ORIGINAL ARTICLE

The use of bovine enamel in bonding studies

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Bovine enamel is commonly used in enamel bonding studies, therefore, a familiarity with some aspects of bovine enamel bonding are important in order to evaluate the studies. Bovine enamel has the advantages of easy attainability and similar microstructure to human enamel. In this study the strength of the enamel bond using an orthodontic adhesive was compared between deciduous bovine, permanent bovine, and human enamel, as well as, the effect on bond strength of multiple rebonding to bovine enamel. This study found that the bond strength to bovine enamel was 21% to 44% weaker than to human enamel, and the bond strength to deciduous bovine enamel was significantly greater than to permanent bovine enamel. Either all deciduous or all permanent bovine incisors should be used, or permanent and deciduous bovine incisors evenly distributed in sample groups. Bovine enamel was rebonded five times without significantly affecting bond strength, thus, bovine enamel can be reused in bonding studies without significantly affecting the results. (Am J Orthod Dentofacial Orthop 1998;113:514-9)

With the near universal use by dentists of direct bonded orthodontic brackets, the study of the factors involved in optimizing bond strength and the bonding process are common in the orthodontic literature. The most ideal tooth for bonding studies is the human maxillary central incisor. It has a nearly flat bonding surface that is usually consistent from incisor to incisor without the concern of fitting a bracket base to a varying curved surface. However, with a continual increase in dental health, more conservative dentistry, and limits on access to human materials being instituted by some hospitals, it is increasingly difficult to get noncarious, sound human incisors for studies. Some studies have used more easily obtainable premolars that were extracted for orthodontic treatment,[1,2] but premolars vary in the curvature of their labial surface and add the additional variable of the bracket base not closely fitting the tooth. Bovine lower incisors are an easily obtainable, inexpensive substitute for human incisors that have been used in a number of past studies. In order for the clinician to adequately evaluate studies, however, he or she must be aware of some of the factors affecting comparability of bovine and human enamel.

This project was supported primarily from a research grant from the University of Colorado School of Dentistry and by donation of materials and supplies by the 3M Unitek Corporation.

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0889-5406/98/$5.00 + 0 **8/1/83879**

Bovine enamel, besides being readily available, is similar to human enamel. The teeth of all mammals appears to be very similar on a histochemical and anatomic basis.[3-5] However, some differences have been reported. Because bovine enamel and dentin develop more rapidly during tooth formation, bovine enamel has larger crystal grains and more lattice defects than human enamel.[6] This may contribute to a reported lower critical surface tension in bovine enamel than in human enamel.[7] The difference between the critical surface tension of bovine and human enamel has been speculated to account for the slightly lower enamel bonding values seen with bovine teeth.[8] Despite the above differences, bovine enamel has been reported to be a reliable substitute for human enamel in bonding studies with no statistically significant difference in enamel bonding values, although the values were all slightly lower.[8,9] Nakamichi et al.[8] did report that the bond strengths to both human and bovine enamel increased significantly when older teeth, as opposed to recently extracted teeth, were tested. More recent research by Barkmeier and Erickson,[10] however, not only found the bond strengths to bovine enamel to be lower than to human enamel, but significantly lower with bovine enamel bonding strengths 35% below that of human enamel. However, Barkmeier and Erickson were using adhesives designed for restorative dentistry.

Other factors in the use of bovine enamel involve the type of tooth used. Cattle are generally slaughtered at approximately 18 months of age. Although the animals are near adult size at this age they still are in the mixed dentition with both deciduous and permanent lower incisors present. The lower incisors are usually

Table I variable factors

Sample	% Teeth permanent	Type test	Tack time	Cure time	Test time
Human	100%	Control	0 sec	0 min	30 min
Bovine 1	40%	Control	0 sec	0 min	30 min
Bovine 2	50%	Control	0 sec	0 min	24 hrs
Bovine 3	50%	Experimental	2 sec	10 min	30 min
Bovine 4	50%	Experimental	2 sec	10 min	24 hrs
Bovine 5	30%	Experimental	2 sec	20 min	30 min
Bovine 6	40%	Experimental	2 sec	20 min	24 hrs
Bovine 7	40%	Experimental	5 sec	10 min	30 min
Bovine 8	50%	Experimental	5 sec	10 min	24 hrs
Bovine 9	50%	Experimental	5 sec	20 min	30 min
Bovine 10	50%	Experimental	5 sec	20 min	24 hrs
Bovine 11	50%	Experimental	10 sec	10 min	30 min
Bovine 12	40%	Experimental	10 sec	10 min	24 hrs
Bovine 13	50%	Experimental	10 sec	20 min	30 min
Bovine 14	40%	Experimental	10 sec	20 min	24 hrs
Bovine Rebond 1	30%	Experimental	0 sec	0 min	30 min
Bovine Rebond 2	30%	Experimental	0 sec	0 min	30 min
Bovine Rebond 3	30%	Experimental	0 sec	0 min	30 min
Bovine Rebond 4	30%	Experimental	0 sec	0 min	30 min
Bovine Rebond 5	30%	Experimental	0 sec	0 min	30 min

chosen for studies because of the greater ease of obtaining mandibles and because the lower incisors are closer to the size of human maxillary central incisors. Deciduous lower bovine incisors are nearly the same size as permanent human maxillary central incisors, whereas, permanent lower bovine incisors are dramatically larger than human maxillary incisors (Fig. 1). One study[11] reported using bovine deciduous incisors, whereas others[12-14] made no mention of whether permanent or deciduous bovine incisors or a mix were used. A question that immediately comes to mind is, how comparable are deciduous and permanent bovine test values? In human beings the more amorphous deciduous enamel requires more etching time to produce a usable bond between enamel and bracket.

The questions asked during this study were:

1. Is the enamel bond strength with orthodontic bonding materials comparable between bovine and human teeth?
2. Is there a difference between the enamel bond strength to bovine deciduous and bovine permanent incisors?
3. Can bovine teeth be bonded multiple times in bonding studies without affecting the bond strength?

MATERIAL AND METHODS

Bovine deciduous and permanent lower incisors, human maxillary central incisors, a standardized orthodontic bracket, and light-cured orthodontic adhesives were used to test the above questions. The

Fig 1. Comparison of bovine permanent lower incisor(*left*), bovine deciduous lower incisor (*center*), and human maxillary central incisor (*right*). Bovine deciduous incisor is closer in size and appearance to the human incisor.

shear/peel bond strength between the bracket and the tooth was tested at specific time intervals with specific light exposures as outlined here and in Table I with the use of an Instron Testing Machine (Instron Corp., Canton, Mass). Test data unique to this study as well as partial data from a previous study[15] were used in this study but analyzed with different criteria than the previous study. Details of the variables are expressed in Table I.

Three control series were tested in which the bonding material was fully cured for 40 seconds and tested after 30 minutes of setting time; two control series used bovine lower incisors, and one control series used human maxillary central incisors. For all test groups there were 10 samples in each cell. Another series of 10 samples was bonded, debonded, cleaned of adhesive, rebonded, and tested a total of five times for each sample, using the same protocol as the control samples. All samples used the same brackets (right maxillary central incisor bracket Mini-Twin .018 bracket, #2017-201 with a bonding surface area of 0.0153 in^2 or 9.8710 mm^2, Unitek Corp., Monrovia, Calif.) prepasted with Transbond Adhesive (Unitek Corp.) enabling comparison of the results of this study with that of a previous study.[16]

The time from the point at which the bracket was tacked in place to the time the adhesive was fully cured was another variable in this study. The bond strength was tested at 30 minutes and 24 hours. Values for the permanent bovine incisors were separated from values for deciduous bovine incisors to observe any differences between the two under other than the control conditions. Variables used for samples taken from a previous study[15] are given in Table I as well as the variables used for the samples specific to this study. The "tack time" was the amount of time that the light was applied to the bracket and tooth to initially "tack" the

Table II Results

Samples	*Mean*	*Standard median*	*Average deviation*	*ARI*
Human 30 minute controls	12.71	11.52	5.00	2.10
Bovine permanent 30 minute controls	7.63	8.27	2.87	1.75
Bovine permanent 30 minute all	7.09	7.14	2.68	1.61
Bovine deciduous 30 minute control	8.29	6.89	4.54	2.17
Bovine deciduous 30 minute all	10.05	9.32	3.65	1.92
Bovine permanent 24 hour control	17.31	16.97	8.59	1.00
Bovine permanent 24 hour all	15.87	13.89	6.50	1.32
Bovine deciduous 24 hour control	12.47	13.58	3.45	1.17
Bovine deciduous 24 hour all	20.40	19.85	8.38	1.68
Bovine rebond 1	9.98	10.77	4.60	
Bovine rebond 2	9.90	10.11	2.11	
Bovine rebond 3	9.06	8.16	3.74	
Bovine rebond 4	9.13	9.35	4.50	
Bovine rebond 5	9.19	9.32	3.39	2.10

bracket into position. The "cure time" as used in the study was the amount of time the tacked bracket was allowed to autocure before the final curing of 40 seconds. The samples that are listed at 0 tack-time and 0 cured time were used as controls.

Standard procedures and manufacturer's instructions were used in preparing the bovine and human enamel. Bovine mandibular incisor teeth were collected within a few hours after slaughtering. The teeth were cleansed of soft tissue and stored in refrigerated Cloramine-T solution. The human maxillary incisors were obtained from adult clinic patients and stored in Cloramine-T solution as soon as obtained by the researchers. The teeth were then mounted in acrylic cylinders and randomly assigned to one of the groups, each group contained 10 teeth. Before bonding, the facial surface of each incisor was cleaned with a mixture of water and fluoride-free pumice. The facial surface was thoroughly rinsed with water to remove any pumice or debris, dried with an oil- and moisture-free air stream, and etched for 30 seconds with the orthophosphoric acid gel supplied by the manufacturer (Unitek Corp.). The teeth were rinsed with distilled water for 20 seconds and dried with warm, oil- and moisture-free air from an air dryer designed for bonding. The tooth surface was inspected for the characteristic dull, white, frosted appearance that reflects adequate etching. The primer provided with the adhesive system was applied and thinned with a gentle stream of oil- and moisture-free air. The bracket and attached adhesive were removed from the light-tight manufacturer's package immediately before bracket placement, positioned on the tooth surface, pressed firmly to place, and the excess adhesive removed before application of the curing light. A Unitek Curing Light (Unitek Corp.) with built-in radiometer was used. The unit was tested for proper light output with the radiometer before each session. The curing light was held as close as possible to the incisal edge of the tooth surface and bracket for the "tack-time" and, during the 40 second final curing, on the mesial and distal of the bracket/tooth interface. All samples were stored in distilled water during the period between final cure and testing, with the 24 hour sample stored in an incubator at 37°C.

Testing was done in a shear/peel mode with an Instron Universal Testing Machine. The specimens were placed in the lower jaw of the Instron Testing Machine such that the bracket base of the sample was parallel to the direction of force. The upper end of a 10 cm long loop of 0.020 inch stainless steel wire was placed in the upper jaw of the Instron Testing Machine to form a suspended loop. The lower end of the loop was positioned under the lower bracket wings of the sample. The specimen was then stressed in a gingivoincisal direction to produce a shear/peel force at a cross head speed of 1 mm per minute. The maximum load required to debond the bracket was recorded. The surface of the tooth was examined under a dissecting microscope to determine the amount of adhesive remaining on the tooth and the amount recorded with the ARI (Adhesive Remaining Index).[12,13] The criteria for the ARI used are as follows: score 0 = no adhesive left on tooth; score 1 = less than half of adhesive left on tooth; score 2 = more than half of adhesive left on tooth; score 3 = all adhesive left on tooth, distinct impression of the bracket base.

For the teeth that were rebonded multiple times, the adhesive remaining on the tooth was removed with a 12-fluted carbide bur in a high-speed handpiece. Light pressure was used to remove the adhesive without overheating the enamel. Attempts were made to avoid visibly scaring the enamel and effecting bond strength.

Statistical analysis of the results was done using an analysis of variance and the three-factor analysis of variance. Analyses were done comparing within the series that was rebonded multiple times; comparing deciduous bovine incisors between the control groups, the 24 hour sample groups, and the control group and the 24 hour group; comparing permanent bovine

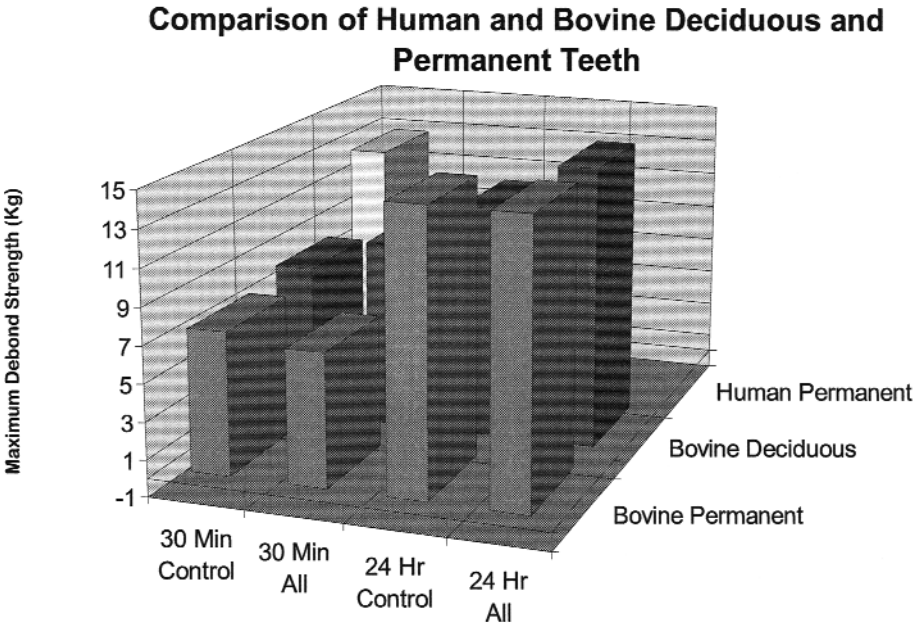

Fig. 2. Comparison of bond strengths found in study between human incisors and deciduous and permanent bovine lower incisors. Values are the maximum strength to debond the bracket in kilograms.

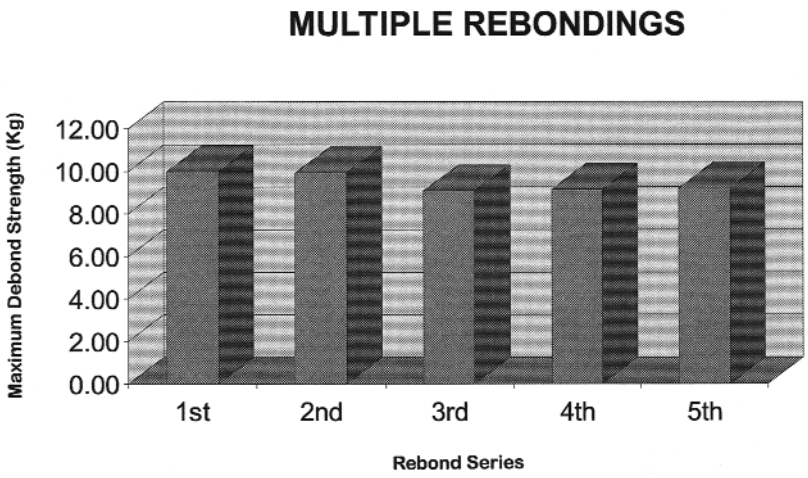

Fig 3. Bond strengths of bovine enamel bonded multiple times. Values are maximum strength to debond bracket in kilograms.

incisors between the control groups, the 24 hour sample groups, and the control group and the 24 hour group; comparing permanent and deciduous bovine incisors between the deciduous and permanent control groups at both 30 minutes and 24 hours and between all groups at 30 minutes and 24 hours; and comparing the human sample to the bovine control samples, all 30 minute bovine samples, and separately for deciduous and permanent bovine samples. Significance was established at the 5% confidence level.

RESULTS

Results are given in Table II and Figs. 2 and 3. Multiple statistical comparisons were made with the results given below. As expected from previous studies, the bond strength of 24 hour samples was greater than for 30 minutes samples. For the 30 minute control samples, there was no significant difference between the bovine deciduous and bovine permanent bond strength. When all of the 30 minute samples were combined, the bond to the deciduous samples was significantly

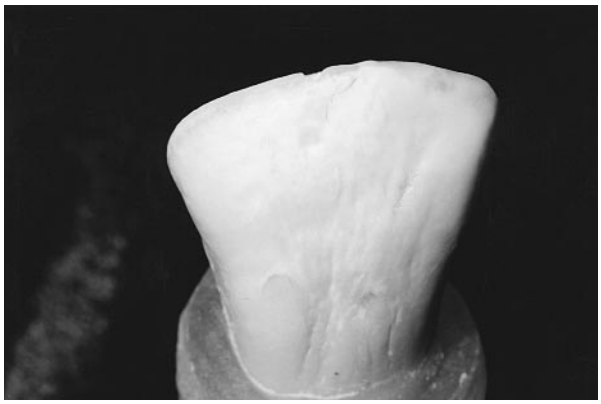

Fig. 4. Bovine permanent lower incisor shows greater surface irregularities than the deciduous bovine incisor (Fig. 5) or human incisors.

Fig. 5. Bovine deciduous incisor shows less surface irregularities than the bovine permanent incisor and is comparable to the human maxillary central incisor.

stronger than the bond to the permanent sample. This same trend was also seen in the 24 hour samples. The 24 hour controls showed no significant difference between deciduous and permanent teeth, but when all of the samples were combined, the bonds to deciduous teeth was significantly stronger than to permanent teeth. In comparing bovine deciduous and permanent samples, no significant difference was found due possibly to the smaller sample sizes in the control groups. In all cases the bovine samples were weaker than the human sample with a statistically significant difference between human and all bovine permanent teeth and marginal significance for all other comparisons (human sample and permanent bovine control sample $P = .08$; human sample and deciduous bovine control sample $P = .10$; and human sample and all 30 minute deciduous bovine samples $P = .08$). There was no statistical significant difference between the multiple rebonding groups. The ARI index was not significantly different between the groups.

DISCUSSION

The results of this study, unlike those of earlier studies,[8,9] did show a weaker bond to both deciduous and permanent bovine enamel than to human enamel. The bovine permanent tooth controls were 40% weaker than the bond to human incisors, all bovine permanent incisors were 44% weaker, bovine deciduous controls were 35% weaker, and all bovine deciduous incisors were 21% weaker than the bond to human incisors. The results of this study compare favorably with the results found by Barkmeier and Erickson.[10] The enamel bond to bovine teeth is significantly weaker than to human teeth. The Barkmeier and Erickson study did not differentiate between permanent and deciduous bovine teeth. This study shows a greater decrease in strength to permanent bovine enamel than to deciduous bovine enamel. Thus, although bovine enamel acts similar to human enamel, the strength of the bond to bovine enamel is lower than to human enamel, probably due to the differences in formation, larger crystal grains, and more lattice defects than human enamel.[8,9]

There are differences in the appearance of bovine deciduous and permanent lower incisors (Figs. 1, 4, and 5). The deciduous bovine mandibular incisors are closer in size to human maxillary central incisors and have a generally smoother labial surface. The bovine permanent incisors are dramatically larger than human incisors and have larger undulations on the labial surface than human incisors (Fig. 4). This has led some researchers to grind the labial surface to create a smoother surface.[8] The somewhat smoother labial surface of the bovine deciduous incisors may account for the slightly higher bond strength of deciduous over permanent bovine incisors. There was a significant difference between the bond strengths to deciduous and permanent bovine enamel when all groups were combined and, hence, permanent and deciduous bovine teeth should not be used interchangeably in bonding studies. If deciduous and permanent bovine teeth are both used in a study, care must be taken to distribute them evenly throughout the sample to negate the effect of the differences in bond strength.

There was not a significant difference between bovine samples that were rebonded up to five times. Although the first bonding had slightly greater bond strengths than subsequent bondings, the difference between the samples was not significant. Therefore, bovine teeth can be reused for bonding studies multiple

times with no significant degradation of the bond strength. The age of the teeth may be a significant factor, however. Nakamichi et al.[8] reported that as the teeth aged bond strengths increased. In this study one permanent bovine incisor collected 2 years earlier was added to each group. Initially it appeared that these teeth fractured more frequently than teeth that were fresher. However, after analyzing the total number of enamel fractures, there were as many fractures within the group of fresh teeth as older teeth.

Fractures occurring in testing may be more a function of very high bond strengths or weakening of the tooth structure that occurred from previous trauma or during tooth removal. The sample of older teeth was too small to statistically analyze.

CONCLUSIONS

Conclusions from this study were:

1. Although bovine lower incisors can be successfully used to study enamel bond strength, the enamel bond to bovine teeth is 21% to 44% weaker to bovine than to human enamel.
2. Deciduous and permanent bovine lower incisors are both usable for testing, however, deciduous bovine incisors have greater bond strengths than permanent bovine incisors. Either all permanent or all deciduous incisors should be used or care taken to distribute permanent and deciduous incisors evenly throughout the samples.
3. Bovine teeth can be used multiple times in bonding studies with no significant decrease in adhesive strength.

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