Validity of using bovine teeth as a substitute for human counterparts in adhesive tests

F. Saleh[1] and N. Taymour[2]

صلاحية استخدام أسنان البقر بدلاً من الأسنان البشرية في اختبارات اللصق

فايز قاسم صالح، نيفين محمد تيمور

الخلاصة: أجريت مقارنة بين المقاومة للقص والمقاومة للشد في إطار دراسة شملت 40 من الأسنان البشرية و40 من أسنان البقر. وتم تقسيم الأسنان عشوائياً إلى مجموعتَيْن: تم تعريض الأولى لراتين مركب مُقَسًّى تقسية خفيفة، أما المجموعة الثانية فتم تعريضها لملاط أيونوميري راتيني مُقَسًّى تقسية خفيفة ومقوى بالزجاج. وتم تقسيم المجموعتَيْن إلى مجموعات فرعية لإجراء اختبارات المقاومة للقص والشد باستخدام ماكنة اختبار متصلة بترجام transducer حاسوبي. وبيَّنت التحاليل الإحصائية وجود فروق يُعتد بها اعتداداً عالياً من الوجهة الإحصائية بين ميناء الأسنان البشرية وميناء أسنان البقر من حيث المقاومة للقص والشد؛ غير أن المعادلات التنبوية للتحوُّف الإحصائي تؤيد استخدام أسنان البقر كبديل جيد لأسنان البشر في الدراسات الخاصة بالتجسير التقويمي للأسنان.

ABSTRACT Shear and tensile bond strengths of 40 human and 40 bovine teeth were compared. Teeth were randomly assigned to group I, which received light-cured composite resin, or group II, which received light-cured resin-reinforced glass ionomer cement. The groups were subdivided for shear and tensile bond strength tests, which were conducted on a universal testing machine installed to a computer transducer. Statistical analysis revealed a highly significant difference between shear and tensile bond strengths of human and bovine enamel; however, regression prediction equations support the use of bovine teeth as a reliable substitute to human counterparts in bonding studies of orthodontic adhesion.

Validité de l'utilisation de dents de bœuf en remplacement des dents humaines dans les tests d'adhérence

RESUME On a comparé la résistance au cisaillement et à la traction de 40 dents humaines et 40 dents de boeuf. Les dents ont été réparties de manière aléatoire entre le groupe I qui a reçu des composites photopolymérisables et le groupe II qui a reçu des ciments de verre ionomère renforcés par résine photo-polymérisable. Ces groupes ont été subdivisés pour les tests de résistance au cisaillement et à la traction qui ont été réalisés sur une machine d'essais universelle installée sur un transducteur informatisé. L'analyse statistique a mis en évidence une différence très significative entre la résistance au cisaillement et à la traction de l'émail humain et celle de l'émail bovin ; toutefois, les équations de prédiction obtenues par régression vont dans le sens de l'utilisation des dents de bœuf comme substituts fiables des dents humaines dans les études concernant le collage orthodontique.

[1]Department of Orthodontics; [2]Department of Dental Biomaterials, Faculty of Dentistry, Beirut Arab University, Beirut, Lebanon.
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Introduction

Many investigators have used extracted human teeth to evaluate the adhesive strength characteristics of dental adhesive and restorative materials [*1,2*]. The widespread use of the teeth in *in vitro* bond strength studies simulates *in vivo* situations. However, because of recent progress in conservative dental treatment, there is great difficulty in procuring sound, non-carious human teeth for *in vitro* bonding studies. This difficulty is more pronounced in cases of evaluating the bond strength characteristics of many direct orthodontic brackets with adhesive materials. Bovine teeth have been considered as a possible substitute for human teeth. Indeed, many researchers [*3 - 9*] are already using bovine lower central incisors because of their similar microstructure to human enamel and because of the ease of obtaining them [*10*].

In this context, both human and bovine teeth are mammalian and they have certain characteristics of mammalian enamel. The enamel forms an outer layer covering the whole or part of the tooth exposed in the mouth cavity. The enamel is highly calcified and its origin is from ectoderm that shows a prismatic structure [*11*].

Several studies have also been conducted to determine the similarity between human and bovine dental enamel in terms of structural and physical properties. In order to investigate the structure and determine the crystal defects and impurities in bovine and human enamel, Spitzer and Bosch [*12*] studied the absorption and scattering of light in thin sections of sound enamel. They concluded that there was no substantial difference between the refractive indices of human and bovine enamel. In another study, the polishing properties of human and bovine enamel were compared and a positive correlation of 0.99 was found, which meant there was a highly significant similarity in the microstructure of both enamel surfaces [*13*].

The present study investigated the possibility of using the enamel of bovine teeth as a substitute for human counterparts in orthodontic bonding tests of recently developed adhesives.

Methods

Forty (40) freshly extracted permanent bovine mandibular incisors were collected from a local slaughterhouse within a few hours after slaughtering. Another 40 recently extracted human maxillary central incisors were collected from the dental school clinics of the Faculty of Dentistry.

Eighty (80) pre-adjusted metal brackets (Adenta, Germany) with retentive mesh back for direct bonding and 13.99 mm^2 nominal areas were used. Two different adhesives were used: light-cured composite resin [Reliance Light Bond, Orthodontic Products, Itasca, Illinois, United States of America (USA)] and light-cured resin-reinforced glass ionomer cement (Fuji Ortho LC, GC Corp., Tokyo, Japan).

The selected human and bovine teeth were thoroughly cleaned and washed under running tap water and all adherent soft tissues were removed. The teeth were stored in distilled water at room temperature until the time of testing.

All human and bovine teeth roots were embedded into an autopolymerizing acrylic resin formed by a standard size plastic ring (15 mm height and 25 mm diameter). The teeth were randomly assigned into two main groups and four subgroups. Teeth were then mounted in acrylic blocks with their labial surfaces in either horizontal shear mode or vertical tensile mode.

After mounting and immediately before bonding procedures, all prepared test specimens were cleaned and polished with a rubber prophylaxis cup using non-fluoridated pumice, then rinsed thoroughly with fresh tap water.

The labial surfaces were etched using 37% orthophosphoric acid for 30 seconds. Each tooth was thoroughly washed to remove the etching remnants and was lightly dried with oil-free compressed air for 30 seconds [*6*]. The light bond composite resin adhesive was applied according to the manufacturer's instructions. The light source was then directed to the bracket and the adhesive material was light-cured for 20 seconds using a visible light-curing unit (Litex 680, Dentamerica, USA) on each of the four sides of the bracket (mesial, distal, incisal and gingival). All test specimens were stored in distilled water at 37 ± 1 °C until ready for testing at 24 hours [*14*].

The shear and tensile bond strength tests were conducted on a universal mechanical testing machine (ELE International Ltd., United Kingdom) installed to a special computer/transducer. A special rigid metal frame was designed to hold the acrylic cylinder carrying the specimen to be tested. The metal frame is composed of two parts: a lower part and an upper part. The lower metal frame is made of an outer part which is rigidly attached to the lower movable compartment of the shear box, and an inner part which surrounds a machined slot of the metallic frame into which the specimen to be tested is placed.

For the shear bond strength test, the labial surface was horizontally seated in the acrylic cylinder and held rigidly. The applied load was directed parallel to the bracket-enamel interface with a crosshead speed of 1 mm/min [*6*]. For the tensile bond strength test, the labial surface was placed vertically in the acrylic cylinder and the tensile load was applied perpendicular to the bracket–enamel interface with a crosshead speed of 1 mm/min [*6*].

All data analyses generated from the shear and tensile bond strength tests were transferred to an integrated computer system through special transducers utilizing software packages. The force in newtons (N) required to cause bonding failure between the enamel–bracket interface was recorded and the shear and tensile bond strength values were calculated in kg/cm^2 by converting force values from N to kg and dividing by the cross-sectional area of the bracket base. Means and standard deviations were calculated for shear and tensile bond strength values and statistical analysis was carried out using the Mann–Whitney Z test.

Results

The shear and tensile bond strength values in kg/cm^2 and in MPa on both human and bovine teeth using the two orthodontic adhesives—light-cured composite resin and glass ionomer cement were collected (Tables 1 and 2). There was a significant difference between the shear bond strength values of human teeth and bovine teeth with the two adhesive systems, the composite resin group (Z = 3.7839, P = 0.0002) and the glass ionomer cement group (Z = 3.6312, P = 0.0003) (Table 1). When comparing the two adhesive groups, the composite resin group showed significant difference of shear bond strength values than that of the glass ionomer cement group in both bovine (Z = 3.4042, P = 0.0007) and human teeth (Z = 3.7839, P = 0002).

The tensile bond strength values followed a similar result pattern to the shear bond strength data (Table 2). The human teeth used in this study showed significant-

Table 1 **Statistical analysis of the shear bond strength values of composite resin and glass ionomer cement on human and bovine teeth**

Serial	Shear bond strength in kg/cm² (MPa) Composite resin		Glass ionomer cement	
	Bovine teeth	Human teeth	Bovine teeth	Human teeth
1	53.30 (5.22)	103.70 (10.16)	36.00 (3.53)	52.60 (5.15)
2	54.70 (5.36)	105.90 (10.38)	39.20 (3.84)	74.20 (7.27)
3	54.80 (5.37)	108.00 (10.59)	40.60 (3.98)	79.70 (7.81)
4	59.00 (5.78)	111.70 (10.95)	43.50 (4.26)	80.0 (7.84)
5	62.50 (6.12)	114.50 (11.22)	44.70 (4.38)	81.40 (7.98)
6	62.70 (6.14)	116.7 (11.44)	46.00 (4.51)	82.20 (8.06)
7	64.20 (6.29)	117.40 (11.51)	47.50 (4.65)	85.70 (8.40)
8	64.20 (6.29)	129.00 (19.65)	53.40 (5.23)	88.60 (8.68)
9	54.60 (5.35)	105.90 (10.38)	57.00 (5.58)	92.20 (9.04)
10	64.30 (6.30)	117.40 (11.51)	44.70 (4.38)	81.40 (7.98)
Mean	59.43 (5.82)	113.02 (11.08)	45.26 (4.43)	79.80 (7.82)
s	4.65	7.64	6.03	10.79
Mann–Whitney *Z*	3.7839, *P* = 0.0002		3.6312, *P* = 0.0003	
Mann–Whitney *Z* between materials	3.4042, *P* = 0.0007		3.7839, *P* = 0.0002	

s = standard deviation.

ly higher tensile bond strength values than their respective bovine counterparts for both the composite resin group (Z = 3.7939, P = 0.0001) and the glass ionomer cement group (Z = 3.7041, P = 0.0002). When comparing the two adhesive systems to each other, the composite resin group showed significantly higher tensile bond strength values than that of the glass ionomer cement group in the human test groups (Z = 3.4838, P = 0.0005) whereas no statistically significant difference was found between the two adhesive systems in the bovine test groups (Z = 1.36632, P = 0.172).

There was a correlation (Pearson's coefficient) between human and bovine teeth for the respective shear and tensile bond strength values using the two orthodontic adhesives (Table 3). A statistically significant Pearson's coefficient ($P < 0.05$) was found between human and bovine teeth for the shear and tensile bond strengths parameters using glass ionomer cement. With the composite resin adhesive, a significant correlation ($P < 0.05$) was found between human and bovine teeth for the shear bond strength parameter, whereas no significant correlation ($P > 0.05$) was found for the tensile bond strength parameter.

From the Pearson coefficients in Table 3 regression prediction equations were set up. These equations supported the use of bovine teeth as a substitute for human teeth in both shear and tensile bond strength tests in cases of direct orthodontic bonding.

Table 2 **Statistical analysis of the tensile bond strength values of composite resin and glass ionomer cement on human and bovine teeth**

Serial	Tensile bond strength in kg/cm^2 (MPa)			
	Composite resin		Glass ionomer cement	
	Bovine teeth	Human teeth	Bovine teeth	Human teeth
1	13.60 (1.33)	26.70 (2.61)	10.60 (1.03)	18.50 (1.81)
2	14.40 (1.41)	93.00 (3.82)	10.80 (1.05)	19.20 (1.88)
3	16.00 (1.56)	30.60 (3.00)	11.20 (1.09)	20.20 (1.98)
4	16.80 (1.64)	33.60 (3.29)	13.00 (1.27)	20.70 (2.03)
5	19.50 (1.91)	37.50 (3.67)	13.20 (1.29)	21.60 (2.11)
6	13.60 (1.33)	26.70 (2.61)	15.20 (1.49)	23.00 (2.25)
7	14.40 (1.41)	39.00 (3.82)	16.50 (1.61)	24.50 (2.40)
8	16.00 (1.56)	30.60 (3.00)	16.60 (1.62)	25.20 (2.47)
9	16.80 (1.64)	33.60 (3.29)	18.00 (1.76)	27.40 (2.68)
10	19.50 (1.91)	37.50 (3.67)	18.70 (1.83)	27.80 (2.79)
Mean	16.06 (1.57)	33.48 (3.28)	14.38 (1.41)	22.81 (2.23)
s	2.17	4.74	3.03	3.32
Mann-Whitney *Z*	3.7939, $P = 0.0001$		3.7041, $P = 0.0002$	
Mann-Whitney *Z* between materials	1.3632, $P = 0.172$		3.4838, $P = 0.0002$	

s = standard deviation.

Discussion

In order to find a substitute for human teeth in adhesion tests of orthodontic brackets, the bond strength to bovine teeth was compared with that of human teeth using two types of orthodontic adhesive materials. Human maxillary central incisors were chosen for the bond strength tests in this study. These teeth are considered ideal for bonding studies because they have a nearly flat bonding surface, which helps in the fitting of a bracket base. Permanent bovine mandibular incisors were used in the present study because of the ease of obtaining them and because these teeth are closer to the size of maxillary central incisors.

The present study found some differences in the bond strengths between human and bovine teeth although they appear to be very similar on a histochemical and anatomic basis [*11*]. The adhesion to enamel showed a statistically significant difference between human and bovine teeth with the two types of orthodontic adhesives used. The strength of the bond to bovine enamel was statistically weaker than to human enamel. Mariwaki et al. reported that the large crystal grains and the lattice defects found in bovine enamel were the results of the rapid development of bovine teeth during tooth formation before and after eruption [*15*]. Moreover, the lower enamel bond strengths reported for bovine

Table 3 **Pearson coefficient correlation between human and bovine shear and tensile bond strengths using composite resin and glass ionomer cement**

Parameter	Composite resin	Glass ionomer cement
Shear bond strength	0.8897*	0.8434*
Tensile bond strength	0.472	0.9864*

*$P < 0.05$.

teeth in this study may be attributed to the difference between the critical surface tension of bovine and human enamel. This finding was consistent with the results of Yu and Chang who reported a lower critical surface tension for bovine teeth than for human teeth [*16*].

Despite the differences between human and bovine teeth in the orthodontic adhesion tests of the present study, bovine enamel may still be a reliable substitute for human enamel in bonding studies designed for orthodontic adhesions. Regression prediction equations of the results of the present study supported the use of bovine teeth as a substitute for human teeth in bonding studies designed for orthodontic adhesion. The present results agreed with the findings of Nakamichi et al. [*17*] who reported that bovine teeth could be used as a substitute for human teeth in adhesion tests to enamel although mean values were lower with bovine teeth. The present study also agreed with the results obtained by Barkmier and Erickson who used adhesives designed for restorative dentistry and found that enamel bonded to bovine teeth was significantly weaker than to human teeth [*18*]. The results of the present study were also consistent with those obtained by Oesterle et al. who found that bovine lower incisors could be successfully used to study enamel bond strength with orthodontic bonding materials although the bond was 21%–44% weaker to bovine than to human enamel [*10*]. Moreover, the shear and tensile bond strengths to both human and bovine enamel increased or decreased simultaneously in the present study, an indication that both human and bovine teeth act in a similar manner and follow a similar pattern of behaviour.

Conclusions

The shear and tensile bond strengths to enamel showed statistically significant differences between human and bovine teeth with the two types of orthodontic adhesive materials used. Bond strengths to human enamel were superior than to bovine teeth. Regression prediction equations used in this study support the use of bovine teeth as substitutes for human teeth in both shear and tensile bond strength tests for direct orthodontic bonding procedures. Further studies are needed to evaluate the use of bovine teeth as substitutes for human teeth in adhesion tests designed for restorative dentistry as well as other dental purposes.

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