

Comparative study of the dental substrate used in shear bond strength tests

Estudo comparativo do tipo de substrato dental utilizado em testes de resistência de união ao cisalhamento

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ABSTRACT: The purpose of this study was to compare shear bond strength values obtained in human enamel and dentin with the values obtained in bovine teeth using two adhesive systems with different actions. Forty human tooth half-crowns and forty bovine tooth crowns were flattened to a minimum plain area of 5 mm in diameter. The samples were divided in four groups of 20 specimens each: 1) human enamel; 2) bovine enamel; 3) human dentin; 4) bovine dentin. The samples of each group were divided in 2 subgroups of 10 samples each, according to the adhesive system used: 1) Scotchbond Multi-Purpose (SBMP); and 2) Clearfil Liner Bond 2V (CLB2V) applied according to the manufacturer's recommendations. Afterwards, restorations of Z100 composite with cylindrical shape (4 mm diameter x 5 mm height) were made using a metallic mold to submit the samples to shear bond testing on an Instron universal testing machine, at a crosshead speed of 0.5 mm/min. The data were submitted to ANOVA and Tukey's test (5%). In enamel, there was no statistical difference between bovine and human teeth for SBMP (7.36 MPa, human; 8.24 MPa, bovine), nor for CLB2V (10.01 MPa, human; 7.95, bovine). In dentin, SBMP showed a statistically lower mean on human dentin (7.01 MPa) than on bovine dentin (11.74 MPa). For CLB2V, there was no statistical difference between human (7.43 MPa) and bovine (9.27 MPa) substrates.

DESCRIPTORS: Dentin-bonding agents; Dentin; Dental enamel.

RESUMO: O propósito deste estudo foi comparar os valores de resistência de união sobre esmalte e dentina humanos com os valores obtidos em dentes bovinos, utilizando dois sistemas de união com princípios de atuação distintos. Para isso, desgastaram-se 20 meias coroas dentais humanas e 40 coroas bovinas, até obter uma área plana de pelo menos 5 mm de diâmetro. As 80 amostras foram separadas em 4 grupos de 20 amostras cada, sendo: 1) dente humano em esmalte, 2) dente bovino em esmalte, 3) dente humano em dentina, 4) dente bovino em dentina. As amostras de cada grupo foram divididas em 2 subgrupos de 10 amostras cada, de acordo com o sistema de união utilizado: 1) Scotchbond Multi-Uso (SBMU); e 2) Clearfil Liner Bond 2V (CLB2V). Em seguida, confeccionou-se um cilindro do composto Z100 (4 mm de diâmetro x 5 mm de altura) utilizando-se uma matriz bipartida, para submeter os corpos-de-prova ao ensaio de cisalhamento numa máquina de ensaio Instron com velocidade de 0,5 mm/min. Os dados foram submetidos à análise de variância e as médias, ao teste de Tukey (5%). Em esmalte, não se verificou diferença estatística entre os dentes humanos e bovinos para os materiais SBMU (7,36 MPa, humano; e 8,24 MPa, bovino) e CLB2V (10,01 MPa, humano; e 7,95 MPa, bovino). Verificou-se que o SBMU apresentou média estatisticamente inferior em dentina humana (7,01 MPa), quando comparado à dentina bovina (11,74 MPa). Para o material CLB2V, não houve diferença estatística entre os substratos humano (7,43 MPa) e bovino (9,27 MPa).

DESCRIPTORIOS: Adesivos dentinários; Dentina; Esmalte dentário.

INTRODUCTION

Since the acid-etch technique was first introduced in dentistry by Buonocore² (1955), phosphoric acid has been routinely used to roughen the enamel surface in order to create micromechanical retention with resin composites. The success obtained with enamel inspired its use on dentin sur-

faces as well. However, with the use of early hydrophobic resins, acid treatment of dentin did not produce bond strengths similar to those obtained on enamel surfaces¹³.

After that, adhesive systems incorporating acid-etching and hydrophilic monomers have been developed. The enamel and dentin acid-etching could be done simultaneously⁸ and there was an

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increase in the adhesive bond strength. With the new adhesive systems, a "primer" became necessary after the acid-etching and before the bonding agent. It increased the wettability of the dentin surface for the penetration of the adhesive agent.

Recently, "one-bottle" adhesive systems were introduced in dentistry. They combined the "primer" and the adhesive functions into a sole solution. These systems require at least two layers of the solution and a previous enamel and dentin acid-etching.

Nowadays, "self-etching" adhesive systems have currently combined the tooth surface etching and primer steps to treat enamel and dentin, which are demineralized allowing the adhesive to infiltrate. They have been developed by raising the concentration of the acid adhesive monomers from their original 5-6% concentration to 20% or more^{11,16}. By dissolving the acid monomers in 2-hydroxyethylmethacrylate (HEMA) and because of its low pH, it resulted in a satisfactory conditioning system^{9,19}.

To evaluate adhesive bond strength, plenty of *in vitro* research has been developed using extracted human teeth, which are, however, more and more difficult to obtain because of preventive dentistry advances. Therefore, it became necessary to look for an alternative substrate. As mammalian teeth are histologically and morphologically similar¹⁷, investigators have turned to teeth from bovine, ovine, equine, or swine to provide quantities of standardized material for their studies. Schilke *et al.*¹⁴ (2000) verified that there were no statistically significant differences neither in the number of tubules per mm² nor in their diameters in corresponding coronal dentine layers of human deciduous and permanent molars, and of bovine central incisor.

The size and availability make bovine incisors preferable for bond strength research^{4,12}. However, there is some concern about whether data obtained from bovine teeth can be applied to human teeth and valid in a clinical situation^{10,12}.

Hence, this study searched for more information about the alternative substrate for human teeth. Therefore, a comparison was made between human and bovine enamel and dentin as regards shear bond strength, using two different adhesive systems with different mechanisms of action.

MATERIALS AND METHODS

Forty freshly extracted, non carious human molars and eighty bovine incisors were obtained and stored in 0.9% sodium hypochlorite for a maximum of 90 days. The roots of the teeth were removed. Human crowns were sectioned in a bucco-lingual direction, amounting to forty half-crowns. The crowns were then embedded with the vestibule (bovine) or proximal (human) surface exposed in plastic tubes with self-cured acrylic resin. The samples were assigned to 4 groups: 1) human enamel, 2) bovine enamel, 3) human dentin, and 4) bovine dentin; with 20 specimens in each group. The vestibule or proximal surfaces were ground wet in a polishing machine (APL-4 Arotec, Cotia, Brazil) with 180 grit, followed by 400 and 600 grit silicon carbide paper to create a 5 mm diameter flat surface on enamel or dentin. To delimitate the testing area, a circular adhesive tape with a central orifice of 4 mm in diameter was applied.

The samples of each group were assigned to two subgroups of 10 specimens each, according to the adhesive system used: 1) Scotchbond Multi-Purpose (3M Dental Products Division, St. Paul, MN, USA), 2) Clearfil Liner Bond 2V (Kuraray Co. Ltd., Osaka, Japan).

After these superficial treatments, a stainless steel mould (4 mm in diameter and 5 mm in height) was placed against the specimen to receive the filling material (Z100, 3M Dental Products Division, St. Paul, MN, USA). The resin composite was inserted in three increments of equal height; each one of them was light-cured (XL-3000, 3M Dental Products Division, St. Paul, MN, USA) for 40 s, with a light intensity of 530 mW/cm². After mould removal, the specimens were stored in distilled water at 37°C for 24 hours. After this period, the specimens were positioned in a universal testing machine (Instron, model 4411) to be submitted to a shear bond strength test, performed at a speed of 0.5 mm/min until breakdown. A stainless steel strip involved the resin cylinder and was fixed in the inferior mordant. A metal jig, which involved the specimen, was fixed in the superior mordant. The obtained data (kgf/cm²) were transformed in MPa and analyzed with ANOVA and Tukey's test ($p = 0.05$).

RESULTS

The shear bond strength results were submitted to ANOVA with factorial schema. The factors

TABLE 1 - Mean of shear bond strength (MPa) for Clearfil Liner Bond 2V, on human and bovine enamel and dentin.

	Enamel (SD)	Dentin (SD)
Human	10.01 a (3.22)	7.43 a (2.09)
Bovine	7.95 a (1.98)	9.27 a (2.69)

Means with same letters on column indicate no significant difference for Tukey's test (5%). SD = standard deviations.

TABLE 2 - Mean of shear bond strength (MPa) for Scotchbond Multi-Purpose, on human and bovine enamel and dentin.

	Enamel (SD)	Dentin (SD)
Human	7.36 a (1.58)	7.01 a (2.26)
Bovine	8.24 a (2.47)	11.74 b (3.78)

Means with same letters on column indicate no significant difference for Tukey's test (5%). SD = standard deviations.

are: the substrate, analyzed for its nature (enamel or dentin); the origin, analyzed for animal species (human or bovine). Two analyses were carried out: one for Clearfil Liner Bond 2V and another for Scotchbond Multi-Purpose. This was done because the purpose of the study was not to evaluate the two adhesive systems themselves, but compare the adhesive systems with dissimilar mechanisms of action in different substrates. The means were submitted to Tukey's test with 5% of significance and they are presented on Tables 1 and 2.

For Clearfil Liner Bond 2V, there was no significant statistical difference ($p > 0.05$) between bovine and human teeth for both enamel and dentin, as can be seen on Table 1.

For Scotchbond Multi-Purpose, there was no significant statistical difference ($p > 0.05$) between bovine and human teeth for enamel, as seen on Table 2. However, on dentin, the bovine tooth shear bond mean was higher than that of the human tooth, and there was some statistical difference ($p < 0.05$).

DISCUSSION

The search for an alternative substrate to human teeth for studies on bond strength and microleakage has increased, arousing the interest of some researchers.

The strength of the adhesive bonds between restorative materials and dentin is affected by the number of dentinal tubules per mm^2 and by their diameter, as well as the relative amount of intratubular and intertubular dentin¹⁰. According to Nakamichi *et al.*¹⁰ (1983), a sufficient area of substrate could be obtained in different dentin depths of bovine incisors, but only the superficial layer could be considered a substitute to human dentin. The dentin in bovine incisors presents larger dentinal tubules and more porous on intertubular dentin than human molars¹².

Bovine enamel is more porous than human enamel, so it presents a higher diffusion and rate of progress of artificial carious lesion^{5,6,7}. Besides, it presents thinner crystallites than human enamel and it has a different structure between prisms¹⁸. Edmunds *et al.*⁵ (1988) using bacterial culture and acid gel studied artificial caries in bovine, equine, and ovine enamel. They verified that the lesions depths in these animal teeth were almost two times bigger than in the human teeth.

The enamel rich in carbonate was found to be particularly vulnerable to acid attack¹⁹. According to Sydney-Zax *et al.*¹⁵ (1991), the carbonate concentration on bovine enamel was higher than in human enamel. It is probable that the same occurs with dentin.

This study found higher shear bond strengths for bovine substrate when SBMP was used. As bovine substrate presents lower mineral quantity and higher carbonate concentration, it is more susceptible to acid-etching; in other words, in the same period of time, the dissolution of enamel crystals, the opening of dentinal tubules and the demineralization of inter and intratubular dentin are more pronounced in bovine teeth than in human teeth. As to enamel, though the statistical analysis showed no difference between bovine (8.24 MPa) and human (7.36 MPa) substrates, higher values were observed for bovine enamel. There was some statistical difference regarding dentin, with higher values in bovine (11.74 MPa) than in human (7.01 MPa) dentin. Maybe these results were obtained because it is easier for the adhesive system to penetrate into the bovine demineralized dentin, because of the greater quantity of dentinal tubules as well as their greater diameter. Their longer and thicker tags may induce higher bond strength values on shear or traction tests.

With Clearfil Liner Bond 2V, there was no difference between human and bovine substrates, both

in enamel and dentin. However, Nakamichi *et al.*¹⁰ (1983) observed a higher bond strength to human enamel than to bovine enamel. In this study, human enamel also presented a few higher values, but without statistical difference. Maybe these results were because self-etching systems do not require a previous acid-etching procedure with phosphoric acid. Hence, the acid monomers of these systems will act on the enamel surface, demineralizing and penetrating it. A close contact with the dental surface is, nevertheless, necessary. According to Yu, Chang²⁰ (1966), bovine enamel presents lower superficial energy than human enamel. This could explain why Clearfil Liner Bond 2V showed smaller results on bovine enamel.

As to Clearfil Liner Bond 2V on dentin, there were a few high values for bovine teeth compared with human teeth, although with no statistical difference. The Scotchbond Multi-Purpose presented the same behavior, albeit with statistical difference. An explanation for these results would be that acid-etching before the application of the bonding agent could permit a higher resin penetration in dentinal tubules and in peri- and intratubular dentin. With the Clearfil Liner Bond 2V system, the resin penetration would be less intense on both substrates. However, when analyzing bond strength values, they are similar to those

of the Scotchbond Multi-Purpose system. Nevertheless, when a shear force is applied, a thicker hybrid layer with larger and longer resin tags, similar to those obtained with Scotchbond Multi-Purpose, may possibly make the difference between the substrates for one system and not for the other.

This study verified that when bovine teeth were used instead of human teeth in laboratory tests of bond strength, it is necessary to be careful because the results will not always be proportional between the two substrates.

CONCLUSION

1. When Clearfil Liner Bond 2V was used, the bovine and human dentin and enamel were equivalent as regards shear bond strength values.
2. The Scotchbond Multi-Purpose system presented no statistical differences on enamel, but on dentin the bovine substrate showed higher bond strength values than the human substrate.
3. The use of bovine teeth in place of human teeth in laboratory tests of shear bond strength seems to be partially valid because the obtained values were not always proportional to the two substrates; this depends on the adhesive system used.

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