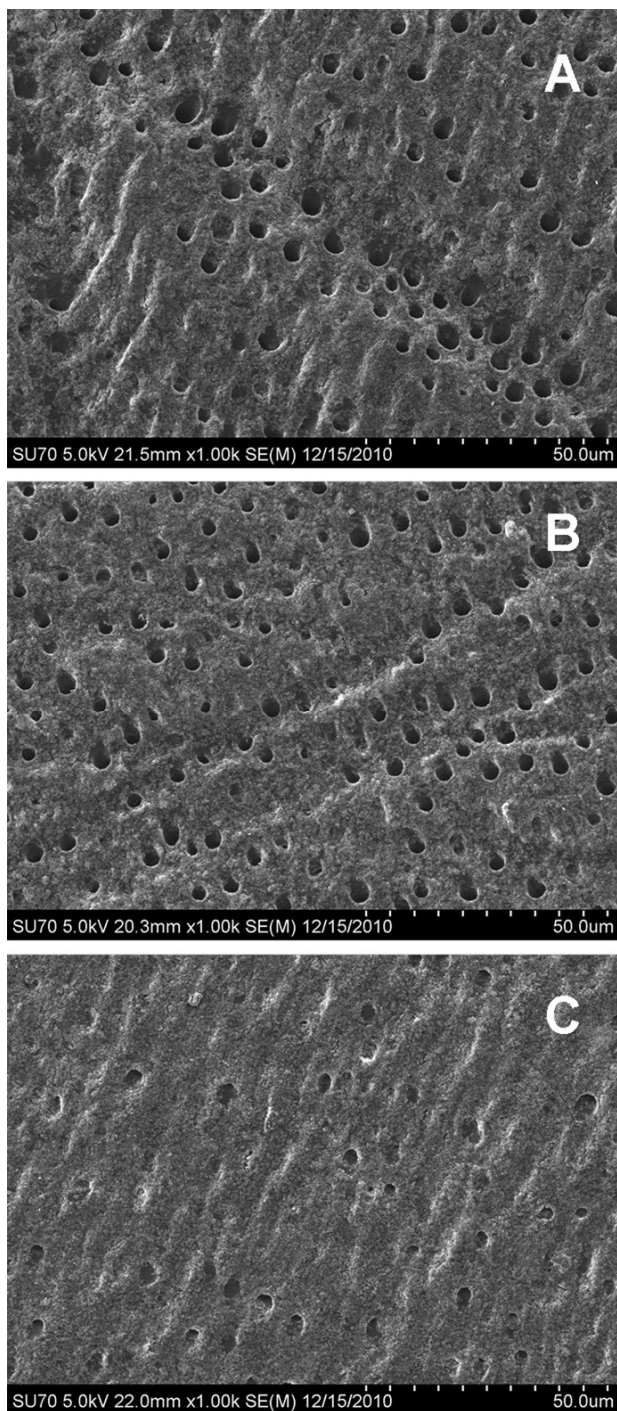
face topography allowed free flow of the filled viscous adhesive into the demineralized dentin matrix creating a strong micro-mechanical interlocking. This is evidenced by the highest bond strengths obtained with 120-grit paper. Conversely, 600-grit abrasive paper yielded the smoothest surface topography of all groups. However, the shallower less wavy surface obtained with this grit also allowed strong hybridization as evidenced by the high bond strength results observed for this group. The observed lowest bond strength results obtained with 320-grit abrasive paper may have been the result of an incomplete infiltration of the adhesive monomers into the demineralized dentin matrix. Although the thickness of the smear layer after preparation with the different abrasive papers was not evaluated as a part of this study, it may have played a role on the observed bond strength results. Differences in smear layer thickness have been reported to affect dentin permeability [23,24] and bond strength of adhesives [24]. The lower bond strengths obtained with 320-grit abrasive paper may have been the combined result of a rather thick smear layer which was

perhaps incompletely removed during acid etching procedures.

It is interesting that ISO Standards for bond strength testing recommend surface preparation of the substrate with 600-grit abrasive paper. While good bond strength results were obtained when the surface was prepared with 600-grit paper, we argue that the surface roughness obtained with this type of surface finish may not always be representative of the real clinical situation when the tooth structure is prepared with different types of carbide or diamond burs. A study compared dentinal surfaces prepared with either a carbide bur or SiC papers and demonstrated that preparation of the surface with 320-grit paper followed by a 254-carbide bur most closely resembled the finish obtained clinically after cavity preparation with a tungsten carbide bur [20].

### Analysis of the Mode of Failure

Our study also evaluated the failure mode for representative specimens for each study group. Although no corre-



**Figure 2.** Scanning electron microscope images in secondary electron mode display surface topography obtained with the different grits of SiC abrasive paper. (A) 120-grit; (B) 320-grit; (C) 600-grit.

lation was observed between bond strength results and surface preparation or location of the bonding, a strong correlation between SBS values and mode of failure was evident with lower SBS values showing predominantly “adhesive” failures between dentin and the adhesive

resin and higher SBS values showing “mixed” failure through the body of the adhesive interface. It appears then that the entire adhesive interface assembly behaves much stronger when acting together as a single body rather than as separate layers. This suggests that the intimacy of the micro-mechanical adhesion between the adhesive resin and the partially demineralized collagen network is such that it surpasses the cohesive strength of each of its individual components and the adhesive strength between the different interfacial layers. Our results are in agreement with studies that have shown a strong positive correlation between strength values and the area of cohesive failure in resin observed in mixed failures has also been demonstrated [2,32].

Nevertheless, there is still minimal understanding regarding the mechanism by which adhesive interfaces fail. The presence of “mixed” failures where there is a combination of cohesive and adhesive failures within the same bonded area, and how an adhesive bond transitions from a strong bond which exhibits cohesive failure to a weak bond which exhibits adhesive failure should be further studied. Failure of adhesive interfaces may be explained by the parameters involved in crack propagation whereby a crack propagates from a critical size flaw found in an area subjected to high stresses [33]. Scanning electron microscope imaging revealed that most failures for the high bond strength specimens were of the mixed type; that is the loading device caused the assembly to split in a cohesive-adhesive fashion, making it a challenge to identify the true plane of separation. A number of aspects play a role in the strength, adhesive or cohesive, of adhesives interfaces making it a challenge to understand its complex nature and behavior when subjected to stress during bond strength testing [4]. This great complexity of the adhesive interface previously reported in the literature [2,34]. The mechanics of the strength testing itself is complex in nature with concentration of forces at the point where the cross-head first contacts the interface. The particular test assembly used in our study, the Ultradent notched rod, uses a larger contact area between the composite and the loading device which theoretically allows better stress distribution than other commonly used assemblies such as the knife-edge chisel which concentrates stress at a single load application point [35,36]. The complex fracture mechanics of adhesive interfaces may be explained by the intricate nature of the test itself. When the cross-head device first contacts the bonded interface, there is a concentration of forces that accumulate on the site where the cross-head first contacts the interface. These forces accumulate until they reach a point of failure which is reported as a nominal bond strength value. Subsequent propagation of the initial fracture will occur cohesively though one of the substrates or adhesively through the

tooth-adhesive or adhesive-composite interfaces, and it will depend on aspects such as the original failure point, the physical and mechanical properties of the individual materials involved in the interface as well as their properties and behavior when combined as a single body.

## 5. CONCLUSIONS

Within the limitations of this study, the following conclusions may be drawn:

- The anisotropic behavior of superficial bovine dentin relative to bond strength was not confirmed.
- Regardless of the location, 320-grit consistently showed the lowest SBS and 120-grit showed the highest SBS indicating that different surface grit preparations have an effect on the *in-vitro* dentin bond strength.
- A correlation between the SBS values and the failure mode was observed. Specimens with high bond strengths showed predominantly “mixed-type” failures and specimens with low bond strengths showed mainly “adhesive” failures.

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