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Comparative study of bond strength to human and bovine dentine at three different depths

Estudo comparativo da resistência adesiva à dentina humana e bovina em três diferentes profundidades

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Abstract

The aim of this study was to compare the bond strength to three different depths of remaining human and bovine dentin, through shear bond strength test, and to establish a possible relationship among the depths of the substrates to contribute for the replacement of human dentin in bond strength tests. Forty-eight human teeth (H) and forty-eight bovine teeth (B), freshly extracted, stored in distilled water and frozen at -18 ° C for at most four weeks were used in this study. 240-, 400-, 600- and 800-grit sandpapers were used to expose the dentin and standardize the smear layer at 0.5, 1.0 and 2.0mm of dentin thickness. Scotchbond Multi-Purpose Plus adhesive system was used following the manufacturer's instructions, on a standardized area of 4mm, followed by the incrementally application of Z100 resin composite (3M). The shear bond test was performed in the Instron Universal machine at cross-head speed of 0.5mm/min. Statistical analysis was performed by ANOVA ($p < 0.05$). There were significant differences in bond strength between H and B teeth, with the highest values for H teeth. There was a significant difference in bond strength to the depths analyzed but there was similarity in behavior between H (0.5mm) and B (2mm) substrates. Bovine substrate can be used for laboratory studies of bond strength as indicative of the initial performance of new products, although the differences related to human substrate should be observed.

Keywords

Human dentin; bovine dentin; adhesive systems; shear bond test.

Introduction

Firstly employed on the enamel surface after the acid etching developed by Buonocore [1], the dental adhesive systems provided a deep modification in the restorative procedures. These procedures became more conservative and durable because of the bonding establishment which reduced microleakage and the possibilities of caries relapse. Bonding to enamel became a reliable and routine procedure in Restorative Dentistry by the characteristics inherent to this tissue: 97% of inorganic content and 3% or organic matter and water; relative inactivity, impermeability and distance from the pulp tissue [2, 3].

However, bonding to dentin has more barriers to surpass: the physical-chemical and morphological characteristics inherent of the dentinal tissue - 70% of mineral content, 30% of organic content, water and e oxygen; the tubular structure with different proportions of peritubular and intertubular dentin, additionally to the permeability and the mineralization degree, which vary as the proximity of the pulp [4-9]. The physical properties of the dentin, such as the modulus of elasticity lower than that of the enamel, acting as a foundation for the enamel; lower compressive force because of its smaller mineral content and higher organic content are very important, once this is the greatest part of the tooth structure.

Currently, the dental adhesive systems have several applications and when associated with resin composites they meet all aesthetic requirements, enabling conservative procedures that protected the tooth structures. With the increase of the interaction between the adhesive materials and the tooth structures, there was an improvement in the marginal and tubular sealing as well as in the protection to the dentin-pulp complex, resulting in the reduction of the post-operative sensibility and staining of the tooth/restorative material interface. Its application followed by the restoration with the current composites, has been used to achieve the aesthetics in posterior and anterior teeth, replace fractured tooth structures, fill erosive defects at the cervical area, reshape the teeth cosmetically, protect the dentin-pulp complex, cement indirect restorations, fix brackets and periodontal splints, and repair porcelain, amalgam, and composite restorations [7].

All this evolution in tooth adhesion and the development of the materials involve several laboratorial and clinical studies. In vivo studies of any dental material are expensive and demand much time for its development. With the advancement in technology and the fast development of the adhesive systems, often, a new material is either launched into or removed from the market before the conclusion of a clinical study. Therefore, in vitro studies are largely employed by the researchers and manufacturers to verify the behavior of these products. Notwithstanding, several factors hinder the correlation of the laboratorial results with the clinical performance of a given adhesive system, such as: difficulty in the collection of sound and freshly-extracted human teeth; the age and storage conditions of the teeth location, depth and mineral content of the dentin; surface roughness; type and duration of the load cell applied onto the specimen; negligence to the hydration conditions, presence of the dentinal fluid, pulp pressure, and ability to flex of the vital dentin; and lack of standardization of the tests to compare the results of different studies with the same objective.

Concerned with the ethical viability of these studies and because of the difficulty in collecting sound human teeth, there is the necessity of finding a substrate similar to human substrate. Human teeth have the morphology and histological characteristics similar to those of other mammals, but the size and availability of the bovine incisors meet the requirements for research. Bovine teeth have been compared to human teeth because of their increasing popularity in bond strength and microleakage studies [2, 10-20], mainly because of the results of Nakamichi et al. [21], who by verifying the possibility of the use of bovine teeth as substrate for bond strength tests, observed values of bond strength to enamel and superficial dentin close to those of the human substrate. Other studies, however, highlighted the need of further studies to improve the understanding regarding the differences and similarities between these biological substrates and their influence on the bond strength [20-22].

A micromorphological study conducted by Dutra-Corrêa et al [22] demonstrated there is differences in the distribution and diameter of the dentinal tubules of the bovine dentin when compared to the human dentin. According to the authors, the region where there is greater similarity between the two substrates is the medium area, between the enamel-cementum junction (ECJ) and the pulp. Consequently, adhesive procedures performed on different areas of the bovine dentin could reach different results of hybridization and bond strength tests. Other micromorphological study [23] which analyzed the hardness of the human and bovine sclerotic dentin revealed a similar number of dentinal tubules of homogenous distribution in the dentin surface of both substrates. Concerning to microhardness, these authors observed that the human sclerotic dentin showed significant higher values than those of the bovine sclerotic dentin.

Considering that several researches on adhesive systems have been conducted in bovine teeth and the need of establishing its relation with human teeth [24], the aim of this study was to compare the shear bond strength of bovine and human dentin in different depths.

Material and Method

This study was submitted and approved by the Ethical Committee in Research of the School of

Dentistry of São José dos Campos – UNESP, under protocols number #050/2000-PH/CEP for research involving human beings and number #021/2000-PA/CEP for research involving animals.

Forty-eight sound lower incisors extracted from cattle immediately after their culling and 48 human upper central incisors, freshly extracted from adults patients exhibiting periodontal disease were used in this study. The teeth were cleaned, stored in distilled water and frozen at –18oC, for at most four weeks. The roots of all teeth were sectioned at the medium third with the aid of a carborundum disc.

To obtain the measurement of the remaining dentin, an opening in the lingual surface of the teeth was made with the aid of a diamond round bur up to the pulp exposure. The pulp tissue was removed and the pulp chamber was carefully washed with distilled water and gently dried. The root orifice was sealed with utility wax to prevent the penetration of acrylic resin from the procedure of fixation of the teeth. Then, the teeth were embedded into acrylic resin with their labial surface parallel to the horizontal plane. The labial enamel was removed with the aid of 80-grit sandpaper (3M ESPE, Brazil) mounted into a plaster trimming machine, under copious irrigation with water, to expose the dentinal surface.

At the center of the tooth crown, the dentin depth was determined with the aid of a thickness gauge (Otto-Arminger&Cia Ltda, RS, Brazil), aiming to obtain three groups of remaining dentin depth, for both bovine and human teeth.

The bovine and human teeth were divided into six groups of sixteen teeth each, as follows: **Group BMM05** – Bovine teeth with remaining dentinal substrate of 0.5mm of depth; **Group BMM1** – Bovine teeth with remaining dentinal substrate of 1.0mm of depth; **Group BMM2** – Bovine teeth with remaining dentinal substrate of 2.0mm of depth; **Group HMM05** –Human teeth with remaining dentinal substrate of 0.5mm of depth; **Group HMM1** – Human teeth with remaining dentinal substrate of 1.0mm of depth; **Group HMM2** – Human teeth with remaining dentinal substrate of 2.0mm of depth.

The dentin exposed was flattened with the aid of 240-, 400- and 600-grit sandpaper (3M ESPE, Brazil), for 20 s for each granulation, under constant pressure and copious water irrigation; next the samples were polished in a polishing machine (DP-10 Panambra Industrial e Técnica SA, Brazil) with a 800-grit sandpaper, also under copious water irrigation to create a standardized uniform smear layer [16].

The dentinal surfaces were cleaned with Robinson brush mounted in contra-angle handpiece at low speed with pumice and water, followed by the cleaning in an ultrasound device with distilled water for 6 min. To standardize the bonding area, the dentinal surface was delimited with the aid of a Teflon adhesive tape with a standardized perforation of 4 mm of diameter.

The adhesive technique employed the Scotchbond Multi-Purpose Plus (3M ESPE, Brazil), and was performed according to the manufacturer's instructions restricted to the area marked by the perforation in the Teflon tape. The dentin etching was executed with 37% phosphoric acid for 15 s, washed with an air/water jet for 15 s and dried with paper filters. A thin layer of Scotchbond Multi-Purpose Primer (3M ESPE, Brazil), was applied onto the etched dentin, and its excess was removed with a gentle air jet. Next, a layer of Scotchbond Multi-Purpose Adhesive (3M ESPE, Brazil) was carefully applied to avoid a thick layer, light-cured for 10 s, with the aid of a light-curing device (Ultralux, Dabi Atlante, Riberão Preto, SP, Brazil) with power constantly calibrated at 600 mW/cm2, through a radiometer (Demetron, Damburry, CT – USA).

To standardize the area and volume of the resin composite, a 4-mm thick bipartite Teflon rectangular strip was used with a central perforation of 4mm of diameter matching the perforation of the Teflon tape on the specimen. After the light-curing of the composite, the two parts of the matrix were separated. To fix the Teflon strip onto the sample, hindering its movement during the composite insertion and light-curing, a customized steel device was used.

The resin composite was inserted with layers of about 1.5mm thick, condensed under constant pressure, light-cured for 40 seconds per layer, according to the manufacturer's instructions. Therefore, composite cylinders were obtained with 4.0mm of diameter x 4.0mm of height, bonded to the dentinal surface.

The samples were identified, immersed into distilled water, and stored in bacteriological incubator at 37oC ± 2oC for 24h. Following, they were submitted to the laboratorial mechanical test to evaluate the shear bond strength with the aid of an Instron 4301 machine, with load cell of 500 Kg at a cross-head speed of 0.5mm/min.

The bond strength values were expressed in MPa when the adhesive union between the dentin and restorative material ruptured Data showed a normal distribution and two-way analysis of variance (ANOVA) and multiple comparison test was used. The level of significance was set at 5%.

Results

Table 1 shows the results with the mean values and standard deviation of bond strength found.

Table 1 – Bond strength mean and standard deviation (MPa)

Groups	Bond Strength (MPa) / Standard Deviation
H MM2	16.88 ± 3.93
H MM1	14.90 ± 4.43
H MM05	12.06 ± 3.48
B MM2	12.64 ± 2.07
B MM1	8.90 ± 2.97
B MM05	7.09 ± 1.83

Table 2 displays the ANOVA results exhibiting the significant differences for the type and depth of the dentin of the groups studied.

Table 2 – Two-way analysis of variance

	Degree Of Freedom	Average Square	F (Snedecor)	P-Level
Var1(H/B)	1	.6168669	58.01801	.000000(*)
Var2 (Depth)	2	216.2224	20.33631	.000000(*)
Interaction	2	.6260090	.58877	.557135

(*) Statistical significance.
Var1 – Tooth type (bovine or human).
Var2 – Depth (mm).
Interaction – Influence of the result of the tooth type on the depth.

Table 3 exhibits the groups (variables) which showed statistical differences and similarities.

According to this analysis, we observed that the measurement of group H at 0.5mm and B at 2mm showed 99.58% of probability of equality.

Figure 1 shows the graphs for the measurement of the interaction according to ANOVA. They exhibit the same tendency for the curves of the groups HUMAN and BOVINE. If there was an interaction, that is, the depths were the main cause of the variability of the groups, there should be a change in the tendency of the lines of one of the groups (HUMAN or BOVINE) at any of the depths. Once this fact did not occur, the graphic result is in agreement with the numeric result obtained by the ANOVA table and aforementioned discussed.

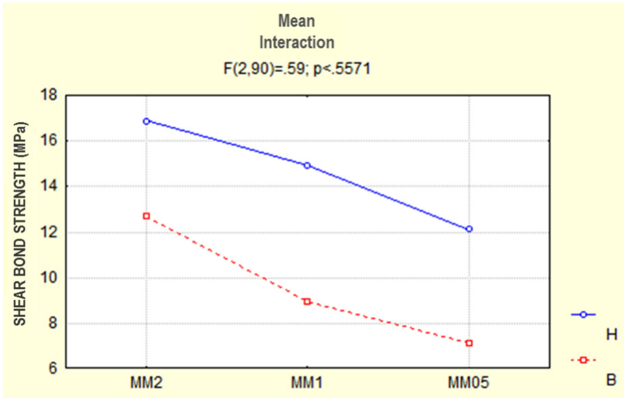

Figure 1 – Interaction between depth and bond strength.

Discussion

The results obtained evidenced that there was a significant difference in the bond strength between human and bovine teeth. However, they both maintained a similar tendency towards decreasing of the bond strength as the depth increased. Previous studies also observed this difference between the two substrates [6, 25-30] but without influence on the dentin depth, fact that may possible be explained by

Table 3 – p-values among the groups tested according to the multiple comparison test

	(1)	(2)	(3)	(4)	(5)	(6)
	16.88	14.909	12.061	12.647	8.9095	7.0921
H MM2 (1)	-	.525815	.001015	.005328	.000122	.000122
H MM1 (2)	.525815	-	.144103	.371900	.000136	.000122
H MM05(3)	.001015	.1441103	-	.995819	.078517	.000688
B MM2 (4)	.005328	.371900	.995819	-	.020130	.000197
B MM1 (5)	.000122	.000136	.078517	.020130	-	.616097
B MM05(6)	.000122	.000122	.000688	.000197	.616097	-

the differences in the methodology employed.

Authors as Tagami et al. [18] suggested an explanation for the highest bond strength values of the superficial dentin than those of the deep dentin, claiming that a greater amount of intertubular matrix - with greater amount of collagen fibers and smaller permeability - would be available for the interlacement with the adhesive monomers, leading to a lower influence of the pulp pressure and of the hydration.

Fogel et al.[31] still suggested that the maximum permeability of the bovine dentin is of 0.02µL cm-2min-1cm H2O-1 while human molars exhibits a permeability of 0.1µL cm-2min-1cm H2O-1, in an attempt of explaining the different bond strength values between the two substrates.

We also observed that according to Table 1, the possibility of a better standardization of the bovine specimens, because they exhibited a smaller standard deviation than the human specimens.

Despite of the higher variability of the bond strength results of the human samples, by analyzing the values of the superficial dentin (mean of 16.88 ± 3.93MPa), we verified that these values are very similar to those obtained by other authors employing Scotchbond Multi-Purpose Plus [25, 32, 33]. Notwithstanding, very different results were also reported [34, 35].

Concerning to the deep dentin, the values obtained (12.06 ± 3.48MPa), were in agreement with those reported in the literature [25]. For the superficial dentine of the bovine samples, the values (12.64 ± 2.07MPa) differed from those of other studies [4, 12, 36].

The results of this present study disagreed with those of the study of Fowler et al.[13] who observed higher bond strength values for bovine dentin (7.1 ± 3.5MPa) than for human dentin (5.4 ± 2.9MPa), although without statistical difference, with the use of Scotchbond 2. These results may be the consequence of the use of the labial surface of the bovine teeth and the occlusal surface of the human teeth; because the labial surface exhibits a smaller variation in the number, size and orientation of the tubules [17]. Moreover, these authors used an adhesive system that did not employed the total etch technique.

Numerous studies established the differences between human and bovine dentin. Retief et al. [37], observed that the bond strength is higher for the human substrate while the microleakage is higher for the bovine substrate, therefore contraindicating the latter for a substitute for this type of tests. Dutra-Corrêa [22], verified that the tubules of the bovine dentin closer to the ECJ have a larger diameter, which decreases as they come closer to the pulp, unlikely to what is seen in the human dentin. Turssi et al. [3], showed that the microhardness of the human root dentin is greater than that exhibited by the bovine root dentin. However, these differences do not seem to justify a change in the behavior of the superficial and deep dentin compared with the human dentin, regarding to the bond strength. This can be explained by the proportion between the inter- and peritubular dentin, presenting a variation in the amount of collagen fibers available for hybridization. As aforementioned exposed, the literature lacks in understanding the differences and similarities between these biological substrates as well as the influence on the bond strength [20, 22]. After a search and analysis of the studies comparing the bovine and human substrate, in different in vitro and in situ studies, Yassen et al. [24] emphasized that the morphology, the composition and the differences in the physical properties of these substrates should be taken into consideration during the interpretation of the data obtained from studies employing bovine teeth.

According to our objective of identifying human and bovine tooth depths exhibiting a similar behavior, it can be observed from Table 1 that the mean bond strength values of HMM05 and BMM2 were similar.

It is important to highlight that the ethical issues has made difficult to conduct studies with human teeth and animals (monkeys and dogs). Thus, despite of the differences in the methodology, bond strength, and microleakage, we should be aware that such differences can be standardized and properly established, in order to make the bovine teeth as substrates capable to be used, firstly in laboratorial tests.

Obviously, the longitudinal clinical studies in humans are still needed to search the ideal material that effectively seals the restoration margins and provides bond strength excellence.

Conclusions

Based on the experimental conditions, evaluation method, statistical analysis, and the aim of this present study, it can be concluded that:

a) there is a significant difference between the HUMAN and BOVINE teeth in the shear bond strength results with the highest values observed for the human teeth;

b) there is a significant difference in the shear bond strength for the dentin depths analyzed, with the highest values for the superficial dentin, followed by the medium and deep dentine for both the HUMAN and BOVINE substrates.

Resumo

O objetivo do presente estudo foi comparar a resistência adesiva da dentina humana e bovina em três diferentes espessuras de remanescente, frente ao teste de cisalhamento, a fim de estabelecer uma possível relação de profundidade entre os substratos visando contribuir para a substituição da dentina humana em testes de adesão. Empregaram-se 48 dentes humanos (H) e 48 dentes bovinos (B), recém-extraídos, armazenados em água destilada e congelados a –18°C, por no máximo quatro semanas. Foram utilizadas lixas de granulação 240, 400, 600 e 800, para expor a dentina e padronizar a smear layer, com espessura de dentina de 0,5, 1,0 e 2,0mm. O sistema adesivo Scotchbond Multi-Uso Plus foi utilizado seguindo instruções do fabricante, em uma área padronizada de 4mm, seguido da aplicação incremental da resina Z100 (3M). O ensaio de cisalhamento foi realizado em máquina Instron Universal à velocidade de 0,5mm/min. Foi realizada análise estatística pelo teste ANOVA a dois critérios ($p < 0,05$). Houve diferença significativa entre a resistência adesiva em dentes H e B, sendo os maiores valores para H; houve diferença significativa de resistência para as profundidades analisadas; houve semelhança de comportamento entre os substratos H 0,5mm e B 2mm. O substrato B pode ser empregado em estudos laboratoriais de resistência adesiva como indicativos da performance inicial de novos produtos, observando-se as diferenças existentes entre eles.

Palavras-chave

Dentina humana; dentina bovina; sistemas adesivos; teste de cisalhamento.

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