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Comparison of hydroxyapatite and dental enamel for testing shear bond strengths

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Aims: To investigate the feasibility of using artificial hydroxyapatite as a future biomimetic laboratory substitute for human enamel in orthodontic bond strength testing by comparing the shear bond strengths and nature of failure of brackets bonded to samples of hydroxyapatite and enamel.

Method: One hundred and fifty hydroxyapatite discs were prepared by compression at 20 tons and fired in a furnace at 1300ºC. One hundred and five enamel samples were prepared from the buccal and palatal/lingual surfaces of healthy premolars extracted for orthodontic purposes. Orthodontic brackets were bonded to each sample and these were subjected to shear bond strength testing using a custom-made jig mounted in an Instron Universal Testing Machine. The force value at bond failure was obtained, together with the nature of failure which was assessed using the Adhesive Remnant Index.

Results: The mean shear bond strength for the enamel samples was 16.62 MPa (95 per cent CI: 15.26, 17.98) and for the hydroxyapatite samples 20.83 MPa (95 per cent CI: 19.68, 21.98). The difference between the two samples was statistically significant ($p < 0.001$). When the nature of failure was assessed with the ARI Index, 83 per cent of the enamel samples scored 2 or 3, while 49 per cent of the hydroxyapatite samples scored 0 or 1.

Conclusions: Hydroxyapatite was an effective biomimetic substrate for bond strength testing with a mean shear bond strength value (20.83 MPa) at the upper end of the normal range attributed to enamel (15–20 MPa). Although the difference between the shear bond strengths for hydroxyapatite and enamel was statistically significant, hydroxyapatite could be used as an alternative to enamel for comparative laboratory studies until a closer alternative is found. This would eliminate the need for extracted teeth to be collected. However, it should be used with caution for quantitative studies where true bond strengths are to be investigated.

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Introduction

Laboratory-based orthodontic bond strength testing has traditionally used natural enamel obtained from healthy, extracted human premolar teeth,[1,2] and the shear bond strength to etched enamel has been typically reported to be within the range of 15–20 MPa.[3,4] The use of extracted human teeth has a number of potential problems. It can be difficult to obtain sufficient numbers of teeth for a satisfactory sample size. There may be variations in tooth morphology, and the fluoride content within the enamel of individual teeth. The retention of extracted teeth is subject to legislation and requires patient consent. There have also been reports of problems related to infection risk and the post-extraction storage of teeth.[5–8]

Previous research suggests that bond failure tends to occur either at the junction between the bracket base and the resin, or within the bulk of the resin itself rather than at the enamel-resin interface.[9] This suggests that the nature of the substrate may not be critical for laboratory testing. Bovine enamel has been used as an alternative to human enamel,[10,11] but has larger crystal grains and more lattice defects than human enamel.[10,12,13] Both the shear and tensile bond strength values obtained with bovine teeth have been shown to be significantly lower than those obtained with human teeth.[14] Plastic cylinders have been used to which brackets could be bonded. However, because the adhesion between the resin and the plastic cylinder was chemical in nature rather than physical through an acid-etch bond, this does

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not reflect clinical bonding to enamel. Nevertheless, the studies showed similar bond strengths to human teeth.[15,16] Other studies have used commercially available glass-ceramic as a substrate and although the surface differed strongly from human enamel, the shear bond strengths achieved were comparable.[17,18]

Despite the fact that results using a range of substrates structurally different from enamel have been broadly comparable, a review of the findings concluded that attempts should be made to develop an artificial substrate which is closer to enamel in terms of its surface features.[9] In response to this, recent research has begun to investigate the use of hydroxyapatite, $Ca_{10}(PO4)_6(OH)_2$ which constitutes the major inorganic component of enamel, and has suggested that it could represent a suitable biomimetic model for orthodontic bond strength testing.[9,19]

The aim of this study was to investigate the use of hydroxyapatite as a potential biomimetic laboratory substitute for human enamel by comparing the shear bond strengths of brackets bonded to hydroxyapatite and enamel from a sample of extracted human premolar teeth, and comparing the nature of failure of the brackets with both substrates using the Adhesive Remnant Index.[20]

The null hypothesis was that there is no difference between the mean shear bond strengths achieved with hydroxyapatite and natural enamel. Hence, a comparable artificial model for bond strength testing in the laboratory can be found.

Materials and methods

Commercially available hydroxyapatite powder Captal R (Plasma Biotal Ltd., Tideswell, UK) was sieved to a grain size of 75 microns using a Fritsch vibratory sieve shaker (Idar-Olerstein, Germany). The powder was weighed out into batches of 4 grams and then compressed in a die under a pressure of 20 tons to obtain circular discs which were then fired at 1300°C in a furnace and left to cool overnight. The cooled discs were placed in 30 mm diameter embedding moulds with lids, and SpeciFix-20 resin (Struers, Denmark) was poured over them to fill the mould to 3 mm from the edge. This was then left to cure overnight. The lid was removed and the embedded discs were pushed out of the moulds. The resin base was smoothed using a lathe, after which the discs were ground and polished using a polishing protocol of 1 minute at 200 micron grit size, 5.5 minutes at 9 micron grit size, 4.5 minutes at 3 micron grit size and finishing with 1.5 minutes at 1 micron grit sizes (RotoPol-11, Struers, Denmark). The samples were then kept aside until ready to use.

Extracted healthy premolar teeth which had been obtained with the patients' consent were stored in water until ready to be embedded. Each tooth was sliced vertically into two halves, using a high-speed handpiece with a straight fissure diamond bur, to obtain separate buccal and lingual surfaces. Small resin blocks of 25 mm were constructed. The teeth were placed on individual blocks and embedded in resin again, ensuring that the resin did not encroach onto the enamel surface. The samples were left for 24 hours to fully cure, before the excess resin was trimmed off to ensure flat surfaces.

Brackets were bonded to the enamel or hydroxyapatite samples using a standardised bonding protocol. The surface to be bonded was polished with pumice and water slurry using a rubber cup in a slow handpiece. The pumice was then washed away and the substrate surface was dried using an oil-free three-in-one syringe. A 35 per cent ortho-phosphoric acid gel (Transbond XT etchant gel, 3M Unitek, Monrovia, CA) was applied for 15 seconds and was then washed away thoroughly. The etched area was dried thoroughly for 20 seconds. Transbond XT Primer (3M Unitek, Monrovia, CA) was applied and cured for 10 seconds using a 3M light-cure unit. A small quantity of Transbond XT light-cure composite resin (3M Unitek, Monrovia, CA) was applied to the bracket base using a plastic instrument. The bracket was then placed onto the primed surface and gently pressed into place to achieve maximal base/substrate adaptation. Visual inspection ensured that the adaptation would be considered clinically satisfactory. Excess resin was removed using a probe. The resin was cured for 20 seconds using visible blue light (3M Unitek Curing Light, XL 3000, B228137). Light intensity was checked between each episode of bonding using the unit's internal light meter. The bonded samples were then incubated at 37°C for 24 hours. For the hydroxyapatite discs, brackets were bonded to each of four quadrants on a single surface such that each disc was used to test four samples. Each tooth specimen was bonded once only at the mid-point of the buccal or lingual surface. Equilibrium 0.022 × 0.030 inch slot MBT prescription stainless steel brackets were



Figure 1a



Figure 1b

Figure 1. The custom made jig with (a) hydroxyapatite and (b) tooth samples.

used (Dentaurum, Ispringen, Germany). One hundred and fifty upper left central incisor brackets with a flat base were used for bonding to the hydroxyapatite discs. One hundred and five upper first premolar brackets with a curved base were used for the tooth surfaces. The base contours of the brackets corresponded with the flat hydroxyapatite disc surface and curved enamel surface respectively in order to obtain comparable film thicknesses of adhesive.

Shear bond strength testing was performed using an Instron Universal Testing Machine (Instron Ltd, High Wycombe, UK). A metal jig was constructed in order to hold the specimen securely in the machine. The jig consisted of a metal holder with an adjustable screw to secure the specimen and an adjustable blade, which was used to shear the bracket from the substrate (Figure 1). The sample for testing was placed in the jig and positioned with the blade at the junction of the bracket and the substrate surface. The jig was secured between the jaws of the Instron, which then applied a shear force at a crosshead speed of 1 mm/minute until the bond failed. For each specimen, the maximum load at failure was recorded using a computer. The force value was obtained in kilonewtons (kN) which was then converted into megapascals (MPa) taking into account the cross-sectional surface area of the bracket bases.

Following bracket failure, the surface of each specimen was examined and the adhesive remnant index (ARI) value was assigned to it.[20]

0, no adhesive left on the tooth
1, less than half the adhesive left on the tooth
2, more than half the adhesive left on the tooth
3, all the adhesive left on the tooth with a distinct mesh pattern visible

Scanning electron micrographs were also taken of etched hydroxyapatite and enamel samples in order to compare them visually.

Results

An initial study was carried out in order to determine the best method to mount and test the samples. Once the method was finalised, a pilot study was carried out on 10 samples in each group, from which the required sample size was calculated. It was agreed with the statistical author (DRM) that means lying within a 10 per cent difference would represent equality between the samples under study. At a significance level of $p \leq 0.05$ the limited availability of extracted teeth meant that with the maximum available 105 enamel samples, the sample size calculation software package (nQuery Advisor version 5.0, Statistical Solutions, Saugus, MA) indicated that

Table I. Shear bond strength data for hydroxyapatite and enamel.

	Mean (MPa)	SD	95% CI	SE
Enamel	16.62	7.03	15.26, 17.98	0.69
Hydroxyapatite	20.83	7.12	19.68, 21.98	0.58

it was necessary to have a hydroxyapatite sample group of 150 in order to obtain a power of 85 per cent.

The results of bond strength testing are presented in Table I. The mean bond strength for enamel was 16.62 MPa (95 per cent CI: 15.26, 17.98) and for hydroxyapatite was 20.83 MPa (95 per cent CI: 19.68, 21.98). The data were found to be normally distributed and so a two-sample *t*-test was used to compare the results and assess their significance. The mean shear bond strength for hydroxyapatite was found to be significantly higher than that obtained with enamel with a mean difference of 4.21 MPa (95 per cent CI: 2.4, 6.00; $p < 0.001$).

For the ARI assessment, each sample was assessed under natural light to determine the amount of adhesive left on the substrate surface. With enamel, 83 per cent of the samples scored an ARI value of 2 or 3, while the hydroxyapatite samples had a more varied result with 49 per cent scoring 0 or 1 (Figure 2).

The fracture sites were assessed and demonstrated that 51 per cent of the enamel samples fractured at the bracket/resin interface, leaving all the resin on the tooth surface, while 3 per cent fractured at the enamel/resin interface, with the rest being within the resin itself. The hydroxyapatite samples showed variation here as well, with 23 per cent failure occurring at the substrate/resin interface and 32 per cent at the bracket/resin interface.

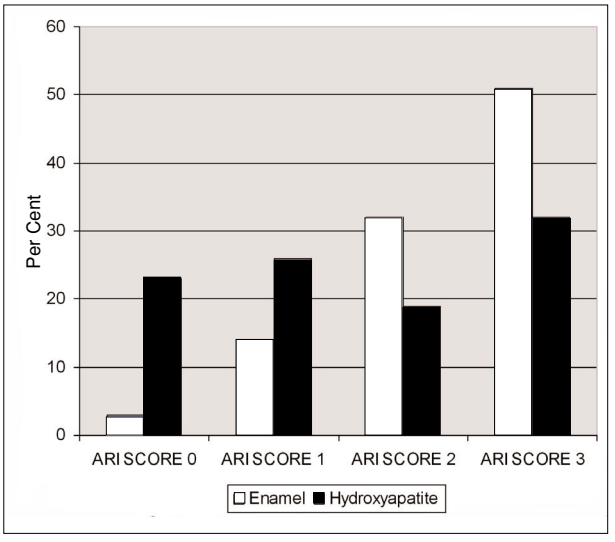


Figure 2. Percentages within each ARI score for hydroxyapatite and enamel.

Discussion

Although different brackets were used for the two substrates, they were similar in all ways except for the base contours, which varied in order to achieve good adaptation to the different substrate surfaces and so minimise variations in adhesive film thicknesses. The differences in base surface area were taken into consideration when debonding force in kilonewtons (kN) was converted to bond strength in megapascals (MPa).

The bonding technique used in this study incorporated a 15 second etch time in line with previous research which showed comparable bond strengths with etch times of 15 or 60 seconds,[2,3] and followed the proposed ideal protocol from a previous review of the available literature on bond strength testing in orthodontics.[21] The protocol suggested that a minimum sample size of 20–30 specimens is necessary in order to obtain significant results, and in this study 105 tooth samples and 150 hydroxyapatite samples were used, giving a statistical power of 85 per cent. To enable comparison with other studies, the protocol suggested that it is preferable to quote bond strength values either in MPa or N,[21] and this study presents data in MPa.

An Instron Universal Testing Machine was used at a crosshead speed of 1 mm/min. Crosshead speeds ranging from 0.1 mm/min to 5 mm/min have previously been investigated in a previous study and variation did not seem to influence the debonding force measurements or mode of failure of brackets bonded to enamel.[22] Shear bond strength testing was performed since this more closely corresponds to the nature of bond failure under the influence of occlusal forces in vivo.[9,23] In this study a custom-made jig with a sliding blade was used in order to direct the debonding force vertically and as close to the bracket/substrate interface as possible. Care was also taken to ensure that the direction of the debonding force was the same for all specimens. This was important because previous work has suggested that changing the point of force application can significantly alter the bond strength and adhesive remnant values achieved.[24]

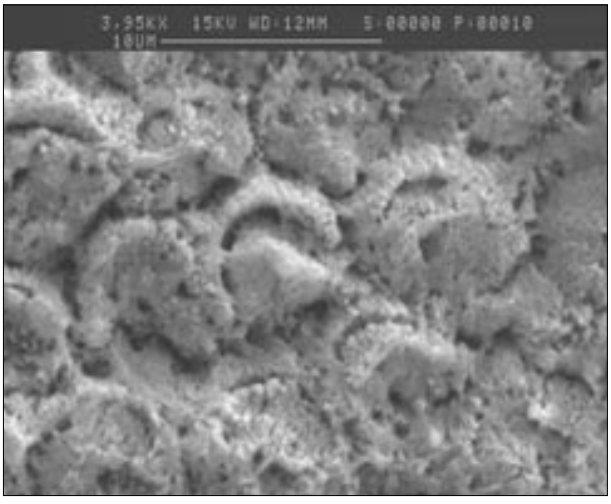


Figure 3. SEM of the etched enamel surface (4000x magnification).

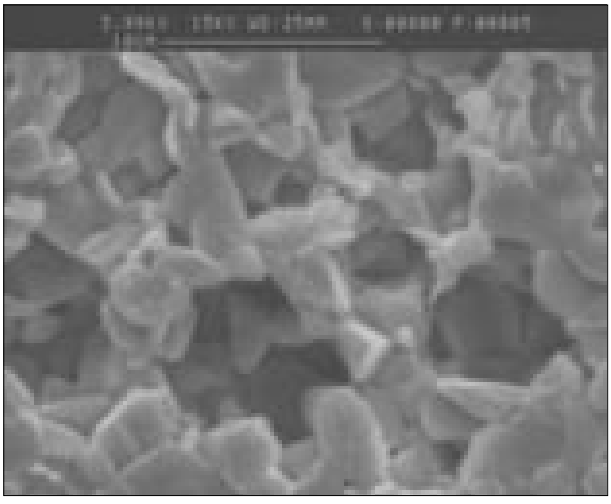


Figure 4. SEM of the etched hydroxyapatite surface (4000x magnification).

In line with all bond strength studies, it is not possible to quantify measurement error by repeating the debonding process. Rebonding results in altered bond strengths so this cannot constitute repetition of the same event.[25] However, the 95 per cent confidence intervals for both enamel and hydroxyapatite were relatively narrow, implying high precision of the estimated means.

The data were found to satisfy parametric assumptions and so a two-sample *t*-test was employed, as has previously been carried out in other studies.[23,26] Although others have recommended the use of a survival analysis such as the Weibull analysis, which they considered more appropriate for bond strength testing, such tests are generally used where the data is found to be non-parametric.[21]

The shear bond strength of resin to enamel has previously been reported to be in the range of 15–20 MPa.[3,4] In the current study, mean bond strengths of both enamel (16.62 MPa) and hydroxyapatite (20.83 MPa) were found to approximate to this range. The value obtained with the enamel specimens was at the lower end of this range, while the value obtained with hydroxyapatite was at the upper end of the range. There was a statistically significant difference in mean shear bond strengths between the two samples ($p < 0.001$). The mean shear bond strength obtained with hydroxyapatite was higher than that demonstrated in a previous study, which reported a mean shear bond strength of 9.55 MPa when using a synthetic hydroxyapatite fabricated through a similar process to this study, but fired at a lower temperature.[9] The different firing temperature may explain the difference in results.

There was a different pattern of distribution of the ARI values between the two groups. The predominant finding in the enamel group was that all, or >50 per cent of the adhesive was left on the enamel surface. This is consistent with the findings of other workers who reported that the most common failure site is at the bracket/adhesive interface.[27,28] With the hydroxyapatite samples, there was a more even distribution of failure sites, with a quarter of the samples showing no adhesive remnants on the substrate surface. This is consistent with the findings of other workers, who reported that as the bond strength increases, failure is found to occur more frequently at the enamel-adhesive interface.[29]

When considering the scanning electron micrographs, well-etched enamel surfaces were seen with both substrates following the 15 seconds etching protocol.[2,3] Although the traditional keyhole etch pattern was seen with enamel reflecting preferential loss of mineral at the prism peripheries, the hydroxyapatite etch pattern was more random. Higher magnifications of the hydroxyapatite surface showed much larger void areas of lost crystals compared to the enamel samples, which may help to explain the higher bond strength values obtained with the hydroxyapatite (Figures 3 and 4).

Conclusions

Hydroxyapatite was an effective biomimetic substrate for bond strength testing with a shear bond strength value (20.83 MPa) at the upper end of the normal range attributed to enamel (15–20 MPa). Although the difference between the shear bond strengths for hydroxyapatite and enamel was statistically significant, hydroxyapatite could be used as an alternative to enamel for comparative laboratory studies until a closer alternative is found. This would eliminate the need for extracted teeth to be collected. However, it should be used with caution for quantitative studies where true bond strengths are to be investigated.

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References

1. Buonocore MG, Matsui A, Gwinnett AJ. Penetration of resin dental materials into enamel surfaces with reference to bonding. Arch Oral Biol 1968;13:61–70.
2. Gilpatrick RO, Ross JA, Simonsen RJ. Resin-to-enamel bond strengths with various etching times. Quintessence Int 1991;22:47–9.
3. Barkmeier WW, Shaffer SE, Gwinnett AJ. Effects of 15 vs 60 second enamel acid conditioning on adhesion and morphology. Oper Dent 1986;11:111–16.
4. Gwinnett AJ. Structure and composition of enamel. Oper Dent 1992;17: Suppl 5:10–17.
5. Shaffer SE, Barkmeier WW, Gwinnett AJ. Effect of disinfection/sterilization on in-vitro enamel bonding. J Dent Educ 1985;49:658–9.
6. Tate WH, White RR. Disinfection of human teeth for educational purposes. J Dent Educ 1991;55:583–5.
7. DeWald JP. The use of extracted teeth for in vitro bonding studies: a review of infection control considerations. Dent Mater 1997;13:74–81.
8. Dominici JT, Eleazer PD, Clark SJ, Staat RH, Scheetz JP. Disinfection/sterilization of extracted teeth for dental student use. J Dent Educ 2001;65:1278–80.
9. Klocke A, Tadic D, Kahl-Nieke B, Epple M. An optimized synthetic substrate for orthodontic bond strength testing. Dent Mater 2003;19:773–8.
10. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res 1983;62: 1076–81.
11. Rueggeberg FA. Substrate for adhesion testing to tooth structure–review of the literature. Dent Mater 1991;7:2–10.
12. Moriwaki Y, Kani T, Kozatani T, Tsutsumi S, Shimode N, Yamaga R. The crystallinity change of bovine enamel during maturation. Jap J Dental Mater 1968;9:78–85.
13. Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. Am J Orthod Dentofacial Orthop 1998;114:514–19.
14. Saleh F, Taymour N. Validity of using bovine teeth as a substitute for human counterparts in adhesive tests. East Mediterr Health J 2003; 9:201–7.
15. Dickinson PT, Powers JM. Evaluation of fourteen direct-bonding orthodontic bases. Am J Orthod 1980;78:630–9.
16. Buzzitta VA, Hallgren SE, Powers JM. Bond strength of orthodontic direct-bonding cement-bracket systems as studied in vitro. Am J Orthod 1982;81:87–92.
17. Wohl T, Rekow ED. An alternative material substrate for bond strength testing. J Dent Res 1996;75:173 (Abstract no. 1244).
18. Martin SE, Wohl T, Rekow ED. Relative bond strength of orthodontic bonding materials to a non-tooth substrate. J Dent Res 1996;75:172 (Abstract no. 1240).
19. Freudenthaler JW, Tischler GK, Burstone CJ. Bond strength of fiber-reinforced composite bars for orthodontic attachment. Am J Orthod Dentofacial Orthop 2001;120:648–53.
20. Årtun J, Bergland S. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. Am J Orthod 1984;85:333–40.
21. Fox NA, McCabe JF, Buckley JG. A critique of bond strength testing in orthodontics. Br J Orthod 1994;21: 33–43.
22. Klocke A, Kahl-Nieke B. Influence of cross-head speed in orthodontic bond strength testing. Dent Mater 2005;21: 139–44.
23. MacColl GA, Rossouw PE, Titley KC, Yamin C. The relationship between bond strength and orthodontic bracket base surface area with conventional and microetched foil-mesh bases. Am J Orthod Dentofacial Orthop 1998;113: 276–81.
24. Klocke A, Kahl-Nieke B. Influence of force location in orthodontic shear bond strength testing. Dent Mater 2005;21:391–6.
25. Bishara SE, VonWald L, Laffoon JF, Warren JJ. The effect of repeated bonding on the shear bond strength of a composite resin orthodontic adhesive. Angle Orthod 2000;70:435–41.
26. Cucu M, Driessen CH, Ferreira PD. The influence of orthodontic bracket base diameter and mesh size on bond strength. SADJ 2002;57:16–20.
27. Reynolds IR, von Fraunhofer JA. Direct bonding of orthodontic attachments to teeth: the relation of adhesive bond strength to gauze mesh size. Br J Orthod 1976;3:91–5.
28. Lopez JI. Retentive shear strengths of various bonding attachment bases. Am J Orthod 1980;77:669–78.
29. Siomka LV, Powers JM. In vitro bond strength of treated direct-bonding metal bases. Am J Orthod 1985; 88:133–6.