VARIATIONS IN HUMAN AND BOVINE SURFACE ENAMEL ACID ETCHING PATTERNS AND RESIN PENETRATION: A SCANNING ELECTRONIC MICROSCOPY *IN-VITRO* STUDY

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ABSTRACT

The aim of this study was to evaluate and compare the different acid etching patterns and resin penetration to human and bovine enamel as indicator of their bond strength properties. Forty human and forty bovine incisors were tested in the shear and tensile bond strength modes using a light-cured composite resin "Reliance Light Bond". Bond strength tests were conducted on a universal testing machine installed to a special computer transducer where bonding failure data were recorded. Statistical analysis of the results revealed a highly significant difference between the shear and tensile bond strengths of human enamel as compared to that of bovine teeth. Scanning Electron Microscopic examination of the enamel surfaces were carried out before and after etching, then resin remnants penetration to enamel after bond failure were also assessed. The results showed a high variability in the etching patterns and penetration depth of the resin tags to human and bovine enamel surfaces which are attributed to the difference in the micro-topography of their enamel surfaces. Despite the overall differences, the consistency of the bovine bonding strength properties suggests their continuous use as a substitute for their human counterparts in similar studies.

Keywords: bovine and human enamel, etching patterns, resin tags penetration, SEM

INTRODUCTION

Since Buonocore (1955) first introduced phosphoric acid etching of enamel to enhance resin adhesion to tooth enamel, a growing number of investigators have used extracted human teeth to evaluate the relationship between structural properties of human hard dental tissue and the bond strength properties of orthodontic adhesives and restorative dental materials (Fusayama *et al*., 1979; Hermsen & Vrijhoef, 1993; Wang & Lu, 1991; Bowen, 1965; Rasmussen *et al*., 1976; Reifies *et al*., 1995; McCarthy & Hondrum, 1994; Aljubouri *et al*., 2004). Today, and because of the great advances in the science and technology of conservative dentistry and the tendency to minimal orthodontic extraction therapy, there is a great difficulty in obtaining sound, non carious human teeth for *in vitro* bonding studies.

Bovine lower central incisors were thought of as a possible substitute to human counterparts, and many researchers are using them in different adhesive tests and comparative etching and bonding studies because of their similar microstructure to human enamel as well as to the ease of obtaining them (Moriwaki *et al*., 1968; Oesterle *et al*., 1998; Miller and Zernik, 1996; Guess *et al*., 1988; Merrill *et al*., 1994; Komori and Ishikawa, 1997; van der Vyver *et al*., 1997; Uno and Finger, 1995; Legler *et al*., 1990)

Spitzer and Ten Bouch (1975), 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.

Putt *et al*., (1980), compared the polishing properties of human and bovine enamel and concluded that both were identical in the degree of polish and yielded a positive correlation of 0.99 to different abrasives, which meant a highly significant similarity in the microstructure of both enamel surfaces.

Nackamichi *et al*., (1983), compared the bond strength properties of human and bovine enamel using five different adhesive cements and two composite resins. They concluded that despite the slightly lower bond strength values recorded for the bovine teeth, they can be used as a substitute for human teeth in adhesive tests.

Eversoll and Moore (1988), examined the penetration of the liquid portions of bonding adhesives to one hundred freshly extracted lower bovine incisors and observed penetration of up to 25µm into the etched enamel surface. Their scanning electron microscopy analyses were in agreement with Buonocore *et al*., (1968) who confirmed that the etching process produces greater potential for resin tags penetration and enhancement of mechanical bonding of adhesives.

Mattick and Hobson (2000), in a comparative micro-topographic study of different tooth enamel surfaces, attributed the high variability in bond strength properties to the difference in the mineral composition of teeth and the micro-topography etched enamel surfaces which may affect acid solubility.

Saleh and Taymour (2003), in an *in-vitro* study tested the shear and tensile bond strengths of human and bovine enamel using two recent orthodontic adhesive materials. Although the adhesion to human enamel was stronger than that of bovine, regression prediction equation in this study supports the use of bovine teeth as substitutes for human counterparts in bonding tests.

Considerable research used the Scanning Electron Microscopy to study the enamel and dentin etching patterns and their influence on the bond strength properties of several adhesives (Whittaker, 1982; Birkenfeld & Schulman, 1999; Diedrich, 1981; Silverstone, 1975; Galil & Wright, 1979; Oliver, 1986; Silverstone *et al*., 1975; Futastsuki *et al*., 1995; Joseph *et al*., 1994; Bowen & Rodriguez, 1962). Only few studies have compared the structural characteristics of bovine and human enamel and their influence on the orthodontic bond strength properties (Scott & Symons, 1971; Barkmier & Erickson, 1994; Yu & Chang, 1966). The present *in-vitro* Scanning Electron Microscopy study aimed to correlate the different acid etching patterns and resin tag penetration to human and bovine enamel after shear and tensile bond failures, with their bond strength properties. Thus, a scientific evidence could be gathered to accept or reject the hypothesis that bovine teeth can be used as a substitute to human counterparts in different adhesive tests can be presented.

MATERIALS AND METHODS

Materials

Teeth: Forty freshly extracted bovine mandibular incisors and forty recently extracted human maxillary central incisors.
Orthodontic brackets: Eighty pre-adjusted metal brackets (Adenta, Germany) with retentive mesh back for direct bonding and 13.99 mm^2 nominal areas were used.
Adhesive materials used in this study: Light - cured composite resin (Reliance Light Bond, Orthod. Products, Itasca, USA)
(Jeol JEM- 5300 Scanning Microscope Japan), was used in this study.

Methods

Details of the sample preparation and testing procedures are best described in a previously published *in-vitro* investigation by Saleh and Taymour (2003). However, a brief summary of the methods employed is appropriate:

Preparation and grouping of test specimens: Human and bovine teeth preparation including cleansing, storage, mounting were randomly assigned into two main groups. Teeth were then mounted in acrylic blocks with their labial surfaces placed either horizontal, shear mode (Fig. 1), or vertical, tensile mode (Fig. 2).

Figure 1. Tooth specimen during shear testing. Load applied perpendicular to the bracket/enamel interface.

Figure 2. Tooth specimen during tensile testing. Load applied perpendicular to the bracket/enamel interface.

Bonding procedures: The labial surfaces of the prepared specimens were etched using 37% ortho phosphoric acid for 30 seconds (standardized for bovine and human enamel surfaces); each tooth was thoroughly washed to remove the etching remnants, and was lightly dried with an oil-free compressed air for 30 seconds (Saleh & Taymour, 2003) . The light bond composite resin adhesive was applied according to the manufacturer's instructions and light-cured for 20 seconds using a visible light- curing unit. All test specimens were stored in distilled water at 37$^oC \pm 1^oC$ until ready for testing at 24 hours (Saleh & Taymour, 2003).

Bond Strength Tests: The shear and tensile bond strength tests were conducted on a Universal Mechanical Testing Machine, (ELE International, Ltd., UK) installed to a special computer/transducer. Each specimen was firmly placed in the machined slot of the metal frame to hold the bracket firmly so that the force was exerted in a uniform direction and the load was applied perpendicular to the bracket – enamel interface with a crosshead speed of 1 mm/min (Saleh & Taymour, 2003).

All data analyses generated from shear and tensile bond strength tests were transferred to an integrated computer system through special transducers utilizing software package (ELE International, Ltd., UK). The force in Newton (N) required to cause bonding failure between enamel/adhesive/bracket interfaces was recorded and the shear and tensile bond strength values were calculated in kg/cm^2 by converting force values from Newton to kilogram and dividing it by the cross-sectional area of the bracket base and statistically analyzed.

Scanning Electron Microscopy (SEM):

(a) Method for examining normal and etched human and bovine enamel morphology before bonding procedures

Extracted human and bovine intact teeth were used, the crowns of which were separated and cut from their respective root portions. Human and bovine enamel surfaces were prepared by grinding flat the palatal surfaces, whereas the labial enamel surfaces were cleaned and smoothed with universal polishing device under running water (Saleh & Taymour, 2003). Progressively, further polishing of the enamel surfaces was performed using waterproof abrasive paper. The enamel surfaces received no further treatment at this stage. The specimens for human and bovine teeth were seated on the prepared flat palatal surfaces and were mounted on aluminium stubs with a vacuum-resistant adhesive. Each specimen was sputter coated with a 10nm of gold-palladium layer prior to examination with Scanning Electron Microscopy (Jeol JEM- 5300 Scanning Microscope, Japan), and photomicrographs were taken for analysis.

(b) Method for examining enamel resin tag penetration

Scanning electron microscopic examination of human and bovine teeth took place after shear and tensile debonding to assess the structural enamel bonding characteristics in the form of adhesive resin tag penetration. The labial surfaces of human and bovine teeth that were provided with bonded brackets (Reliance Light Bond composite) were cut longitudinally with a diamond disc under copious water-cooling. Each specimen was labelled to compare resin tags penetration with the bond failure pattern and strength. The mesial and distal surfaces of each tooth specimen were then ground flat and all cut surfaces were polished. A subsequent etching for 15 seconds with nitric acid and 5% sodium hypochlorite for 12 hours resulted in partial demineralization and deproteinization of the surface enamel, thus revealing the enamel/resin junction showing the resin tags. The specimens were seated on either the prepared flat mesial or distal surfaces and were mounted on aluminium stubs. Following desiccation for one hour, the specimens were sputter coated with gold-palladium at 10nm for two minutes and then examined in the Scanning Electron Microscope.

RESULTS

Data including statistical analysis for bond strength tests were taken from the in-vitro study by Saleh and Taymour (2003).

Shear and Tensile Bond Strength values

Table 1 and Table 2 illustrate the shear and tensile bond strength values (mean standard deviation, and Mann - Whitney Z test) in kg/cm^2 and in MPa on both human and bovine teeth using the Reliance Light Bond orthodontic adhesive.

TABLE 1

Statistical Analysis of the Shear Bond Strength Values in kg/cm^2 (MPa) of Reliance Light Bond on Human and Bovine Teeth

Shear Bond Strength in Kg/cm^2 (MPa)	(Reliance Light Bond)	
Serial	Bovine teeth	Human teeth
1	53.30 (5.22)	103.70 (10.16)
2	54.70 (5.36)	105.90 (10.38)
3	54.80 (5.37)	108.00 (10.59)
4	59.00 (5.78)	111.70 (10.95)
5	62.50 (6.12)	114.50 (11.22)
6	62.70 (6.14)	116.7 (11.44)
7	64.20 (6.29)	117.40 (11.51)
8	64.20 (6.29)	129.00 (19.65)
9	54.60 (5.35)	105.90 (10.38)
10	64.30 (6.30)	117.40 (11.51)
Mean	59.43 (5.82)	113.02 (11.08)
SD	4.65	7.64
Mann-Whitney Z	3.7839, P = 0.0002	
Mann-Whitney Z Between materials	3.4042 P = 0.0007	3.7839 P = 0.0002

According to Table 1, human teeth showed significantly higher shear bond strength values than bovine teeth (Mann - Whitney Z = 3.7839, P = 0.0002)...

The tensile bond strength values demonstrated in Table 2 follow a similar result pattern to the present shear bond strength data. The human test groups used in this study showed significantly higher tensile bond strength values than their respective bovine counterparts (Mann - Whitney Z= 3.7939 P = 0.0001).

TABLE 2

Statistical Analysis of the Tensile Bond Strength Values in kg/cm^2 (MPa) of Reliance Light Bond on Human and Bovine Teeth

Tensile Bond Strength in Kg/cm^2(MPa)	**Reliance Light Bond**	
Serial	**Bovine teeth**	**Human teeth**
1	13.60 (1.33)	26.70 (2.61)
2	14.40 (1.41)	93.00 (3.82)
3	16.00 (1.56)	30.60 (3.00)
4	16.80 (1.64)	33.60 (3.29)
5	19.50 (1.91)	37.50 (3.67)
6	13.60 (1.33)	26.70 (2.61)
7	14.40 (1.41)	39.00 (3.82)
8	16.00 (1.56)	30.60 (3.00)
9	16.80 (1.64)	33.60 (3.29)
10	19.50 (1.91)	37.50 (3.67)
Mean	16.06 (1.57)	33.48 (3.28)
SD	2.17	4.74
Mann-Whitney Z	3.7939, P = 0.0001	
Mann-Whitney Z Between materials	1.3632 P = 0.172	3.4838 P = 0.0002

Table 3 illustrates a correlation (Pearson's coefficient) between human and bovine teeth for the respective shear and tensile bond strength values using the Reliance Light Bond adhesive, a significant correlation ($P<0.05$) is found between human and bovine teeth for the shear bond strength parameter, whereas no significant correlation ($P > 0.05$) is found for the tensile bond strength parameter.

TABLE 3

Correlation (Pearson Coefficient) between Human and Bovine Shear and Tensile Bond Strengths using Reliance Light Bond

Parameter	Reliance light Bond
Shear bond strength	**0.89***
Tensile bond strength	**0.47**

Level of significance at (P<0.05)

Figures 3 and 4 depict the behaviour of shear load - bond failures as illustrated in Table I.

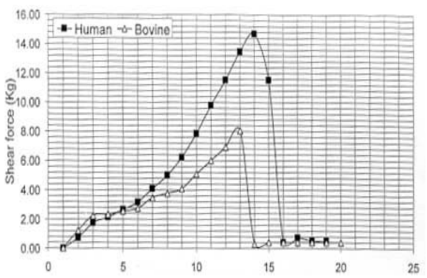
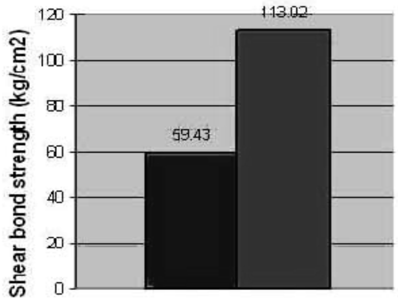


Figure 3. Typical load (Shear) displacement curve of human and bovine teeth using Reliance Light Bond.



Bovine Human

Figure 4. Mean Shear bond strength values (Kg/Cm^2) of human and bovine teeth using Reliance Light Bond.

Morphology of etched enamel and resin tags penetration

Acid etching patterns of human and bovine enamel surfaces:

Selective solubility of enamel due to acid etching produces characteristic patterns, which expose the prism structure, and roughness of the enamel surface with deeply penetrating micro clefts. Figures 7 to10 show normal and etched human and bovine enamel surfaces. The human etching patterns followed the central etching type where dissolution of central prism portions took place (Pattern 1 of Silverstone's classification) (Birkenfeld &

Schulman, 1999), the bovine enamel followed the peripheral etch type with little dissolution of the prism centers (Pattern 2 of Silverstone's classification) (Birkenfeld & Schulman, 1999).

Figures 5 and 6 depict in the same manner the tensile bond failures as per Table II.

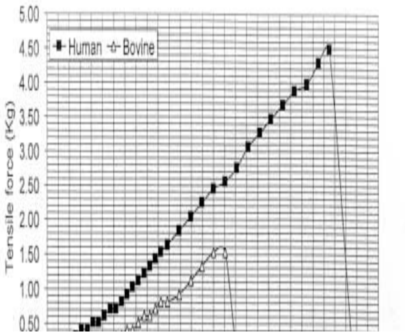


Figure 5. Typical load (tensile) displacement curve of human and bovine teeth using Reliance Light Bond.

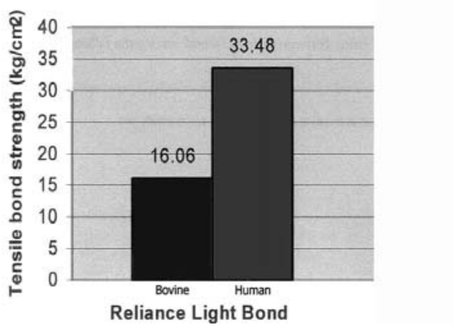
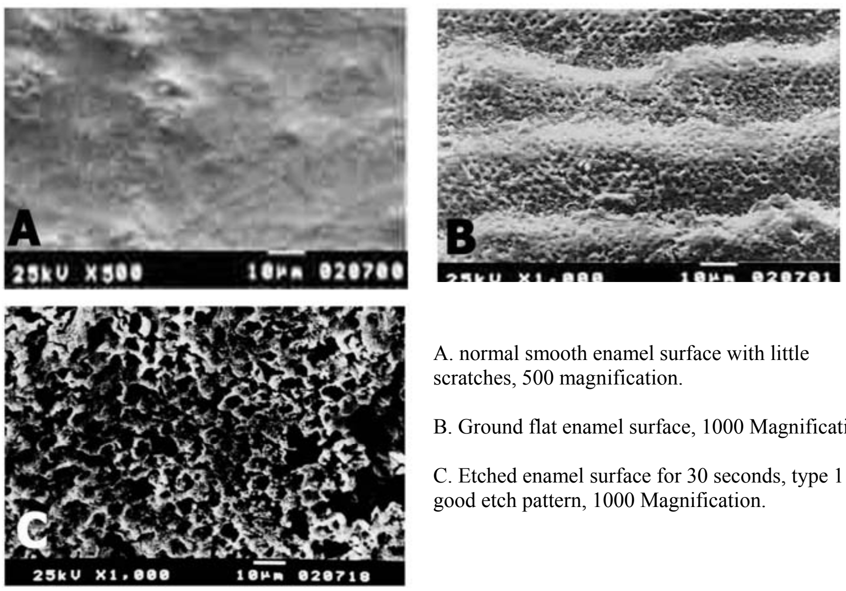


Figure 6. Mean tensile bond strength values (Kg/Cm^2) of human and bovine teeth using reliance light bond.

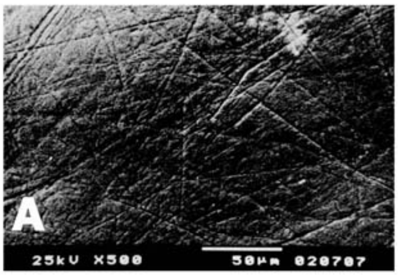


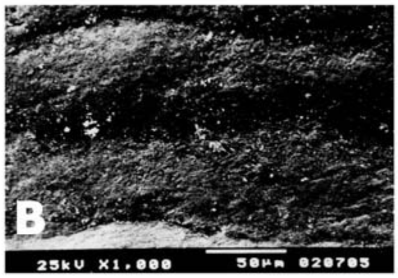
A. normal smooth enamel surface with little scratches, 500 magnification.

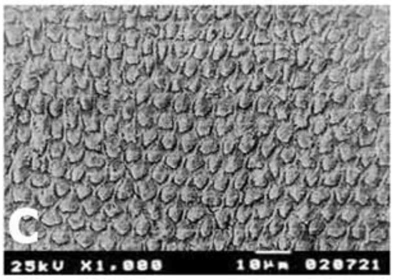
B. Ground flat enamel surface, 1000 Magnification

C. Etched enamel surface for 30 seconds, type 1 good etch pattern, 1000 Magnification.

Figure 7. SEM photographs showing etching pattern of human enamel.







A. Normal bovine enamel, showing a slightly rough enamel surface traversed by minor surface irregularities**.**

B. Ground rough enamel surface, 1000 Magnification

C. Type 2 etching pattern affecting the prism boundaries rather than the prism centers (30 seconds).

Figure 8. SEM photographs showing etching pattern of bovine enamel.

Resin tags depth of penetration to human and bovine etched enamel:

Figures 9 to 12 show the enamel/adhesive interface in shear and tensile testing modes for both human and bovine teeth. The human shear resin tag penetration was between 70-80µm as compared to only 30-40µm for bovine. In tensile testing modes the human resin tags penetration was relatively consistent 20-40 µm in contrary to bovine which revealed a highly variable tag penetration ranged from 5 to 30 µm.

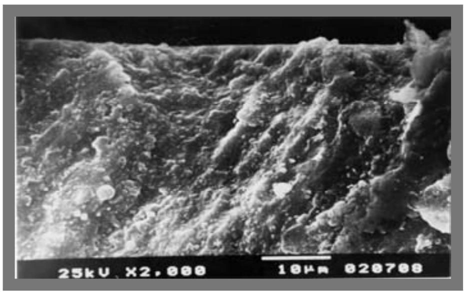


Figure 9. Scanning electron micrograph (Magnification X 2000) showing numerous deep resin projections of Reliance Light Bond penetrating the enamel surface.

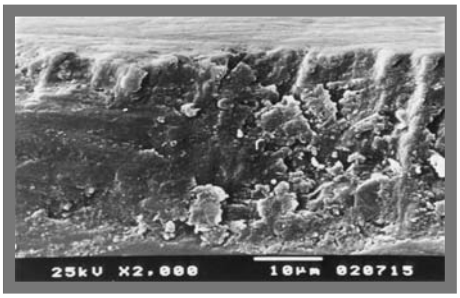


Figure 10. Scanning electron micrograph (Magnification X 2000) showing few resin projections of Reliance Light Bond into the bovine enamel surface.

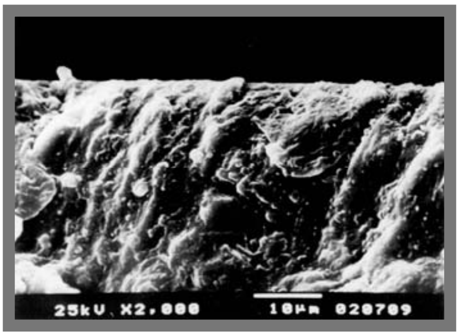


Figure 11. Scanning electron micrograph (Magnification X 2000) showing numerous deep resin projections of Reliance Light Bond penetrating the enamel surface.

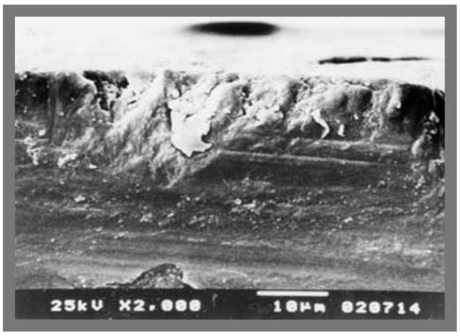


Figure 12. Scanning electron micrograph (Magnification X 2000) showing slender and short resin projections of Light Bond penetrating the bovine enamel surface.

DISCUSSION

Because of the inconsistencies in the published reports examining correlations between enamel micro-topography and bond strength properties, this Scanning Electron Microscopic examination was carried out to determine the relationship between the enamel morphology, etching patterns, and resin tags penetration to both human and bovine teeth as indicators of their bond strength properties.

Based on the data from a previous investigation (Saleh & Taymour, 2003), a statistically significant difference was recorded between the weaker bond strength of bovine enamel as compared to its stronger human counterpart although; several published works have reported a great histochemical and anatomic similarity between human and bovine enamel (Moriwaki *et al.*, 1968; Oesterle *et al.*, 1998; Miller & Zernik, 1996). Other researchers quoted differences in the micro-topography of bovine and human enamel surfaces; Moriwaki *et al*. (1968) attributed the reason for the lower adhesive strength of bovine teeth to the large crystal grains and the lattice defects found in bovine enamel that were the result of the rapid development of bovine teeth during tooth formation before and after eruption. Yu and Chang (1966) stated that the lower enamel bond strengths reported for bovine teeth in their study may be due to the lower critical surface tension of bovine than human enamel.

The findings of this SEM investigation showed that bovine enamel micro-topography has a poorer definition than that found on human prismatic enamel, the enamel etching patterns of human enamel followed the central etching type or dissolution of central prism portions took place (Pattern 1 of Silverstone's classification) (Silverstone, 1975; Galil & Wright, 1979; Oliver, 1986; Silverstone *et al*., 1975); the bovine enamel followed the peripheral etch type with little dissolution of the prism centers (Pattern 2 of Silverstone's classification) (Silverstone, 1975; Galil and Wright, 1979; Oliver, 1986; Silverstone *et al*., 1975). Numerous resin tags with a greater depth of penetration were seen on the larger well defined etched enamel surfaces of human teeth as compared with fewer and more superficial resin tags on less defined smaller etched surfaces of bovine enamel. These results explain the higher bond strength values of human enamel as compared to bovine enamel in shear and tensile testing modes. Silverstone (1975) and Mattick and Hobson (2000) found that there were considerable etch pattern differences not only between different groups of teeth but also within groups of similar teeth, another possible explanation to the high variability in the depth of resin tag penetration (5 to 30 µm) encountered with the bovine enamel surfaces.

The present findings are in agreement with those of Nakamichi *et al.* (1983); who reported that bovine teeth could be used as a substitute for human teeth in adhesion tests to enamel, although the mean values were lower with bovine teeth, and coincide with the results found by Barkmier and Erickson (1994) who used adhesives designed for restorative dentistry and found that enamel bond to bovine teeth was significantly weaker than to human teeth. The results of this study are also consistent with those obtained by Oesterle *et al.* (1998) who found that bovine lower incisors could be successfully used to study enamel bond strength with orthodontic bonding materials although the bond to bovine teeth was 21% to 44% weaker to bovine than to human enamel. Moreover, the authors in the present study reported that the shear and tensile bond strengths to both human and bovine enamel increased or decreased simultaneously, an indication that both human and bovine teeth act in a similar manner and follow a relatively similar pattern of bond failure curve.

CONCLUSION

Despite the above mentioned differences in micro-topography and bond strength values between human and bovine teeth in the orthodontic adhesion tests of the present study, bovine enamel failure pattern followed a lower but similar curve in shear and tensile bond strength tests.

Bovine teeth may still be considered a reliable substitute for human enamel in the bonding studies designed for orthodontic attachments.

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