A STUDY OF THE SUITABILITY OF PRE-MIXED SELF-ETCHING PRIMERS AS ORTHODONTIC BONDING AGENTS

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ABSTRACT

Aims

This study aimed to examine the suitability of single-component self-etching primers (1-SEP) as orthodontic bonding agents. A further aim of the investigation was to establish whether 1-SEP could be used successfully to bond brackets when the pre-cure step in the bonding protocol of 1-SEP was omitted.

Method

Four hundred and twenty human premolars were cleaned, mounted and divided into seven groups by random allocation. Pre-coated stainless-steel brackets were used. The seven protocols were: Groups 1-3: Pre-cure (PC) protocol: Xeno V+, Clearfil S3 and iBond SE were applied to enamel, light cured, brackets placed and light cured again; Groups 4-7: Co-cure (CC) protocol: the same 1-SEPs as the Pre-cure group were used, however, the light curing step prior to bracket placement was omitted. A multi-step SEP, Transbond Plus, was used as a co-cured control (group 7). Bonded specimens were subject to three experimental conditions: debonding within 30 minutes from initial bonding, thermocycling between 5 ºC and 55 ºC for 500 cycles and water storage at 37 ºC for 7 days. The teeth were debonded using a Universal Testing Machine. The amount of residual adhesive on the tooth surface was determined by visual examination under x 10 magnification.

Results

Mean shear bond strengths (SBS) of brackets bonded with Xeno V+, Clearfil S3 and iBond SE were 7.86 +/- 2.27 MPa, 5.71 +/- 2.43 MPa and 6.24 +/- 1.96 MPa respectively when

debonded within 30 minutes. After thermocycling (Xeno V+ 2.11 +/- 1.73 MPa; Clearfil S3 4.36 +/- 3.31 MPa; iBond SE 0.66 +/- 0.62 MPa) and water storage (Xeno V+ 3.10 +/- 3.08 MPa; Clearfil S3 5.16 +/- 3.43 MPa; iBond SE 4.48 +/- 1.85 MPa) the mean SBS of 1-SEP were below acceptable clinical levels. Co-curing improved bond strengths for aged specimens in all cases. Highest bond strengths were recorded for co-cured Xeno V+ (13.09 +/- 4.01 MPa) and Clearfil S3 (13.07 +/- 4.03 MPa) after water storage which were not significantly different to the control (12.68 +/- 4.74 MPa) (P<.05). Predominant failure mode for 1-SEP was at the enamel-adhesive interface, however Clearfil S3 that was co-cured left significantly more adhesive on enamel compared to adhesive that was pre-cured (P=.006).

Conclusion

Early shear bond strengths of 1-SEP within 30 minutes may be clinically acceptable. However, after ageing with thermocycling or water storage, bond strengths were unsatisfactory. Co-curing improved bond strengths, elevating the performance of Xeno V+ and Clearfil S3 after ageing, to clinically acceptable levels which may occur due to enhanced interaction between 1-SEP and enamel. Most of the adhesive remained on the bracket, although Clearfil S3 left more adhesive on the tooth surface when co-cured.

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CHAPTER ONE: INTRODUCTION

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1.1. Introduction

Effective adhesives play an integral role in the success of contemporary orthodontic dentistry (1). Direct bonding techniques are used to attach orthodontic components to the labial enamel tooth surfaces which, when coupled with an orthodontic archwire, facilitate the alignment of teeth (2). Frequent bracket failures frustrate the efficient progress of treatment, are an inconvenience both to the patient and the operator and can extend treatment times (3). Currently, there is no consensus for ideal orthodontic bond strength values although researchers have used different quantitative and analytical methods to explore this topic. Reynolds (4) (1975) suggested that the optimal tensile bond strength for brackets is between 6.0-8.0 Megapascals (MPa), which is adequate to withstand masticatory forces and most clinical orthodontic needs although, for in vitro testing, it was recommended that bond strengths may be sufficient at 5 MPa. Meanwhile, a contemporary view was reported by Littlewood (5) (2000) who stated that minimum bond strength values for orthodontics may be more appropriately determined using a suggested 5% chance of failure during clinical treatment. Extrapolations of data by Littlewood (5, 6) using the Weinbull Analysis, indicates that the bond strength of an adhesive with a 5% chance of failure should be at least 5.4 MPa.

Traditional methods for preparing teeth for orthodontic bonding involves the use of three different agents, an enamel etchant, primer and adhesive resin (7). The enamel etchant is usually phosphoric acid, a technique pioneered by Buonocore (8) to increase the retention of acrylic buttons to enamel and subsequently applied by Newman (9) to bond orthodontic attachments to teeth. Application of 37% phosphoric acid to enamel is found to cause a selective dissolution of hydroxyapatite crystal formations creating a roughened, porous

surface in readiness for a mechanical retention between the bracket adhesive and tooth (10). After etching, the teeth are washed and dried, creating a high energy surface that is receptive to wetting by resins (11, 12). By tradition, the tooth surface is then brushed with low viscosity priming and adhesive resin solutions which allows the flow of resin components into enamel micro-porosities (13). To complete the conventional orthodontic bonding procedure, a resin composite material is applied to the bracket base before positioning the bracket onto the tooth surface, where a light-cure polymerisation secures the retention of the bracket to the tooth with a mechanical bond (4). Evidently, the multiple stages of conventional acid-etch bonding practices (AE) are time-consuming to perform, thus the aim of research for many years has been to streamline the process without compromising bond reliability (14). Advances in adhesive technologies have since created innovative "self-etching" solutions that combine etchant, primer and adhesive resin into single products which require fewer procedural steps, in the development of self-etching primers (SEP) (15). The reactive components in these unified SEP materials are aqueous mixtures of esters from bivalent alcohols, alongside methacrylic acid and phosphoric acid or derivatives that dissolve and remove calcium ions from the hydroxyapatite (16). Etching and priming stages occur simultaneously (17) for SEP and there is no need to rinse the product away to remove reaction products or residual acid esters because, unlike conventional etchants, the acid in SEP is neutralised during function, and subsequent polymerisation incorporates residual products into the bonding layer (18, 19).

The chemical and procedural distinctions between "self-etch" and "total-etch" systems present a number of advantages which favour the use of SEP in preference to AE for orthodontic bonding. Clearly, a key advantage of SEP over traditional etchants remains the simplified application methods for SEP where conventional two-step etch and prime stages are combined into a single step (20). Minimum steps for application of SEP is likely to lead

to fewer procedural errors and, thus, decrease the technique sensitivity for bonding brackets using SEP compared with conventional AE systems (21). Multi-procedural steps for AE are also time-consuming, and it has been reported that a benefit of using SEP over AE is that average bonding times per tooth using SEP are significantly reduced (22, 23). Faster bonding times and reduced chair-time are attractive propositions for operators and according to Banks et al. (23) the mean bracket placement time per bracket using SEP was 75.5 seconds compared to 97.7 seconds for AE, producing a time-saving of 22.2 seconds per tooth for SEP. It is important to realise, however, that given time-savings for application of SEP are tempered by the fact that an additional pumice prophylaxis step is advised to remove the acquired dental pellicle before bonding with SEP (24). This additional pumicing step is not required for AE bonding (25) and therefore overall bonding times for both AE and SEP are likely to be similar.

Another advantage for use of SEP over conventional AE techniques is the milder etching patterns demonstrated for the majority of SEP products and, thus, the potential for SEP to be less destructive to the enamel surface (26, 27). Orthodontic bracket bonding is a temporary procedure since mechanical debonding of the bracket from the tooth surface is undertaken at the completion of fixed appliance treatment. There is keen interest, therefore, in performing procedures which are as least destructive to the enamel surface as possible. Orthodontic bonding (28), debonding (29) and clean-up of remnant adhesive from the enamel surface (30) is known to be associated with irreversible iatrogenic damage (31) and it is necessary to consider all aspects to this provisional bonding procedure when considering the superiority of one adhesive product over another. During the bonding procedure, the strong acids typically used for conventional bonding, most commonly 37 per cent phosphoric acid (pH 0.1-0.4), have been shown to cause permanent surface enamel loss and damage to the enamel surface (28), which can render the enamel more susceptible to decalcification

during and after orthodontic treatment. Furthermore, aggressive etching treatments may weaken subsurface enamel (28) and increase the risk of enamel fractures at debonding. It has been extensively reported that self-etching primers produce a more conservative etch pattern than 37 per cent phosphoric acid, which is less destructive to the enamel surface (16, 18, 19, 26, 27, 32). Cal-Neto (2006) (18) used scanning electron microscopy (SEM) to observe enamel etching depth and resin tag penetration for AE compared with relatively milder SEP (Transbond Plus, pH 1.0). Cal-Neto showed that the application of SEP produced less aggressive enamel demineralisation and shorter resin tags than the AE control group. A similar pattern was confirmed by Hannig et al. (16) who measured the thickness of the resin-infiltrated hybrid layer, at 1.5 - 3.2 micrometres (μm) for SEP and 6.9 μm for AE. Thus, the potential for SEP to promote successful orthodontic bonds which are less destructive and produce fewer irreversible changes to the enamel surface than traditional etchants may be a particular advantage for these combined systems.

Further advantage provided by using SEP over a conventional bonding process is that self-etching systems are less sensitive to moisture contamination (33). Obtaining the complete isolation necessary for traditional AE bonding using hydrophobic monomers and solvents is clinically challenging, and any possible contamination with water or saliva can compromise bracket retention (34). By contrast to AE, SEP formulations are hydrophilic and their moisture-insensitive solvents displace water from the surface to allow successful resin penetration into enamel micro-porosities in the absence of a completely dry environment (35). The hydrophilic features of SEP have been observed in bonding experiments where it was demonstrated that application of SEP can achieve high bond strengths when even contaminated with saliva (34, 36). Therefore, the moisture-tolerant characteristics of SEP provide valid everyday benefits for operators over traditional AE adhesives by reducing the

technique sensitivity of orthodontic bonding procedures which may, in turn, improve bracket failure rates.

Following bracket debonding, evaluation of failure surfaces is often performed to provide information on the manner in which the adhesive fails (37, 38). Both the magnitude and location of the adhesive remnants, namely the extent to which the cleaved adhesive material resides either on the enamel surface or on the bracket base, is an important determinant of the functionality of the material in failure (39). Comparison of the failure characteristics between brackets bonded using SEP and AE have demonstrated differences in the failure modes between these two methods (40). It has been widely reported that SEP tend to leave less adhesive on the tooth surface after bracket debonding than AE (7, 40-43) and require less extensive clean-up by the operator (44). The removal of adhesive remnants from the enamel surface after debonding is most often performed mechanically and is associated with permanent damage to enamel (45). In particular, the popular practice of using rotary instruments with tungsten carbide burs to clean adhesive remnants from the tooth surface can be harmful by removing the surface layer of enamel as well as producing enamel scratches and deeper grooves (45, 46). A certain advantage of using SEP over AE is that bonding with SEP often requires less extensive clean-up of adhesive remnants at the end of treatment which reduces chair-time and lessens the opportunity for iatrogenic damage. However, careful debonding techniques need to be employed to remove brackets which are bonded using materials that are known to fail at the adhesive-enamel interface since this mode of failure can elevate the risk of enamel fracture compared with other failure profiles that leave more adhesive on the tooth surface. The fracture point of enamel is around 14 MPa (47, 48), however, in vitro bonding experiments often have large ranges to their data (29) which makes it difficult for clinicians to judge the associated risks to enamel. Ultimately, it remains at the discretion of the clinician to consider the relative advantages and

disadvantages attributed to the failure mode of either SEP or AE when resolving which approach to use for their own bonding procedures.

Given the broad array of benefits offered by SEP in comparison to AE, there has been keen interest to scrutinise bond strengths. Many available laboratory and clinical studies have assisted investigators in understanding the efficacy of SEP as orthodontic bonding agents (20, 49, 50). The greater part of published literature for bonding brackets with SEP surrounds analysis of the performance of the popular orthodontic self-etching product, Transbond Plus Self Etching Primer (3M Unitek), of which there are extensive reports available from higher quality randomised controlled clinical trials (RCT) and systematic reviews to evaluate its effectiveness (22, 23, 51-53). The Transbond Plus SEP bonding system is a widely-available orthodontic product which presents as three individual foil compartments which contain key ingredients that remain separated from one another until the point of use. Methacrylated phosphoric acid esters and initiators are stored in one compartment whilst, in an adjacent compartment, there is contained water, fluoride complexes and stabilisers. The operator is required to press to combine the solutions of each compartment prior to application to form a unified, activated liquid capable of initiating the bond (49). Most RCTs comparing Transbond Plus SEP to traditional AE methods have reported no difference in bond failures between these two bonding modalities (22, 23, 51, 54). Strict inclusion criteria and rigorous examination of methodology for a Cochrane Systematic Review and Meta-Analysis by Hu (2013) (53), led the author to conclude that there was insufficient high-quality evidence to conclude whether or not there was a difference in bond failure rates between SEP and AE. Higher-than-acceptable risk of bias and imprecision of results were specified as major incumbrances and the report appealed for higher quality RCTs to be able to inform future systematic reviews. However, the meta-analysis for the Cochrane report incorporated five RCTs for quantitative evaluation

(22, 23, 54-56), separating the studies into two subgroups of parallel-designed RCTs (n=2) and split mouth designed RCTs (n=3), and found no evidence that the proportion of bond failures was different for SEP or AE in either subgroup. Therefore, it was acknowledged by the Cochrane report that the limited body of evidence to ascertain the superiority of one etching method over another may encourage the routine clinical use of SEP in preference to AE due to the reduced clinical steps, lower risk of salivary contamination and shorter bonding application times known for SEP (53). A similar systematic review conducted by Fleming in 2012 (52) examined the evidence comparing the clinical failure rates of brackets bonded with either SEP (Transbond Plus) or AE. Five randomised controlled trials (22, 23, 42, 51, 54) were accepted for the review and two studies were different from those selected for the Cochrane report in 2013. The meta-analysis performed by Fleming detected a slightly higher risk of bond failure with SEP compared to AE over 12 months, although the increase was statistically insignificant. In agreement with the findings of the Cochrane report, Fleming expressed a need for additional high quality RCTs to validate the hypothesis around this particular treatment intervention and, in the absence of clear evidence to favour one bonding modality over another, concluded that the choice to bond orthodontic brackets to teeth using either SEP or AE could be left to the discretion of each operator.

Dental bonding technologies continue to advance and recently the next generation of adhesives have come to the fore, borne of the desire to simplify a bonding procedure to one of ultimate ease. The complex formulations of these latest self-etching adhesives arrive pre-mixed in a single flask which, unlike Transbond Plus SEP, require no activation prior to use (57, 58). These innovative ‘all-in-one’ self-etching primer systems (1-SEP) perform simultaneous etching, priming and bonding, customary to self-etch formulations, whilst their activated solutions remain suspended in a single bottle, ready to use (59). Relatively high concentrations of acidic monomers and water initiate the self-etching character of these

1-SEP solutions, whilst hydrophilic primer and hydrophobic adhesive rein components are dissolved in solvent to suspend them in a homogeneous state (60). Whilst the 'one-step' bonding procedure for 1-SEP is undoubtedly the most convenient, the behaviour and performance of these combined adhesives is likely to be product-specific, ascribed in the main to the various proprietary blends of different acidic functional monomers and solvents (15). The character of the new 1-SEP adhesives can be defined according to levels of acidity, hydrolytic stability and notably, whether these products have the capacity for chemical interaction to tooth tissue to enhance the bond.

Regarding acidity, one-bottle self-etching primers present more broadly across the pH spectrum than for previous adhesive generations following a keen interest to develop increasingly milder formulations. Researchers have sought to categorise 1-SEP according to the acidity levels of their active solutions as 'strong' ($pH \leq 1$), 'intermediate' ($pH \sim 1.5$), 'mild' ($pH \geq 2$) and 'ultra-mild' self-etching adhesives ($pH \geq 2.5$) (61, 62). Levels of acidity for SEP are significantly correlated with the etching aggressiveness of their solutions and the subsequent interaction depth of the adhesive bond (32, 63). Studies have observed the adhesive interface of various 1-SEP adhesives of differing acidity and found that interaction depth ranges from a few hundred nanometres for 'ultra-mild' 1-SEP procedures, which can be termed 'nano-interaction' (64), up to an interaction depth of several micrometres for 'strong' 1-SEP procedures (60). Whilst the activated solution of the established Transbond Plus SEP is strongly acidic (pH 1.0), the prospective use of milder operative 1-SEP adhesives for the impermanent bonding of orthodontic brackets provides a new and appealing opportunity for a less harmful bond to enamel, provided that the thinner resin lamination produced by milder 1-SEP can generate the required bond strengths and durability suitable for orthodontic treatment.

The blended 1-SEP formulations are operative adhesives and as such, are designed to adhere to the inherently moist surfaces of dentine substrate as well as to enamel. In general, for 1-SEP, the hydrophilic ends of functional monomers encourage interaction and 'wetting' of tooth surfaces to promote the adhesive bond. However, there is some concern that excessive hydrophilicity of 1-SEP may be problematic, whereby heightened water affinity may be able to continue to attract moisture even after polymerisation (65). It is possible that the cured resin bond of these combined adhesives may act like a semi-permeable membrane which leaves the bond interface vulnerable to hydrolytic degradation over time (66). These matters are of particular interest for operative bonds to dentine whereby moisture from the dentine substrate may continue to seep into the adhesive layer and compromise the bond (67). A retained hydrophilicity in the cured adhesive material may also bear some consequence for the integrity and durability of orthodontic bonds to enamel in aqueous conditions although there is relatively little evidence exploring this topic.

Residual water droplets have also been observed in the uncured resins of selected 1-SEP adhesives after air-drying which may compromise bonding performance (68). The integration of water within 1-SEP formulas is essential for the ionisation of functional acidic monomers, however, phase separation of water molecules from solvent and monomer components during air-drying has been demonstrated by some studies (68, 69) and may be associated with polymerisation inhibition, hydrolytic instability and reduced shelf life which is of consequence to both operative and orthodontic bonding techniques alike (68).

The desired character of single bottle self-etching adhesives can be assisted through controlled titrations of other hydrophobic and hydrophilic monomers (15). The hydrophobic end of functional monomers serve to interact with methacrylate resin and composite to form the adhesive bond, whilst other adhesion-promoting hydrophobic and hydrophilic

monomers are added in varying quantities to proprietary requirements. Still, it is a delicate challenge for the manufacturers of 1-SEP to hold antagonistic hydrophilic and hydrophobic elements juxtaposed in one solution. Typically, one of the most hydrophobic functional monomers used in dental adhesives, due to its long carbon backbone, is 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP). 10-MDP is known to become more hydrophobic after polymerisation, which is useful in fortifying the adhesive layer against hydrolytic degradation over time as well as increasing bond durability. The hydrophobic nature of 10-MDP also means that it is relatively more stable in solution, which promotes shelf life. The 10-MDP monomer is also one of only a few functional monomers which can chemically adhere to tooth tissue to enhance the bond (70). The chemical interaction of 10-MDP monomer with calcium hydroxyapatite of tooth substrate generates a strong ionic bond, in which MDP-calcium salts (MDP-Ca) are laid down in organised, self-assembling nano-layers (71) which positively bond strength, durability and survival of the adhesive layer (72). As an alternative to 10-MDP, the 4-methacryloyloxyethyl trimellitic acid (4-MET) monomer is also known to bond ionically to calcium hydroxyapatite, but the bond produced by 4-MET is less powerful than that produced by 10-MDP (70). Moreover, the Ca-4-MET salt is highly soluble which renders it more unstable and prone to hydrolytic attack compared to the hydrophobic MDP-Ca salt.

Initial reports from laboratory studies examining the performance of 1-SEP for orthodontic bonding have been encouraging, and many reports from in vitro investigations have found clinically acceptable mean bond strengths for various different 1-SEP products (7, 37, 43, 58, 73-75). Scougall-Vilchis (2009) (57) observed that shear bond strengths of brackets bonded using 1-SEP, Bond Force (Tokuyama, Japan), were not significantly different from those bonded with Transbond Plus SEP. Other studies (37, 58, 74) also concluded that there were no significant differences in bond strengths of brackets bonded with either AE or 1-SEP

systems, Scotchbond Universal (3M ESPE), Xeno IV (Dentsply Caulk) or Ideal 1 (GAC Orthodontic Products). Furthermore, Clearfil S3 Bond (Kuraray, Frankfurt), One-Coat Bond (Coltène Whaledent) and Xeno formulations V and V+ (Dentsply, UK), have also used demonstrated clinically useful bond strengths during in-vitro tests (7, 43, 73, 75). While most studies have reported effective bond strengths for 1-SEP, a less compelling performance was given by AdheSE One (Ivovlar Vivadent), which produced significantly lower shear bond strengths than Transbond Plus SEP during an investigation by Bishara (76) in 2008. Also, the clinical trial conducted by House and Ireland (2006) (3) found unacceptably high bond failure rates of 72 per cent for 1-SEP, Ideal 1.

Further in vitro investigations are needed before the use of one-bottle self-etching primers, which are designed for restorative adhesive procedures, may be advocated for routine orthodontic bonding. This present study will seek new knowledge to understand the suitability, for bonding orthodontic brackets, of three commonly-available 1-SEP systems, Xeno V+, Clearfil S3 Bond and iBond SE (Heraeus Kulzer, UK), by expanding on the scope and range of testing seen in previously published works. The shear bond strength of each adhesive will be determined within half-an-hour after bonding to simulate the clinical conditions when the first archwires are ligated to freshly bonded teeth. As yet, limited data exists describing the orthodontic bond strengths of 1-SEP after thermocycling or prolonged water storage. Bishara (2003) (77) suggested that thermocycling tests should be routinely included in the laboratory experiments for new adhesives, because it is paramount to establish whether temperature variation could induce stresses which may compromise bond durability in vivo. One available study by Pithon (2010) (58) found that bond strengths for Xeno IV remained clinically acceptable for orthodontic use after thermocycling. In other restorative dentistry studies where 1-SEP was bonded to dentine, Clearfil S3 proved resilient to thermocycling effects (78, 79) ascribed to the efficient bonding capabilities of 10-MDP,

the milder acidity, homogeneity of solution and lack of phase separation demonstrated for Clearfil S3 (80, 81).

A secondary aim of this investigation is to investigate whether altering the curing technique for 1-SEP adhesives, rather employing just one rather than two light-cure applications, has any effect on bond strengths or failure profiles. In a study by Bishara (2007) (82), simultaneous co-curing of Clearfil S3 with composite after bracket placement, did not adversely affect orthodontic bond strengths. Moreover, the use of a single light cure application is already an established technique for bonding brackets using the verified orthodontic SEP, Transbond Plus, which saves considerable clinical time (54). This investigation aims to evaluate whether the simplified practice of co-curing 1-SEP and composite could be successful for bonding brackets, broadening the scope of previous research to the three 1-SEP selected for this study.

Transbond Plus SEP will be used as the control adhesive in this study. A reliable performance for Transbond Plus under the highest academic scrutiny has persuaded many operators to adopt this adhesive into their clinical practice as an excellent alternative to conventional etchants (52). Accordingly, the orthodontic SEP, Transbond Plus, which requires mixing just prior to use, is used here as the benchmark for comparison against the new single-bottle 1-SEP adhesives.

1.2. Human Dental Enamel: Physical Characteristics

1.2.1. Enamel Colour

Dental enamel is translucent and varies in colour from yellow-white to bluish-grey-white (83, 84). Enamel colour is affected by a chromatic influence from the underlying yellow dentine and by intrinsic optical properties of the enamel itself, such as the ability to reflect light (light scattering) at the tooth surface, transmit light through its tissue and to absorb and scatter light within its tissues (85). It is the opalescent quality of enamel, that is the ability to scatter shorter wavelengths of visible light, which is seen in the blue hues of incisal tips (86). Variation in mineral content and the size of hydroxyapatite (HA) crystals, the dominant crystal in enamel, affect the parameters of enamel colour (hue, lightness and chroma) (87). An ingress of fluoride ions into the developing enamel will affect the local refractive index (n), which is associated with light scattering properties inside the tissue and results in a more opaque mature enamel (85). Larger hydroxyapatite crystals also appear darker than smaller crystals, and is one of the factors responsible for age-related changes in enamel colour – becoming darker and more yellow with increasing age – based on evidence that there is a regulated increase in the mean size of enamel apatite crystals over time (87).

1.2.2. Enamel Thickness

The thickness of the enamel outer layer is between 1.0 mm and 2.5 mm on average (88). There is variation across the tooth surface, being thinnest at its border with the cementum, the cemento-enamel junction (CEJ), and increasing in thickness towards the cusp or incisal edge where functional demands are greatest (89). There is wider morphological variation in enamel thickness such as sex differences (females having greater average enamel thickness than males), population differences and trends of increasing enamel thickness along the

molar row M1 > M3 (88, 90, 91). The degree to which these differences may be attributable to environment or genetic factors is unclear, but influencing variables include the length of the enamel-dentine junction (EDJ), dentine area, population disease and the promotion of X and Y chromosomes (88, 90).

1.2.3. Enamel Hardness

Enamel is the hardest known biological tissue (92). Hardness values of enamel using indentations techniques range from approximately 270 to 360 VHN (Vickers Hardness Number) that is between the VHN values given for iron and carbon steel (93, 94). The hardness property of enamel is affected by its structural anisotropy – the property of being directionally dependent – due to the many different crystallographic orientations which exist within its structure (95). Enamel is hardest in the transverse section, parallel to the occlusal plane, providing it the ability to withstand the high forces of mastication (96).

1.2.4. Enamel Crack Resistance

Enamel displays a rising crack growth resistance with crack extension, from the tooth surface inwards towards the dentine (97). Optical micrographs of crack propagation through enamel specimens reveal two distinct zones, each with a unique morphology (98). The initial crack increment in the 'outer' enamel, closest to the tooth surface is a distinct, continuous straight path over a comparatively long distance, travelling parallel to the long axis of enamel prisms (99). Midway between tooth surface and EDJ, the crack encounters the inner 'decussated' enamel, where enamel rods are orientated obliquely to one another in alternating 'bands'. Contact with decussated enamel forces the propagating crack into bifurcations, with deflections of up to 70 degrees and twists of up to 45 degrees (97). On reaching the inner enamel, the crack encounters a steep rise in crack growth resistance with crack extension

(beyond a factor of three over less than 1.6 mm of extension) which is largely attributed to the tortuous crack path within the region of rod decussation, and subsequent energy dissipation (100). This mechanism of toughening is so effective, that crack growth is frequently arrested. Outer enamel offers the lowest resistance to crack extension along its straight prisms - the functional significance of which is to guide surface cracks inwards to prevent chipping of the exterior enamel and to direct it inwards, towards the decussated inner enamel, where it can be halted (101).

1.2.5. Enamel Ultimate Tensile Strength

Ultimate tensile strength (UTS) is defined as "the maximum stress a material can withstand while being stretched or pulled" (102). Enamel is anistropic and, therefore, values given for enamel UTS are directionally dependent (103). When a tensile stress is applied to enamel, either transversely or perpendicular to the orientation of its prisms, it requires a significantly lower load to cause fracture (11.4-11.5 MPa) when compared to the same force applied parallel to the prisms (24.7-42.2 MPa) (104). This is because only a few enamel prisms will be seen to fracture when enamel is subjected to stresses in transverse or perpendicular directions, as the fracture propagates along the weaker interprismatic substances, resulting in a relatively lower UTS value. However, when a tensile force is applied to enamel parallel to the orientation of its prisms, the prisms are forcibly separated from one another by a fracture line running vertically down the interprismatic substance, but the prisms remain largely intact individually (105). In other words, a fracture would require entire prisms to be cleaved from one another, resulting in a much higher UTS for enamel generated by parallel-directed loads (104).

1.3. Structure and Composition of Enamel

Mature enamel consists of 95-97 per cent carbonated hydroxyapatite, $Ca_5(PO_4)_3OH$, (HAP) by weight and approximately 3-5 percent organic material and water (106). The high mineral content makes it the hardest substance in the body. The basic structural elements of enamel are rods (prisms) and interrod enamel (interprismatic substance). Enamel rods are long, cylindrical or hexagonal structures, 3-6 micrometres (µm) in diameter and up to several millimetres (mm) in length (107). Each enamel rod consists of bundles of ribbon-like crystals of HAP which individually measure between 40-150 nanometres (nm) in width and 15-50 nm in thickness (108, 109). The length of crystallites was shown to be at least 100 µm, although some may extend to the full length of the rod (110). The long axis of the crystals mainly run parallel to the long axis of the enamel rod. HAP crystals consist of grouped and stacked hexagonal calcium phosphate unit cells, which initially assemble to impart a hexagonal outline to the crystal (111). Each rod contains about 40,000 HAP crystals at a density of roughly 500 crystallite cells/μm^2 (109). As the unit cells are compressed during the final part of their growth, their shape definition is somewhat lost and the resulting HAP crystal takes on a more cylindrical form (110). The intercrystallite space is occupied by soft organic matter consisting of proteins and firmly- and loosely-bound water, as well as lateral branches (bifurcations) from adjacent crystallites and areas of crystallite fusion, which help to hold crystallites together (108, 112). These intercrystallite spaces have been demonstrated to be ion-rich, with increased concentrations of sodium, carbon, hydrogen, nitrogen and magnesium (113). Magnesium ion concentrations are particularly high in these spaces (15 times that which are found within HAP crystals) and recent observations of their distribution alongside HAP crystals, also detected quantities of amorphous calcium phosphate (ACP), the precursor to enamel (113). The significance of this magnesium-rich

interphase is unclear, but it is recognised that magnesium ions play a critical part in the stabilisation of the ACP phase during amelogenesis (114).

The interrod region surrounds each rod and its HAP crystals run in a different orientation to those which make up the rods. The orientation is significantly different around three quarters of the rod circumference (115), and in these areas the rod and interrod enamel are separated by a thin shell of organic material, approximately 2 nm thick, known as the rod sheath – the main components of which are non-collageneous proteins such as amelogenins, enamelin and ameloblastin (116-118). In the small portion of the rod circumference, where the orientation of rod and interrod crystals are confluent, there is no rod sheath between them and rod crystals flare out into the interrod enamel. Cross-sectional observations of these two related components have compared their outline to that of a keyhole (119).

Examination of the hierarchical structure of enamel reveals a spatial variation in enamel types. Radial enamel contains rods which lie parallel to each other and occupies the outer enamel, whilst decussation enamel contains rods which cross each other in alternating bands and occupies the inner enamel (115, 120). It is recognised that decussation constitutes an evolutionary toughening mechanism for enamel, playing a critical role in crack resistance and arrest (100, 121).

1.4. Acquired Enamel Pellicle

The acquired enamel pellicle (AEP) is a thin organic layer, which adheres quickly to the enamel surface of teeth, following exposure to fluids of the oral cavity, to perform a critical role in oral health and prevention of disease (122). The AEP consists of salivary proteins, non-salivary proteins, carbohydrates and mucins (123-125). Mass spectrometry techniques

have characterised over three hundred and fifty different pellicle proteins embedded within its structure, which appear to be tooth- and site-specific, and the AEP may have a composition which is unique to the individual (123). Proteins are known to adsorb selectively onto the tooth surface from oral fluids including saliva, gingival crevicular fluid and blood, under the influence of complex electrostatic interactions (126-129). Formation begins immediately, and the pellicle has been shown to offer protective effects after just three minutes (122). The pellicle is inhomogeneous and, initially, a dense basal layer known as the inner pellicle is formed on the enamel surface. Subsequent pellicle layers are more loosely arranged than the basal pellicle layer, and are termed outer pellicle. Adhesion of the inner layer to enamel increases once the outer pellicle is present, in a process of maturing pellicle form and function (129).

The role of the enamel pellicle is manifold. Its physical contributions are to offer a lubricant surface to the teeth to prevent tooth wear and trauma of mucosal surfaces. Enamel pellicle thickness acts as a natural barrier, which inhibits contact between the enamel surface and dietary acids in preventing tooth erosion (130). The enamel pellicle also has biochemical capabilities to modulate demineralisation and remineralisation processes at the enamel surface, conferring protection against acidic dissolution (131). More specifically, the AEP contains calcium- and phosphate-binding proteins and peptides, which lessen the diffusion rate of phosphate and calcium from enamel into the surrounding area following an acid challenge, thus inhibiting microhardness loss (132, 133). It is also known that intact histatins, low molecular weight salivary proteins, are found in the AEP and whose major property is antifungal activity against Candida albicans. In whole saliva, cleavage products of histatin are detected, but not the intact molecule, and it may be that the adsorption of histatin to the tooth surface offers some protection against degradation (134).

1.5. Dental Plaque Biofilm

Dental plaque is a complex community of micro-organisms enmeshed in a self-produced, three-dimensional matrix of exogenous polysaccharides, proteins and DNA which adheres to the surface of teeth as a natural biofilm (135). Co-localised microcolonies of bacteria within the matrix,communicate with each other through multiple interactive networks, facilitating synergistic activity and bestowing emergent properties on the biofilm, that is properties which are greater than the sum of the component species (136). The role of dental plaque biofilm is protective, and resident microorganisms have the capacity to protect against foreign microbes and contribute to the development of the host's defences (137). However, if environmental pressures exceed biofilm defence capabilities, dental plaque will become a ready vehicle for pathogenicity and disease (138).

Transcription profiling has allowed the identification of over seven hundred bacterial species within dental plaque samples, and a functional core microbiota of nearly sixty species (139, 140). Streptococci species are dominant in the biofilm, but include a variety of other genera such as Veillonella, Campylobacter, Neisseria, Fusobacterium, Rothia, Granulicatella, Gemella and Capnocytopha. It appears that dental plaque biofilm compositions may display interpersonal variation (135). Streptococci, as well as Neisseria, Rothia and Veillonella species, are the predominant early colonisers and in the earliest phase of biofilm formation, these pioneer species adhere to the glycoprotein molecules of the acquired enamel pellicle via specific adhesion-receptor mechanisms (139, 141). Some species of oral bacteria, especially Streptococcus mutans but also Streptococcus sobrinus, possess the enzyme Glucosyltransferase (Gft) in three isoforms B, C and D, which utilise sucrose to synthesise glucan polymers, which may be water insoluble or soluble. Water-insoluble and partly-soluble glucans are generated by GftB and GftC enzymes respectively which play key

roles in creating the structural carcass of the exopolysaccharide matrix (142, 143). Water-soluble glucans, synthesised by GftD enzymes, are employed as GftB primers and facilitate a significant increase in the population of extracellular polysaccharides. The resultant 'sticky' meshwork of dental plaque provides additional binding sites for bacteria, thereby increasing the bacterial biomass, whilst glucan polymers on the surface of microbes facilitates co-adhesion between organisms (144, 145). It is established that sucrose is a major factor for development of bacterial biofilm, where it is utilised as a substrate for enzyme-derived glucan synthesis (138). Investigations into the effect of sucrose concentration on dental plaque biofilms have shown that increases in concentration of sucrose, particularly between 1-10 per cent, generate large increases in exopolysaccharide production and bacterial aggregation (146). The natural tendency for microorganisms to stick to one another, is central to biofilm development, and is fundamentally how biofilms can manifest the optimised properties of a single entity (147, 148). Cell to cell signalling occurs via the quorum-sensing system which utilises small diffusible, effector molecules called autoinducers (especially autoinducer-2 [AI-2]) to regulate diverse mechanisms involved in biofilm formation such as gene transcription and protein synthesis, as well as bacterial synthesis and autolysis (136, 149, 150). It may be that efficiency of signalling may be enhanced by co-adhesion (147, 151). The close proximity of physiologically relevant organisms also permits nutritional cooperation, food chains and horizontal gene transfer between organisms, which generate group interdependencies and confer resistance and stability to the biofilm, against environmental disturbance (141), (152). Expansion of the maturing dental plaque biofilm gradually restricts oxygen diffusion into its colonies and anaerobic species begin to proliferate (153). At the same time, consumed fermentable carbohydrates, such as sucrose or starch, are metabolised by plaque microbes and produce organic acids which lower the pH of the biofilm (154). Protective buffering mechanisms within the biofilm respond to acidic change by synthesising base, via the arginolytic and

ureolytic activity of plaque bacteria such as Actinomyces naeslundii and Staphylococcus epidermidis (136, 155). Where consumption of sucrose and/or starch is frequent and/or excessive, the dental biofilm is subject to prolonged periods at a lower pH which, below a critical pH value, allows lactic acid to penetrate enamel between hydroxyapatite crystals amongst the aqueous phases, dissolving calcium and phosphate ions to cause a loss of surface hardness and eventual cavitation (138).

1.6. Chemical Composition of Self-Etch Adhesives: Resin Components

The main characteristic of adhesives can be considered its monomers.

1.6.1. Methacrylic Acid (MA)

Methacrylic acid (MA) is a colourless, viscous liquid which is soluble in warm water and miscible with most organic solvents (156). One of the oldest monomers in production, MA is often created industrially as a precursor to its esters, especially methyl methacrylate (MMA) and polymethylmethacrylate (PMMA). Methacrylic acid is strongly acidic, corrosive and will rapidly penetrate through gloves to elicit allergic reactions on the skin (157). It is now very sporadically used in restorative composites, however the hydrolysis of ester groups in other more commonly included monomer constituents, means that MA is probably still present to some degree in most adhesive resins. Hydrolysis of methacrylate monomers is typically a concern in 1-SEP adhesives which are acidic aqueous solutions (158).

1.6.2. Methyl Methacrylate (MMA)

Methyl methacrylate (MMA) is the colourless methyl ester of MA (156). Like MA, the small molecular dimension of MMA has the potential to induce skin sensitisation in susceptible individuals and has been banned for use in cosmetics for this reason (159). MMA is still used in dental adhesives, but its function is restricted to the dissolution of other monomers (17). The principal application of MMA is the industrial manufacture of polymethyl methacrylate acrylic plastics (PMMA) (156).

1.6.3. 2-Hydroxyethyl Methacrylate (HEMA)

2-Hydroxyethyl methacrylate (HEMA) is a monomer based on MA, and is in widespread use in adhesive solutions (160). Liquids containing hydrophobic and hydrophilic components can be stabilised using HEMA, which allows the constituents to be kept in solution (68). A foremost attribute of HEMA is its hydrophilicity (161). HEMA has good solvability, and can even evaporate in small amounts from adhesive solutions. The inclusion of HEMA to an adhesive solution containing water and/or alcohol, will lower the water vapour pressure and therefore, high amounts of HEMA can hinder the evaporation of the solvent (162). The hydrophilic nature of HEMA means that it is often included in adhesives as an adhesion-promoting monomer for dentine bonding. HEMA enhances the wetting of dentine and its inclusion to adhesives resin products significantly improves dentine bond strengths (163). Either cured or uncured, HEMA will also readily absorb water (164). Fixed after polymerisation, HEMA still shows hydrophilic properties, remaining vulnerable to water contamination and consequential expansion and discolouration (165). High quantities of HEMA also introduces flexibility to the polymer and inferior strength, and the concentration of HEMA in adhesives need to be moderated to preserve mechanical

properties (62). The uncured monomer is notoriously allergenic, but the cured polymer is widely used in medical applications and may be considered relatively biocompatible (166).

1.6.4. 4-Methacryloyloxyethyl Trimellitic Acid (4-MET)

4-Methacryloyloxyethyl trimellitic acid (4-MET) is a functional acidic monomer with adhesion-promoting properties (167). The 4-MET monomer has the capacity to demineralise the enamel surface, due to its two carboxylic groups, which provide acidic and thus demineralising properties, as well as promoting monomer infiltration and adhesion (168). It has been documented that addition of 4-MET improves the bond to enamel and dentine (169). The two carboxylic groups in 4-MET are attached to an aromatic group which is hydrophobic and tempers the hydrophilicity of the 4-MET molecule (170). Thus, 4-MET is hard to dissolve in water, but is still somewhat solvable in ethanol and very solvable in acetone. Yet, if ethanol is used as a solvent particularly in acidic conditions, esterification can occur, and so the use of ethanol as a solvent for 4-MET is not recommended (68). 4-MET is readily available in its anhydride form as a crystalline powder, 4-methacryloyloxyethyl trimellitate anhydride (4-META) which can be hydrolysed to the 4-MET monomer with the addition of water (168). 4-MET is also commonly used with MMA to produce the tri-n-butyl borane (4-META/MMA-TBB) adhesive (163). Some functional acidic monomers, including 4-MET, can chemically interact with the tooth surface. 4-MET will bond ionically to calcium hydroxyapatite to create Ca-4MET salt (171). The ionic bond formed by 4-MET to hydroxyapatite is less intense than that formed by other monomers like 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), and the Ca-4MET salt is highly soluble and, therefore, also quite unstable (172).

1.6.5. 10-Methacryloyloxydecyl Dihydrogen Phosphate (10-MDP)

10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP) is another functional acidic monomer capable of etching the tooth surface (171). Developed and patented by Kuraray (Osaka, Japan) (now expired), the 10-MDP monomer was initially synthesised for Panavia cement which was clinically adept in adhesion to metals and ceramics as well as tooth tissue (173). The in vitro and clinical success of 10-MDP, led to its inclusion in virtually all subsequent Kuraray adhesives (174, 175). The dihydrogen phosphate group, a hydrophilic polar phosphate group, will dissociate in water to create two protons which will instigate a mechanical and strong chemical bond to tooth tissue, metals and zirconia (176). The strong ionic bonds formed with the calcium of hydroxyapatite at tooth surfaces, produces MDP-calcium salts which are deposited as nano-layers (177). This layered structure is approximately 4 nm in depth and contains two 10-MDP molecules that have opposing methacrylate groups which face one another, and their hydrogen phosphate groups which face outwards, away from one other (70, 178). Deposition of calcium salts occurs between the layers. This type of self-assembled layering is a distinctive feature of 10-MDP and is not seen in other functional monomers such as 4-MET. Agitation of the 10-MDP monomer on enamel intensifies this nano-layering, and could be one of the reasons why this type of active application technique increases bond strengths (179). The 10-MDP-Ca-salt also has low solubility in its own solution, which gives stability and durability to the ionic bond (70). Moreover, 10-MDP has a long carbonyl chain backbone which renders the monomer hydrophobic. In fact, 10-MDP is the most hydrophobic functional monomer commonly used in dental adhesives and therefore, acetone or ethanol are appropriate solvents (180). The hydrophobicity of 10-MDP is likely to favourably influence durability, as the monomer will discourage water sorption even after polymerisation, and impart a hydrolytic resilience in the cured polymer (181-183).

1.6.6. 2-Hydroxyethyl Methacrylate Phosphate (HEMA-Phosphate)

In aqueous solutions, 2-hydroxyethyl methacrylate phosphate (HEMA-phosphate) and di-HEMA-phosphate will dissociate into HEMA and phosphoric acid (60). HEMA-phosphate and di-HEMA-phosphate monomers are hydrolytically unstable functional monomers, and the creation of phosphoric acid molecules means their adhesive solutions may be typically quite acidic (184). Water will, therefore, act a medium for ionisation and activation of HEMA-phosphate and di-HEMA-phosphate monomers.

1.6.7. Di-Methacrylates

The most frequently used di-methacrylate monomers in self-etching adhesive solutions are bisphenol A-glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA) (17). The role of di-methacrylate monomers in adhesive solutions is to provide mechanical strength, through the formation of dense, cross-linking polymers and are, thus, termed cross-linking monomers. Bis-GMA or 'Bowen's resin' is named after its creator who, in 1956, synthesised the hybrid monomer by attaching methylmethacrylate groups to epoxy monomer. Bis-GMA is a large, high-molecular weight molecule with high viscosity and maintains several attributes which account for its popularity as a cross-linker in contemporary composites, as well as adhesive resins (15). High molecular weight provides the Bis-GMA monomer with rapid hardening and low polymerisation shrinkage (around one third that of MMA) to impart superior mechanical qualities to the final polymer (185). The Bis-GMA monomer is quite rigid, due to the two aromatic rings within its spacer and methacrylate groups can struggle to locate a corresponding group for polymerisation, which can restrict conversion rates (17). Lower viscosity monomers like MMA, ethylene glycol dimethacrylate (EDMA) and most commonly TEGDMA, are frequently added to Bis-GMA as diluents, optimising the

viscosity of adhesive systems for clinical application and increasing conversion rates (186). The addition of TEGDMA results in greater tensile, but lower flexural, strength of the subsequent polymer (187). UDMA is also commonly found in contemporary resins, and although its molecular weight is comparable to Bis-GMA, UDMA has lower viscosity and greater flexibility, thus allowing easier rotation of methacrylate groups during polymerisation (185). As a group, dimethacrylate monomers are usually characterised by hydrophobic behaviour especially in comparison to mono-methacrylate monomers. Therefore, dimethacrylates are limitedly solvable in water and less vulnerable to water contamination over time. Nevertheless, the polarity of hydroxyl groups and ether-links make some water sorption inevitable. The greatest water sorption is expected to occur for TEGDMA followed by Bis-GMA, and the least water uptake is suggested for UDMA (185, 188). Concerns have been raised about the potential cytotoxicity and oestrogenic activity of these monomers, particularly Bis-GMA. It is speculated that Bis-GMA can be metabolised to Bisphenol A, a compound with known oestrogenic activity (189).

1.7. Initiators

Initiators are compounds whose molecules can generate free radicals with unpaired electrons under certain conditions (190). In adhesive systems, initiators are incorporated so that free radicals may be released for the purpose of initiating a polymerisation reaction (191). A variety of stimuli are capable of inducing free radical formation from initiator molecules. Most commonly for dental adhesives, initiation of free radicals is by light irradiation but chemical initiation (redox initiation or 'self-cure') is commonplace in dental resin cements, where the monomers may be inaccessible to light (192). There may also be a combination of these two methods in some adhesive systems, where the polymerisation technique would be referred to as 'dual cured' (193). Generally, the molecules of initiator compounds possess

atomic bonds with low dissociation energy which can readily generate the necessary radicals with their unpaired electrons. Only small amounts of initiator are required in adhesive solutions to induce polymerisation, around 0.1-1.0 per cent weight and these compounds are consumed during the polymerisation process (194).

1.7.1. Photo-activation

Photo-initiators absorb photons from light irradiation which 'excites' its molecular structure as a first step in the process of dissociating into free radicals (195). This type of photoinitiation activation occurs at specific wavelengths, which should correspond to the spectrum of a dental light curing unit (196). Photo-initiators absorb the electromagnetic energy inherent in radiation to promote their electrons into higher orbital 'excitation'. At this point, the radiation can either induce homolytic bond-cleavage in the initiator molecule to produce two free radicals (photofragmentation), or the excited electrons will interact with a co-initiator to generate the free radicals by electron transfer (190, 191).

1.7.2. Co-initiator Systems/Camphorquinone (CQ)

Camphorquinone (2,3-bornanedione, CQ) belongs to the aliphatic α-diketones and is among the most popular visible light photoinitiators (197). At room temperature CQ is a powder, with only limited solubility in water (198). CQ compound absorbs over a wide spectrum (360-510, peak 474 nm) which is one of its most useful properties (190). The polarity of solvent used to dissolve CQ can affect its absorption peak. Dissolution of CQ in water will lower the absorption spectrum towards 457 nm, whereas in solvents with less polarity e.g. TEGDMA, CQ maintains a higher absorption peak at around 474 nm (198). CQ has a strong yellow-brown colour which fades partially after curing but can, nevertheless, complicate the fabrication of high aesthetic tooth-coloured restorations. Even in miniscule

amounts (0.03- 0.1 per cent) CQ will have a significant impact on the colour of the final adhesive (199).

Camphorquinone can initiate polymerisation, however, only at an insufficiently low rate. A co-initiator e.g. tertiary amine is, therefore, required with CQ systems to accelerate the process and improve polymerisation efficiency (200, 201). After blue light irradiation, an excited-state CQ interacts with the tertiary amine molecule to abstract a hydrogen atom, yielding aminoalkyl radicals by electron-proton transfer. These radicals are then able to initiate polymerisation with methacrylate monomer units (202). The trouble with using amines as co-initiators, is that amines are bases and in acidic monomer solutions, such as 1-SEP systems, they can give rise to an acid-base reaction. A progressive deactivation of amines in acidic solutions can occur, decreasing the available amine to form free radicals (15, 203). The addition of anionic resins may be helpful in overcoming this incompatibility issue (204). Degraded tertiary amines are also notorious for causing discolouration, and their doses must be considered carefully (199). More recently, a derivative of CQ, carboxylated-camphorquinone (CQCOOH), with an amine co-initiator and accelerator has been proposed as a water soluble, visible light photoinitiating system (205). This compound, CQCOOH, has revealed significantly higher photoreactivity compared to CQ when using the same amine co-initiator system, proven by a quick formation of crosslinked polymer, higher crosslinking density, higher double bond conversion and superior mechanical properties (higher storage modulus) (205). Importantly, CQCOOH may demonstrate good biocompatibility (206). The improved water solubility of CQCOOH provides an important distinction from CQ, which is only limitedly solvable in water, and may present an opportunity for this compound to supersede conventional CQ in aqueous adhesive systems (205).

1.7.3. 1-Phenyl-1,2-Propanedione (PPD)

Photoinitiators such as the more recent 1-phenyl-1,2-propanedione (PPD) has a much less intense yellow colour compared to CQ and has been introduced in response to the growing demand for more aesthetic adhesive systems (207). PPD is more compatible with resins than CQ since it presents as a viscous fluid at room temperature (195). PPD has peak absorbance near 400 nm and yields radicals by cleavage, as well as amine co-initiator proton transfer. The photosensitisation efficiency of CQ and PPD are comparable but PPD yields better mechanical strength. When used in combination, CQ and PPD demonstrate synergistic effects (195).

1.8. Inhibitors

Inhibitors are antioxidant compounds which are added to dental adhesives to scavenge prematurely formed free radicals (191). Particularly in severe conditions, such as the higher temperatures which adhesives may experience in transit or storage, initiators may spontaneously dissociate into free radical fragments. Inhibitors will suppress small quantities of emerging radicals, thus preventing spontaneous instigation of free radical polymerisation and promoting shelf life (208). The impact that inhibitors have on intentional photoinduced polymerisation is insignificant, as the quantities of inhibitors are very low and the exponential rise in the number of radicals formed during the final polymerisation reaction easily outweighs inhibitor capability (191). Great quantities of inhibitors will, however, affect the cure rate and, consequently, the levels of inhibitors in dental adhesives are controlled (209). Established examples of inhibitors in dental adhesives are butylhydroxytoluene (BHT) or monomethyl ether hydroquinone (MEHQ). MEHQ may be selected for hydrophilic systems but BHT is hydrophobic and is commonly found in

restorative composite or hydrophobic resins (210). The biocompatibility of inhibitors is still under scrutiny as they have been demonstrated to leach from cured polymer complexes, and cytotoxic and mutagenic behaviours of inhibitor molecules have been documented (211-213).

1.9. Solvents

Solvents are added to adhesives to dissolve monomers and other solutes, and to lower the viscosity of the resins, for an improved diffusion into the micro-porosities of etched enamel (214). Upon contact between a solvent and the solid or liquid solute, a mixing of the two substances occurs, whereby the molecules of the solvent arrange around the molecules of the solute. In addition to mixing, substances in a solution interact with each other at a molecular level. Bonds between the molecules of the solute are broken and particles of the solute are allowed to form new bonds with solvent molecules. The reaction between solute and solvent is not a chemical reaction, but rather a type of complex-formation, which does not cause any chemical configuration changes in the participating molecules (15). Solubility characteristics are determined by polarity, that is, the difference in electronegativity between bonded atoms, as indicated by the dipole moment and dielectric constant. The dipole moment is a measure of the electric polarity of a molecule, based on the geometry of the molecule and the distance between charged atoms within that molecule; the dielectric constant, in comparison, is an indication of the polarity of a substance, by measuring the resistance it provides towards the flow of electric charge through its matter (permittivity) in comparison to the permittivity of a vacuum (214). Solvents with a dielectric constant greater than 15 may be considered polar. The strong polarity of water, for example, is indicated by its high dielectric constant of 88 at 0 °C (215). Non-polar or weakly polar organic solvents such as hexane, have a low dipole moment and low electric constant and dissolve non-polar

and weakly polar substances, such as oils and waxes. Highly polar solvents such as water, have higher dipole moments and higher dielectric constants, and will dissolve highly polar compounds, such as sugar or table salt (216). In broad terms, "like dissolves like". Polar solvents can be further divided into protic and aprotic, based on their hydrogen bonding capacity. Polar protic solvents (e.g. water, ethanol) solvate anions (negatively-charged solutes) by donating a hydrogen atom from their hydroxyl group (O-H) to form strong hydrogen bonds with solute molecules. Aprotic solvents (e.g. acetone), by comparison, do not have a hydroxyl group and therefore are not hydrogen bond donors, but may accept hydrogen atoms from positively charged compounds upon solvation (215). Water, acetone and ethanol are the most commonly-used solvents in adhesives (15). In self-etch adhesives, water is also essential as an ionising medium for monomers (217). Other solvents include lower viscosity monomers, such as MMA and HEMA, which are used as diluents for higher viscosity monomers (218).

The vapour pressure and boiling point of a solvent are important characteristics to understand when considering the nature of an adhesive system, because of the desire to evaporate solvent from the solution prior to photopolymerisation (219, 220). Manufacturers specify an air-drying stage following application to the tooth, the purpose of which is to evaporate solvent from the adhesive and render the adhesive layer uniform and even. Complete evaporation of solvent is quite difficult, because as the water evaporates, the monomer to water ratio increases and lowers the vapour pressure of water, thus rendering it difficult to evaporate the final quantities of solvent (15, 220). Residual water and solvents in the adhesive, prior to photo-polymerisation, are thought to produce localised areas of incomplete monomer polymerisation which can generate voids in the cured material. The presence of voids in polymerised resin can impact the mechanical properties of an adhesive and affect the integrity of the bond (221, 222). It may also be, that areas of deficiency at the bond

interface increase the porosity of the cured material, allowing greater diffusion of oral fluids into the adhesive over time (223, 224).

1.9.1. Water

Water is responsible for the ionisation of monomers in self-etch adhesives and is an essential ingredient in these adhesives (217). However, organic monomers are poorly solvable in water because of their general hydrophobic nature, and a secondary co-solvent in self-etching systems is often deemed necessary. The complete and necessary evaporation of water from adhesive systems prior to photo-polymerisation, is particularly difficult to achieve due to the high boiling point and low vapour pressure of water (220). In addition to water, low viscosity monomer HEMA is commonly used as a co-solvent in self-etch adhesives to facilitate dissolution of more viscous monomers. However, the presence of HEMA as a co-solvent, lowers the vapour pressure of water ever further, which renders the complete evaporation of water even more challenging (162). Water is a strongly polar solvent and miscible with highly polar compounds. Proficient at donating hydrogen atoms, water can form strong hydrogen bonds with negatively-charged solute molecules upon solvation (215).

1.9.2. Ethanol

Ethanol can dissolve both polar and non-polar molecules and is thus a useful co-solvent, often to water, in adhesives (216). The ethanol molecule is able to form hydrogen bonds with polar solutes via its hydroxyl (OH) group and will attract non-polar molecules by means of its non-polar ethyl (C_2H_5) group. Hydrogen bonding also occurs between the water and ethanol molecules to create a positive azeotrope, whereby the boiling point of the solvent/co-solvent mixture is lowered to 78.2 °C, which is below the boiling points of either

of its constituent parts (ethanol 78.4 °C; water 100 °C) (214). The lower boiling point increases the vapour pressure and volatility of the liquid which, in turn, facilitates evaporation upon air-drying. Thus, the addition of ethanol as co-solvent to water in self-etch adhesives, aids the removal of water (and ethanol) from adhesives during the air-drying step (220). It should be noted that ethanol is not a suitable solvent for monomers containing carboxylic acids because in the presence of alcohol, the carboxylic acids will esterify and a deactivation of the monomer will occur (216).

1.9.3. Acetone

The very high vapour pressure of acetone, around four times that of ethanol, allows excellent evaporation from solution with air-drying (216). Like ethanol, acetone will also interact with water molecules to form an azeotrope, which facilitates a rapid and effective removal of water from solution when air-dried, and describes the renowned water-chasing abilities of acetone. Also, the high dipole moment and relatively low dielectric constant of acetone enables dissolution of both polar and non-polar solutes. This makes acetone a useful solvent for types of adhesives such as SEP, which contain both hydrophilic and hydrophobic molecules (17).

1.10. Filler

Adhesive resins which contain filler particles and are referred to as ‘filled’ resins compared to ‘unfilled’ resins (225). Nano-sized filler particles, typically 7 nm and smaller, are included in dental adhesives to develop the material properties of unfilled resin, which has a relatively low tensile strength and elastic modulus compared to its filled restorative counterparts (226). Commonly found filler materials include silicon dioxide, zirconia, hydroxyapatite, ytterbium trifluoride and tantalum oxide (227-231).

Filler particles may be used as thickening agents for resins since overly-thin adhesive layers, particularly after air-drying, are at risk of incomplete polymerisation due to oxygen inhibition (232). Thicker polymerised resin layers are also more able to resist the contraction stresses of filled restorative composites, compared to thinner adhesive layers, due to their inherently greater elasticity (232, 233). Another feature of fillers is their usefulness to provide radio-opacity to the resin, which facilitates the diagnosis of recurrent caries beneath restoration and enables good definition of root canal systems (234-236). Filler particles with radio-opaque properties include calcium tungstate, ytterbium trifluoride or silicate glass which contain heavy metal ions like barium or strontium. Ytterbium trifluoride is among the filler particles which also act as fluoride-releasing agents (237).

Agglomeration of filler particles remains a challenge for adhesives due to the high surface charge of particles. Phase separation, impaired conversion and voids in the polymeric matrix can all act to reduce bond strengths because of particle clustering (238). Silanisation of filler particles is an approach frequently seen in efforts to reduce agglomeration, since the creation of a chemical bond between filler particles and resin matrix, promotes resin-filler coupling rather than particle-particle interactions (239).

1.11. Glutaraldehyde

Glutaraldehyde is included in the formulations of self-etching primers as an anti-bacterial agent, the main aim of which is to prevent caries beneath composite restorations or around the base of orthodontic brackets (240). The glutaraldehyde complex can also occlude dentine tubules upon application and, hence, other indications for its use are as a desensitising treatment for hypersensitive roots or for reducing post-operative pain after

dental restoration placement (241-243). Glutaraldehyde may also be added to self-etch adhesives in an effort to increase the durability of dentine bonds by stabilising the collagen fibres in the hybrid layer (244).

1.12. Polymerisation Kinetics

1.12.1. Free Radical Polymerisation – Monomer to Polymer

The formation of polymer from monomer in adhesives is via free radical polymerisation which is a type of chain growth polymerisation through which no by-products are formed, and is termed 'addition polymerisation' (190). The well-documented stages of this type of polymerisation are known as initiation, propagation and termination (191). Following exposure of the photo-initiator to radiation, free radicals bear their unpaired electrons and are referred to as 'initiator fragments'. The unpaired electron of initiator fragments attacks the double bond of a monomer unit, pairing with one of the electrons from that double bond to create, instead, new bonds between carbon atoms of the monomer and the initiator fragment (245). The new initiator-monomer chemical bond, leaves one electron now unpaired, which proceeds to associate itself with the carbon atom which is not bonded to the initiator fragment, turning the entire molecule into another free radical. This begins the polymer chain and is the initiation phase of the polymerisation reaction. The monomer-free radical is then free to attack, and subsequently add itself to another monomer unit, thus generating a polymer chain. This self-perpetuating chain growth defines the propagation stage (190). A variety of systems can terminate chain propagation. The simplest method of chain termination is for two polymeric free radical chain ends to meet each other (194). Other methods include a more complex reaction referred to as 'disproportion', whereby two growing polymer chains exchange a hydrogen atom, resulting in two terminated chains, one

saturated and one with a terminal double bond (191). Interaction of free radical chain ends with impurities or inhibitors will also stop chain propagation (208).

1.12.2. Degree of Conversion

The conversion of monomer to polymer during polymerisation observes a transition from carbon double bonds of monomer to a network of single carbon bonds in the polymer, and is known as the degree of conversion, DC (246). The DC of a photoactivating adhesive resin is affected by a number of factors including light irradiation intensity and exposure time, adhesive type including monomeric systems, initiator and solvent concentrations as well as the amount and type of particles (196, 247, 248). The level of conversion achieved during polymerisation has a critical impact on the material properties of adhesive resins. Previous investigations have demonstrated a direct relationship between the DC of a resin and its hardness, wear and tensile and compressive strength (249, 250). Ideally, the resin adhesive would have all monomers converted to polymer, but it is apparent that di-methacrylate monomers show substantial residual unsaturation ranging from 25-45 per cent (251-254). During the polymerisation process, unconverted methacrylate groups are entrapped within the polymer matrix by a rapid increase in viscosity (250). Unreacted species may exist within the polymer as residual monomers, or alternatively as pendant side chains that extend out from the principle polymer chain, following reaction at just one end of the molecule (255). Unconverted methacrylate species within polymer materials can function as a plasticising agent to the polymer and critically reduce the properties of the adhesive. It has been shown that there is a direct correlation between the DC of a resin and shear bond strength (256-258). The plasticising effect of residual monomer may diminish with a gradual

leaching of monomer from the composite resin into the mouth, but pendant side chains have been considered permanent plasticisers (255).

1.12.3. Polymerisation Shrinkage

The polymerisation of photo-curable methacrylate dental resin adhesives and composites is accompanied by shrinkage (255). Proceeding from monomer to polymer during the polymerisation process, most shrinkage in mono-methacrylates is the result of the conversion of carbon double bonds to single bonds, where the larger van der Waals intermolecular spaces are substituted for smaller intra-molecular covalent bonds. The result is a molecular densification in the material which is correlated to volumetric shrinkage (259, 260). Contraction stress is generated in composite resin during polymerisation, the magnitude of which is governed by the complex interaction of numerous material and environmental factors, including the amount of volumetric shrinkage, the viscoelastic behaviour of the material and its flow capacity (260-262). In general, greater volumetric shrinkage in methacrylates expedites the acquisition of elastic behaviours during polymerisation, which often increases the contraction stress (261). Other factors which influence the magnitude of shrinkage stress include the quantity and type of monomer, monomer molecule size, quantity of inert material (filler), DC, irradiation exposure time and light intensity, composite layer thickness, c-factor (the ratio of unbonded to bonded surface areas at the adhesive interface) and the degree of compliance within proximate structures (263).

Contraction forces generate stress along bonded surfaces and may cause cohesive or adhesive fractures leading to microcracks within the material (cohesive failure) and/or

interfacial gap formation (adhesive failure). If a void exists between the adhesive and tooth surface at the margin of orthodontic brackets, this could lead to microleakage and ingress of cariogenic bacteria (264). Such voids are responsible for some cases of white spot decalcification lesions in orthodontic patients (265). Propositions to eliminate or reduce polymerisation shrinkage have included incremental curing protocols or the use of "soft start" irradiation methods (266). In recent years, the most innovative attempt at compensating polymerisation shrinkage has been the synthesis of monomers that expand upon polymerisation for use in "low shrinkage" composites (267).

1.12.4. Post-cure Polymerisation

The polymerisation of light-activated resin adhesives and composites continues even after light irradiation has ceased (250). A strong relationship exists between the progressive post-polymerisation phenomenon and attendant network formation, shrinkage stress development and intensification of bond strength across interfacial surfaces (255, 268, 269). Post-polymerisation may be considered a function of the mobility of reactive species within the polymer. Unreacted molecules containing free radicals require the freedom to move and connect with other reactive species in the polymer. Following photo-activation, free radicals and molecules containing double bonds that have not reacted, remain entrapped in the polymer network without the ability to flow (270, 271). Some 'free spaces' exist within the matrix following polymerisation. After the exothermic reaction of polymerisation, the composite reduces in temperature, to that of the environment, to facilitate a relaxation process and reduction in 'free spaces'. The smaller 'free spaces' in the cooling composite material may lead to extemporaneous connection of residual double bonds to free radicals, which could react and generate a rise in conversion degree over time (271).

A direct correlation exists between the post-polymerisation level of conversion of an adhesive and shear bond strength, with significant increases in bond strength of composite adhesives after 24 hours (269, 272). An awareness of the emergent properties of cured composite materials can be useful to guide appropriate levels of force in the earliest stages of orthodontic and prevent premature debonding of brackets. High initial forces at the bracket-adhesive interface may overpower the preliminary bond and debond the bracket (273). Modification of force intensity can avoid these untimely and inconvenient failures, pending a maturation of bond strength.

1.13. Orthodontic Brackets – General Characteristics

Orthodontic brackets are passive components bonded on to the labial (or lingual) enamel surfaces of teeth which, when coupled with a wire, facilitate the orthodontic alignment of teeth (274). Orthodontic brackets may be narrow mesio-distally with a vertical slot (ribbon arch brackets e.g. Begg bracket) or wider mesio-distally with a horizontal slot (edgewise brackets e.g. Straight-Wire bracket). Edgewise brackets are by far the more commonly used brackets in contemporary clinical practice, and are generally preadjusted to deliver specific degrees of in-out, tip and torque to individual teeth, known as the 'bracket prescription' (275). Once the brackets are bonded to the teeth, a starting wire (commonly a round diameter nickel-titanium wire) is placed into the bracket slot and held into position using elastomeric modules or by a closing 'gate mechanism,' in the case of self-ligating brackets. The ligation process is complete when all the teeth intended for alignment are attached to the wire. Observation of the nickel-titanium wire at this point will reveal varying points of distortion along its length, dependent on the level of tooth malalignment within the dentition. Over the coming months, during the initial stage of orthodontic treatment, the distorted aligning wire recovers its shape utilising its inherent shape memory capability, bringing the teeth

along with it into alignment (274, 276, 277). Thereafter, the orthodontic appliance is progressed through further stages where wires of increasing dimensions are selected. Increasing the diameter of the wire, and managing a subtle transition from round to rectangular wire shape, brings about an increasing engagement of the wire within the rectangular slot of the bracket. The use of successively larger wires delivers a controlled, incremental bracket prescription to the teeth which can be manipulated during the course of treatment to pursue individualised functional and aesthetic treatment goals (277).

1.14. Orthodontic Bracket Material

Orthodontic brackets can be fabricated from a range of materials including metals, plastics, ceramics or a combination of these materials (278). An important requirement is that the material is of adequate hardness and strength to be able to deliver the three-dimensional bracket prescription effectively to the tooth (279). Unacceptable distortion within the bracket will reduce the amount of prescription transferable to the tooth, and can result in a less than ideal positioning of the teeth (280). Positioning errors can be time-consuming for the orthodontist to address later. Another key characteristic to consider when choosing a bracket material, is the amount of friction the material will create at the bracket slot-wire interface. Tooth movements can be seriously impeded by undue amounts of frictional resistance within an orthodontic appliance system (281). A bracket material with a smooth surface minimises unwanted friction and increases the speed of treatment (282). Naturally, a bracket material also needs good biocompatibility and high corrosion resistance (283).

1.14.1. Metal Brackets

Austenitic stainless steel is the most common metal to be used in the fabrication of orthodontic brackets. American Iron and Steel Institute (AISI) grades 304L, 316, 316L and

martensitic 17-4 PH are commercially available choices with varying nickel, molybdenum, chromium and carbon content (284). The superior mechanical properties of stainless steel, namely its appropriate hardness, high deformation resistance and high fracture resistance permit excellent transference of force from wire to tooth, when the wire is engaged in the bracket slot (285). The smooth surface of stainless-steel also minimises frictional resistance to optimise orthodontic tooth movement (286). Stainless steel has good biocompatibility and notably, the nickel component of the alloy is rarely reported to elicit allergic reactions within the oral mucosa, even in hypersensitised individuals (287). Alternative material choices such as titanium, cobalt chromium or specialised coatings of gold or platinum, over stainless-steel brackets, are available for situations where either wholly nickel-free brackets are required or a different aesthetic is sought by the patient (288). Traditionally, metallic brackets were cast or milled but metal injection moulding is now the most common method of fabrication.

1.14.2. Ceramic Brackets

Tooth-coloured ceramic brackets may be offered to patients as an aesthetic alternative to traditional metallic appliances (289). Ceramic brackets are made from single-crystal or polycrystalline aluminium oxide and are classified according to differences in their manufacturing process. Monocrystalline brackets are fabricated from a single crystal of aluminium oxide, which accounts for their most notable difference in comparison to polycrystalline brackets - their optical clarity (290). In the first stages of manufacturing monocrystalline brackets, aluminium oxide particles are fused at high temperatures (2100 °C) into a molten mass which is then cooled slowly to control the crystallisation process. The gradual lowering of the temperature of the molten material generates a single-crystal, translucent sapphire (alumina) from which the mono-crystallisation brackets

are then milled (291). In contrast, polycrystalline brackets are manufactured from a moulded mixture of aluminium oxide particles and a binder, cut into bracket shapes and heated to temperatures in excess of 1800 °C to fuse the aluminium oxide particles together and burn out the binder (290). Polycrystalline brackets begin as aluminium oxide particles of about 3 microns which are fused to produce ceramic grains of 20-30 microns. Grain boundaries refract light and therefore, the resulting polycrystalline bracket is an opaque, less aesthetic bracket than its mono-crystalline counterpart (292, 293). Machine-induced surface imperfections, impurities, pores and propagation lines along grain boundaries are disadvantages which may also compromise the physical properties of polycrystalline brackets (294). The lower manufacturing costs of polycrystalline brackets, however, make these brackets the more widely available. Injection moulding has also now become a common fabrication method for polycrystalline alumina brackets, as machining is not required and thus eliminates structural imperfections created by the cutting operation (291).

The favourable characteristics of ceramic brackets are their aesthetics, dimensional stability, durability and biocompatibility (295). The hardness of ceramic also means these brackets deliver an effective level of prescription to the tooth (280). Clinical complications, however, have challenged the constructors of ceramic brackets - the most serious being the breakage of brackets and a higher incidence of enamel fracture upon debonding the brackets (296, 297). Breakage of ceramic brackets is a consequence of their inherent brittleness which can result in tie-wing fracture to render the bracket ineffective, or a shattering of the bracket upon debonding (296). Early designs of ceramic brackets and specialised bonding techniques with silane coupling agents, resulted in very high bond strengths and posed a significant, unacceptable risk of enamel fracture upon debond (298). Modifications to the ceramic bonding pads have assisted in reducing the risk of enamel fracture, but it remains a clinical concern (299, 300).

1.14.3. Plastic Brackets

Contemporary plastics used for orthodontic bracket fabrication include polyurethane, polyoxymethylene and calcium-aluminium-silicate fibreglass-reinforced polycarbonate (291, 301, 302). Traditionally, pure plastic brackets have exhibited a number of problems including a lack of strength and stiffness, creep deformation when transferring torque loads, tie-wing fracture, slot wear, water uptake and increased levels of friction (301, 303, 304). Despite efforts to overcome these difficulties, mainly by utilising reinforced plastics or incorporating metal slots, many of the problems persist (305).

1.15. Bracket Base Morphology and Adhesion: Mechanical Retention

To increase the mechanical retention of adhesive to the base of orthodontic brackets, there exists many morphological variations to bonding pads (306).

1.15.1. Metal Mesh Bases

A metal mesh base is constructed of many fine stainless-steel wire strands set at 90 degrees to each other (307). The mesh is available as a laminate which may be braised, laser-welded or laminated onto a foil on the bracket profile. Many variables influence the retention of adhesive within the meshwork including the mesh number (number of openings per linear inch), the wire diameter and the volume of the aperture (307, 308). For resin to pass into the mesh base, air must be displaced from the contact surface and this is influenced by the available space between mesh and bracket base. Also, if the mesh wires are too thick they can limit the penetration of resin, too thin and they are at risk of fracture (309). A variety of mesh designs are marketed as using either a single or double mesh with available mesh gauge sizes (number of wires per linear inch) 40-, 60-, 80-, 100-, 200-gauge (306). Previously

popular finer meshes (e.g. 100-gauge) have been much superseded by contemporary meshes which are now usually less dense, following demonstrations of higher bond strengths with larger apertures (e.g. 40- to 80-gauge) (309, 310).

1.15.2. Mechanical Undercuts

Grooves, pits, dovetails, serrations, replicates of mesh architecture or indeed any variety of shaped undercuts are available from many manufacturers to provide recesses for the mechanical retention of resin to the bracket base to promote bond strength (307, 308, 311). Retentive characteristics may be cast, moulded or milled into the base. Recent advancements have been laser-structured bases of injection-moulded metal brackets, whereby a computer-aided laser (CAL) cutting process utilises Nd-YAG laser beams to evaporate and melt part of the metal base, leaving behind numerous grooves to increase the retentive mechanism (312).

1.15.3. Micro-mechanical Retention

The application of surface treatments or coatings is available to modify the surface of bracket bases with the objective of enhancing micromechanical retention and improving bond strengths (313). Various techniques and materials have been suggested to increase active surface areas for bonding. Chemical micro-etching is a commonly-used technique to improve retention, however, innovative approaches include particulate-coated bases (314, 315). Irregular microscopic metallic elements (spheres, rods, crystal protrusions or similar) may be fused together and to the bracket base, to create a network of pores for a strong adhesive grip (316). Similarly, thermal plasma sprays of finely ground metallic or ceramic particles may cover the bases to improve the surface properties for bonding (317, 318). One advantage of some porous metal powders, which may be sintered onto the bracket bases, is

that capillary action will draw resin up into the metalwork to create a robust, interlocking bond (319).

1.16. Chemical Adhesion of Bracket Bases

Silane coupling agents have been used as adhesion promotors to enhance the bond between the base of aesthetic ceramic orthodontic brackets and resin adhesives during bonding procedures (320, 321). However, it was demonstrated that bond strengths of silane-treated chemically retentive bracket bases were excessively high, making enamel fractures more likely to occur at debonding (322-324). The more desirable ceramic brackets now rely on mechanical retention and avoid chemical pre-treatments (325).

1.17. In Vitro Bond Strength Testing

In vitro bond strength testing offers the opportunity for investigators to examine the performance of orthodontic adhesives under a series of controlled conditions. The results of in vitro studies can be of some assistance to clinicians in their selection of materials (326, 327). Lower in vitro bond strengths can indicate that brackets will be too easily debonded by clinical therapeutic force or by the patient's masticatory forces or habits. Inadvertent debonding of brackets hinders treatment, is costly in terms of time and materials and is inconvenient to the patient. On the contrary, bond strengths which are relatively much higher can indicate that the brackets will be more difficult to remove at the end of treatment which can be associated with an increased risk of enamel fracture (328).

An orthodontic bracket within an active appliance can be subjected to six different load components. Forces in mesio-distal, occluso-gingival or bucco-lingual directions, together with first order (in-out), second order (tip or torsion) and third order (torque) moments may

be produced during treatment (278). It is likely that the bracket experiences simultaneous combinations and fluctuations of these six load components which is, as yet, unfeasible to replicate in the laboratory. Efforts have been made instead to provide useful experimental simulations of clinical debonding from isolated load components, most commonly shear-peel, but also tension and occasionally torsion (329, 330). During treatment, inadvertent debonding of orthodontic brackets is more likely to occur from excessive shearing forces to the tie-wings from mastication or undue orthodontic force and, therefore, shear bond strength tests tend to predominate in orthodontic studies.

Common in vitro methods for shear force application is by means of a wire loop or a shear blade, as illustrated in Fig. 1.1. (331). A wire loop may be placed in the groove beneath the bracket tie-wings before a vertically-directed force imparts a shear-peeling force to the bracket-adhesive border (332). Debonding forces that are parallel to the base of the bracket are required to impart a shearing force to the adhesive interface and authors have emphasised that test specimens using wire loops must be meticulously aligned (332, 333). A systematic review by Finnema et al. (334) described the major impact that angulation of the wire loop can have on shear bond strength values and the use of a rectangular wire has been recommended for wire loop testing to provide a more stable point of force application (333). By contrast, shear force application with a blade is made directly at the adhesive interface. Criticisms for shear blade debonding methods have focused on larger dispersions to the data produced by this method and abnormal wedge-shaped opening forces which lack clinical relevance and may misrepresent material behaviour (332). Results obtained with shear blades have also been reported to be relatively higher when compared with systems using wire loops (335).

Another experimental condition shown to significantly affect outcomes is crosshead speed (334). From the in vitro studies included in the meta-analysis by Finnema et al. (334), cross-head speeds ranged from 0.1-5.0 mm/min with most researchers using speeds of 0.5 mm/min and where it was shown that an increase in crosshead speed of 1 mm/min yielded an increase in mean bond strengths of 1.3 MPa.

There is currently no standard for evaluating bond strengths and the diverse test designs found within the literature make comparison between studies very challenging (331, 336). There have been specific attempts to standardise bond strength testing for orthodontic studies but none has yet been universally accepted (333, 337).

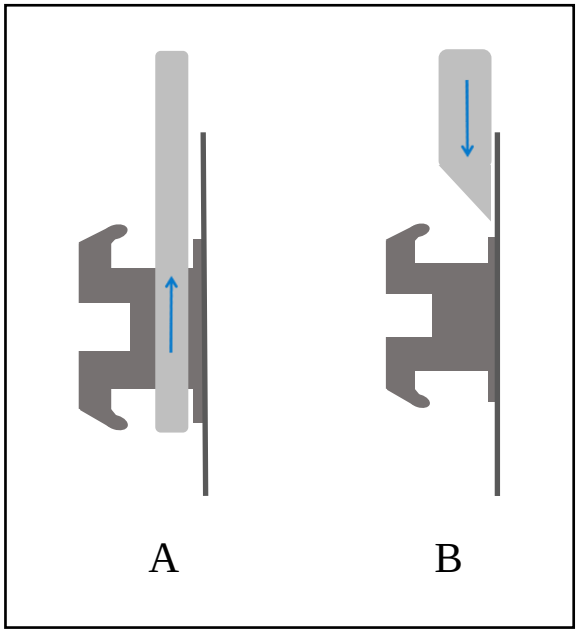

Fig 1.1 Diagrammatical representation of in vitro shear bond strength testing using either a wire loop (A) or a blade (B) to debond an orthodontic bracket from the tooth surface.

1.18. Thermocycling

Thermocycling is a widely used in vitro thermal fatigue methodology which simulates the fluctuations in temperature that occurs in the mouth to evaluate bond durability (81, 338, 339). A thermal cycling regimen is defined by the water temperature in each of two baths, the dwell time of the tooth in each water bath, transfer time between the two baths and the number of thermal cycles. The International Organisation for Standardisation (ISO/TR 11405E) (340) have indicated that thermocycling regimens of 500 cycles in water between 5 °C and 55 °C are suitable aging tests. Dwell times are recommended to be at least 20 seconds per bath and shorter transfer times between baths of 5 to 15 seconds may best simulate the abrupt changes in temperature that occur in the mouth (340, 341).

The artificial ageing effects produced by thermocycling are created through two principle approaches. Firstly, thermocycling utilises differences in the thermal coefficients of expansion of composite, resin and tooth tissue to stress the adhesive bond (342, 343). Fluctuations in temperature cause repeated cycles of contraction and expansion stress at the enamel-adhesive interface resulting from the higher thermal coefficients of expansion of restorative materials relative to tooth tissue (344). Thermal stresses generate mechanical stresses which can disrupt the tooth-adhesive interface and propagate cracks along bonded surfaces. Once a gap opens up, changes in gap dimension allows the in- and out-flow of fluid in a process called percolation, which can disrupt the adhesive interface and facilitate bond failure (81). Secondly, the effects of thermocycling in water remain inseparable from the impact of hydrolysis on the adhesive bond and it is likely that heated water accelerates the process of hydrolytic degradation, facilitating water sorption and the leaching of resin components at elevated temperatures (223, 345, 346).

1.19. Water Storage

Water sorption may be one of the most important factors responsible for adhesive degradation (347). Consequently, varying water sorption programmes are found in many studies as an artificial ageing technique, and investigations of this type may often use bonded tooth specimens stored in water at 37 °C for specified periods of time (182, 348, 349). Absorbed water in polymer matrices exists as either 'unbound' or 'bound water' (350, 351). 'Unbound' water inhabits the free space between polymer chains formed by polymerisation; 'bound' water attaches itself to polymer chains via hydrogen bonding to hydrophilic or ionic functional groups (351). 'Bound' water sorption into a polymer may be significantly influenced by resin polarity (223, 352, 353). It is speculated that ionic attraction between water molecules and polymer enables successive binding of water molecules to hydrophilic groups (354). Resin polarity also impacts the amount of hydrogen bonding sites available to 'bound' water molecules (353). Studies have demonstrated that increasing the hydrophilicity of a resin can increase water sorption and may negatively affect the mechanical behaviour of the resin after water ageing (170, 223).

To consider the uptake of 'unbound' water, it is reasoned that this type of absorbed moisture may be taken up into the resin matrices by a pattern of diffusion through the nanopores of the polymer with no association with the polar molecules of the resin (350). Chain topology may influence the pattern of absorbed 'unbound' moisture by determining the spatial configuration of polymer molecules and nanopores available for water molecules to inhabit (352). Uptake of water will initially soften the polymer material by swelling the system. Hydrolysis effects at the filler matrix interface lead to separation of filler particles and the formation of gaps, causing the polymer to swell. Infiltration of water reduces friction between chains of the polymer which acts to decrease the material properties of the polymer

matrix, in a process known as ‘plasticisation’ (355). Saturation eventually reaches an equilibrium between bound and free sites and water sorption stabilises (356, 357). Over time, resin components are further degraded and leached as the polymer infrastructure deteriorates (81, 355).

1.20. Adhesive Remnant Evaluation

Various quantitative and qualitative methods have evaluated the amount of adhesive remnant on the tooth surface after debonding an orthodontic bracket (358-362). Årtun and Bergland (358) developed the Adhesive Remnant Index (ARI) which was a 4-point scale based on a pilot study of 20 extracted teeth and the criteria were set out as: score 0= no adhesive left on the tooth; score 1 = less than half of the adhesive left on the tooth; score 2 = more than half of the adhesive left on the tooth; score 3 = all the adhesive left on the tooth with a distinct impression of the bracket mesh (Fig. 1.2.) Later attempts to provide more accurate, qualitative evaluations of the remnant offered a modified ARI system in which the scoring criteria was expanded into a 5-point scale (Oliver 1988) (359). Visualisation of the adhesive remnant in laboratory studies is usually facilitated by magnification, typically x 10 or x 20, using stereomicroscopy (363, 364). Both the 4- and 5-point ARI systems continue to be used in contemporary orthodontic bonding studies (365-367).

To address the qualitative and subjective nature of existing ARI scoring systems, O’Brien (360) introduced a quantitative method of adhesive remnant determination which proposed that the amount of adhesive remaining on the bracket base be calculated as a percentage of the base surface area using a magnified and digitised image. More advanced technologies also now offer precise imaging and analysis of adhesive remnant, such as scanning electron microscopy (SEM) with quantitative image analysis techniques, finite

element analysis and three-dimensional profilometry (312, 361, 362, 368). The quantitative evaluation of the adhesive remnant is the more sophisticated methodology, however, requires specialised equipment, is more time consuming to apply than qualitative evaluations and it is especially difficult to apply clinically (44).

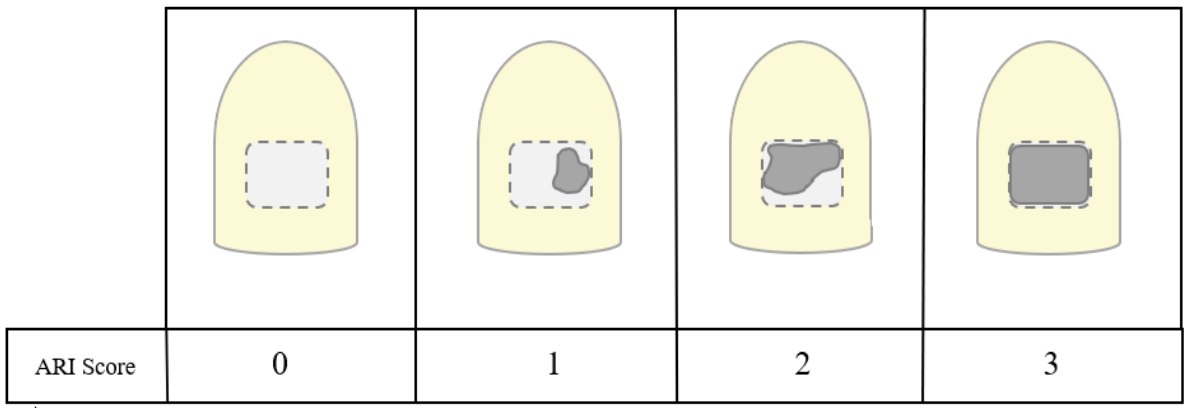

Fig. 1.2. Diagrammatical representation of the Adhesive Remnant Index (ARI) developed by Årtun and Bergland (1984). After debonding an orthodontic bracket, the adhesive remaining on the tooth surface is allocated a score based on a 4-point scale as: score 0= no adhesive left on the tooth; score 1 = less than half of the adhesive left on the tooth; score 2 = more than half of the adhesive left on the tooth; score 3 = all the adhesive left on the tooth with a distinct impression of the bracket mesh.

1.21. Classification of Failure Mode

Following bracket debonding and examination of the adhesive remnant at the failure surfaces, a classification of failure mode can be performed (369). The mode of failure for an orthodontic adhesive may be selected from one of three following categories: adhesive-enamel failure, adhesive-bracket failure or cohesive failure (370). Bracket failure at the adhesive-enamel interface may be classified as such if the tooth surface is clean, with no adhesive remaining on the enamel after debonding (ARI score 0). At the opposite end of the spectrum, an adhesive-bracket failure is recorded if there is total absence of material on the bracket base and all the adhesive remains on the tooth surface after debonding

(ARI score 3). Cohesive failures are defined by evidence that the bond has separated within the adhesive and are categorised between two scores on that basis (ARI scores 1 and 2) (39, 308, 369).

Adhesive-bracket failures, which leave the entire adhesive on the enamel surface, can be considered undesirable because removing the adhesive with carbon-tungsten burs can lead to enamel damage and takes considerable chairside time (44, 45, 371). On the other hand, adhesive-enamel failures, which leave no adhesive on the enamel for clean-up, may seem advantageous but observations of surface enamel removal as well as more significant enamel fractures have occurred at higher stress (29, 371). Cohesive failures, by definition, require less adhesive removal from the tooth surface than complete adhesive-bracket failures with a lower propensity for iatrogenic damage however, concerns of the risk of enamel fracture persist even with these 'mixed' categories of failure (29, 44).

1.22. Aims and Objectives

The research aims and objectives are:

1. To obtain knowledge and understanding about the suitability of pre-mixed, self-etching primer adhesives (1-SEP) as orthodontic bonding agents.
    a) Data sets for shear bond strength will be collected from three 1-SEP adhesive types: Xeno V+, Clearfil S3 and iBond SE.
    b) Data sets will be collected after bonded teeth specimens are assigned to three groups: immediate debonding (within 30 minutes), thermocycling between 5 ºC and 55 ºC for 500 cycles and water storage at 37 ºC for 7 days.
    c) Data sets will be obtained for the mode of failure of 1-SEP adhesives.

2. To investigate whether omitting the pre-cure step in the bonding protocols has any effect on the performance of 1-SEP as orthodontic bonding agents.
    a) Data set collection will be repeated, as above, with omission of the pre-cure step for 1-SEP.

1.23. Null Hypotheses

The null hypotheses for this study were:

1. There would be no statistically significant relationship between the type of adhesive used to bond orthodontic brackets to tooth enamel and mean shear bond strength, when the bracket is debonded either immediately, following thermocycling or following water storage.

2. Omitting the pre-cure step from the bonding protocol of 1-SEP adhesives Xeno V+, Clearfil S3 and iBond SE when bonding orthodontic brackets would have no statistically significant effect on the mean shear bond strength, when the bracket is debonded either immediately, following thermocycling or following water storage.

3. There would be no statistically significant relationship between adhesive type and amount of adhesive remaining on the tooth surface, following the debonding of orthodontic brackets either immediately, following thermocycling or following water storage.

4. Omitting the pre-cure step from the bonding protocol of adhesives when bonding orthodontic brackets would have no statistically significant effect on the amount of adhesive remaining on the tooth surface, when brackets were debonded either immediately, following thermocycling or following water storage.

CHAPTER TWO: MATERIALS AND METHOD

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2.1. Selection and Preparation of Tooth Specimens

2.1.1. Selecting Premolar Tooth Specimens

Four hundred and twenty extracted human premolar teeth with sound, unblemished enamel were obtained from Birmingham Dental Hospital (St Chad's Queensway, Birmingham, UK). Teeth with enamel defects (such as hypoplastic or hypomineralised lesions), or teeth containing restorations, cracks or caries were excluded. No teeth were subject to chemical pre-treatments. Birmingham RM&G Consortium awarded Permission for Research for this study.

REC Ref: 09.H0405.33 Consortium R&D No: 1467 (Appendix 1)

2.1.2. Tooth Decoronation and Mounting

Selected premolar teeth were decoronated by manually feeding horizontal tooth specimens against a custom-built vertical, water-cooled, diamond-edged rotatory blade under a load sufficient to section the tooth 2 mm below the cemento-enamel junction. The roots were discarded. The crowns were kept and placed into deionised water at room temperature (23 °C +/-1 ºC) for no longer than 7 days before mounting, to prevent growth of known oral bacteria, such as Streptococcus and Actinomyces species. To mount the premolar tooth crowns, thick consistency cold-cure acrylic (Paladur, Heraeus Kulzer Ltd, Berkshire) was placed in cylindrical copper holding rings (43 mm high x 14 mm diameter) which contained a wax base insert (Tenatex wax 16-364, Kemdent, Wiltshire). The teeth were embedded horizontally into the acrylic to half of their available depth, keeping the buccal surfaces

facing upwards. Once set, the tooth-acrylic samples were then removed from the copper rings and stored in deionised water at room temperature (23 ºC +/- 1 ºC) for a maximum of 2 weeks. Deionised storage water was changed after 7 days to prevent bacterial growth.

2.1.3. Prophylaxis and Drying of Tooth Surfaces

Prepared tooth-acrylic samples were retrieved from deionised water storage and the buccal surfaces cleaned for 10 seconds using a 6 mm diameter, latch-grip silicone rubber prophylaxis cup in a low-speed dental handpiece (5000 revolutions per minute, rpm) with a slurry of distilled water and an oil- and fluoride-free, extra-fine pumice stone. No additional water was recruited from the handpiece during the cleaning process and after every 5 prophylaxes, a new rubber cup was used to standardise the procedure. After pumicing, the tooth surfaces were washed with distilled water for 10 seconds using a 500 ml wash bottle and then dried for an additional 10 seconds with a compressed, oil-free air stream from a dental air compressor unit with a disposable plastic 3-in-1 air/water syringe tip.

2.2. Materials and Curing Protocol Group Allocation

After prophylaxis and drying, 420 embedded tooth-acrylic specimens were randomly allocated to 7 test groups (n= 60), corresponding to 3 single-component self-etching primers (1-SEP) which were further subdivided into 2 curing groups and 1 control group self-etching primer (SEP). Group allocation information is displayed in Table 2.1. and the manufacturer's information for adhesive composition and bonding procedures are shown in Table 2.2.

The 1-SEP systems were the following:

Xeno V+ (Dentsply Ltd, Weybridge, Surrey, UK Lot: 1404000473)

Clearfil S3 Bond (Kuraray Europe GMBH, Hattersheim am Main, Germany Lot: 420004)

iBond Self Etch (Heraeus Kulzer Ltd, Newbury Berkshire, UK Lot: 010702)

The control SEP system was Transbond Plus Self Etching Primer (3M Unitek UK, Loughborough, UK Lot: DA2FQ).

The curing protocol subgroups were:

Pre-cure (PC) protocol: 1-SEP was cured prior to bracket placement, in accordance with time periods specified by the manufacturer of each material.

Co-cure (CC) protocol: 1-SEP was cured simultaneously, with composite pre-coated on the bracket base, after bracket placement. The pre-curing of 1-SEP, prior to bracket placement, was omitted. The control SEP, Transbond Plus, was cured in accordance with instructions provided by the manufacturer which recommends a co-cure (CC) protocol.

Group	*n*	*Adhesive*	*Curing Protocol*
1	60	Xeno V+ (Dentsply Ltd, Weybridge, Surrey, UK)	PC
2	60	Clearfil S3 Bond (Kuraray Europe GMBH, Frankfurt)	PC
3	60	iBond Self Etch (Heraeus Kulzer Ltd, Newbury, UK)	PC
4	60	Xeno V+ (Dentsply Ltd, Weybridge, Surrey, UK)	CC
5	60	Clearfil S3 Bond (Kuraray Europe GMBH, Frankfurt)	CC
6	60	iBond Self Etch (Heraeus Kulzer Ltd, Newbury, UK)	CC
7	60	Transbond Plus Self Etching Primer (control) (3M Unitek UK, Loughborough, UK)	CC

Table 2.1. Table showing the 7 experimental groups corresponding to three 1-SEP (groups 1-6) and the control SEP (group 7). Experimental groups were subdivided into two curing protocols: PC= Pre-cure (groups 1-3) and CC=Co-cure (groups 4-7). The control SEP was cured according the manufacturer's specifications (CC).

Adhesive Systems	Components	Bonding Procedure
Xeno V+ (Dentsply Ltd, Weybridge, Surrey, UK) Lot: 404000473	Bifunctional acrylate, acidic acylate, functionalised phosphoric acid ester, water, tertiary butanol (75-65-0-t-butyl alcohol)	Apply Xeno V+ sufficiently, wetting all cavity surfaces uniformly. Then gently agitate the adhesive for 20 seconds. Begin evaporation with a moderate stream. Continue with more forceful air until there is no more movement of the adhesive, but for at least 5 seconds. Cure for at least 10 seconds.
Clearfil S3 Bond (Kuraray Europe GMBH, Hattersheim am Main, Frankfurt, Germany) Lot: 420004	10-Methacryloyloxydecyl dihydrogen phosphate (MDP), bisphenol A diglycidylmethacrylate (Bis-GMA), 2-Hydroxyethyl methacrylate (HEMA), dl-camphorquinone, ethanol water, colloidal silica	Apply Clearfil S3 Bond and leave it in place for 10 seconds. Dry the entire surface sufficiently by blowing mild air for more than 5 seconds until the bond does not move. Light-cure bond for 10 seconds with a dental curing light.
iBond Self Etch (Heraeus Kulzer Ltd, Newbury Berkshire, UK) Lot: 010702	Orthophosphoric acid, 4 – methacryloxyethyltrimellitic acid anhyrdride, butyl-hydroxy-toluol, dl-camphorquinionone 1,4-Dioxane, acetone, water	Apply iBond Self Etch with the applicator tip or brush. Agitate the adhesive slightly for 20 seconds to improve demineralisation and diffusion. Carefully air-dry iBond Self Etch with a flow of oil-free air (may take 5-10 seconds or longer depending on the geometry of the surface). The aim is to evaporate the solvent and water from the bonding layer without removing the active ingredients from the tooth surface. The surface must be visibly glossy. If the surface does not appear shiny, apply iBond Self Etch as described above a second time. Light cure for 20 seconds.
Transbond Plus Self Etching Primer (3M Unitek UK, Loughborough, Leicestershire, UK) Lot: DA2FQ	A: Methacrylated phosphoric acid ester Initiators Stabilisers dl-camphorquinone B: Water Fluoride complexes Stabilisers	Mix A and B blisters, according to the manufacturer's recommendation. Rub the saturated tip of applicator onto tooth surface. Continue rubbing liquid onto enamel while applying some pressure for a minimum of 3-5 seconds per tooth. Use an oil and moisture-free air source to deliver a gentle air burst for 1-2 seconds to each tooth to dry primer into a thin film.

Table 2.2. Composition and bonding procedures of adhesives as specified by the manufacturer of each material.

2.2.1. Curing Protocol: Pre-cure (PC) Group

In groups 1, 2 and 3, shown in Table 2.1., each 1-SEP adhesive type was applied to the clean, dry tooth surface and irradiated with a quartz-tungsten-halogen (QTH) light curing unit (Optilux 501 910070 Kerr USA), 1 mm from the curing surface, according to the time periods specified by the manufacturer's instructions for each material: Xeno V+ 10 seconds; Clearfil S3 10 seconds; iBond SE 20 seconds (Table 2.2.). The light intensity was measured prior to the fabrication of each specimen set between 650 and 680 milliwatts per centimetre square mW/cm^2) using the built-in radiometer. An 11 mm guide tip diameter was employed to ensure adequate irradiation of the entire bonded surface.

After photopolymerisation of 1-SEP in the Pre-cure group, stainless steel Siamese twin premolar orthodontic brackets (Victory Series Metal Bracket APC II Adhesive Coated Appliance System 3M Unitek, Monrovia, Calif. USA Lot: DA2FQ) were held in 14cm metal spring-loaded, direct bond bracket holders (RMO Europe, Strasbourg, France) in preparation for placement on the tooth surface. The bracket bonding base comprised an 80-gauge, woven stainless steel single-mesh, pre-coated with filled diacrylate composite resin adhesive - a modified, more viscous version of Transbond XT (3M Unitek, Monrovia, Calif. USA), as received. Pre-coated adhesive brackets were employed in the present study to standardise the amount of composite on the bracket base. Each bracket was seated in accordance with Andrews' (372) recommended standard technique on the buccal tooth surface, that is, the Long Axis Point (LA) defined as "the midpoint of the long axis of the clinical crown." To seat the bracket, a 300g load was applied to a central point on the bracket with a Correx Tension Gauge (Haag Streit Diagnostics, Switzerland). Any excess flash composite was removed from around the bracket with a No. 9 stainless steel handled probe (Dentsply, Surrey UK). Once the bracket was seated, each specimen was examined visually to ensure

the bracket was a close fit against the tooth surface. In this way, variations in adhesive thickness were minimised. The tooth-bracket specimen was then irradiated for 10 seconds on both the mesial and distal edge of the bracket (total 20 seconds), 1 mm from the curing surface, using the quartz-tungsten halogen (QTH) light curing unit as described. A schematic illustration of specimen preparation and adhesive curing protocols is presented in Fig 2.1.

2.2.2. Curing Protocol: Co-cure (CC) Group

Cleaning of the tooth surface prior to placement of adhesive was undertaken using the same method as for the Pre-cure group. In groups 4, 5 and 6 the 1-SEP adhesive was applied to the clean, dry tooth surface using the technique specified by the manufacturer of each material (Table 2.2.). However, the curing method recommended by the manufacture, was not followed for 1-SEP in Co-cured groups – 1-SEP was not cured prior to bracket placement and, therefore, the recommended pre-cure step was omitted from the bonding protocol. The bracket, pre-coated with composite (3M Unitek, Monrovia, Calif. USA), was sited directly onto the un-polymerised, primed tooth surface using the same seating technique as for Pre-cure group specimens. Irradiation was initiated once the bracket was correctly positioned and seated, using the quartz-tungsten-halogen (QTH) light curing unit for 10 seconds mesially and 10 seconds distally as described. The control adhesive, Transbond Plus SEP (group 7), was applied according to the method specified by the manufacturer and recommended curing procedures were followed, which adhered to a co-curing procedure for the control.

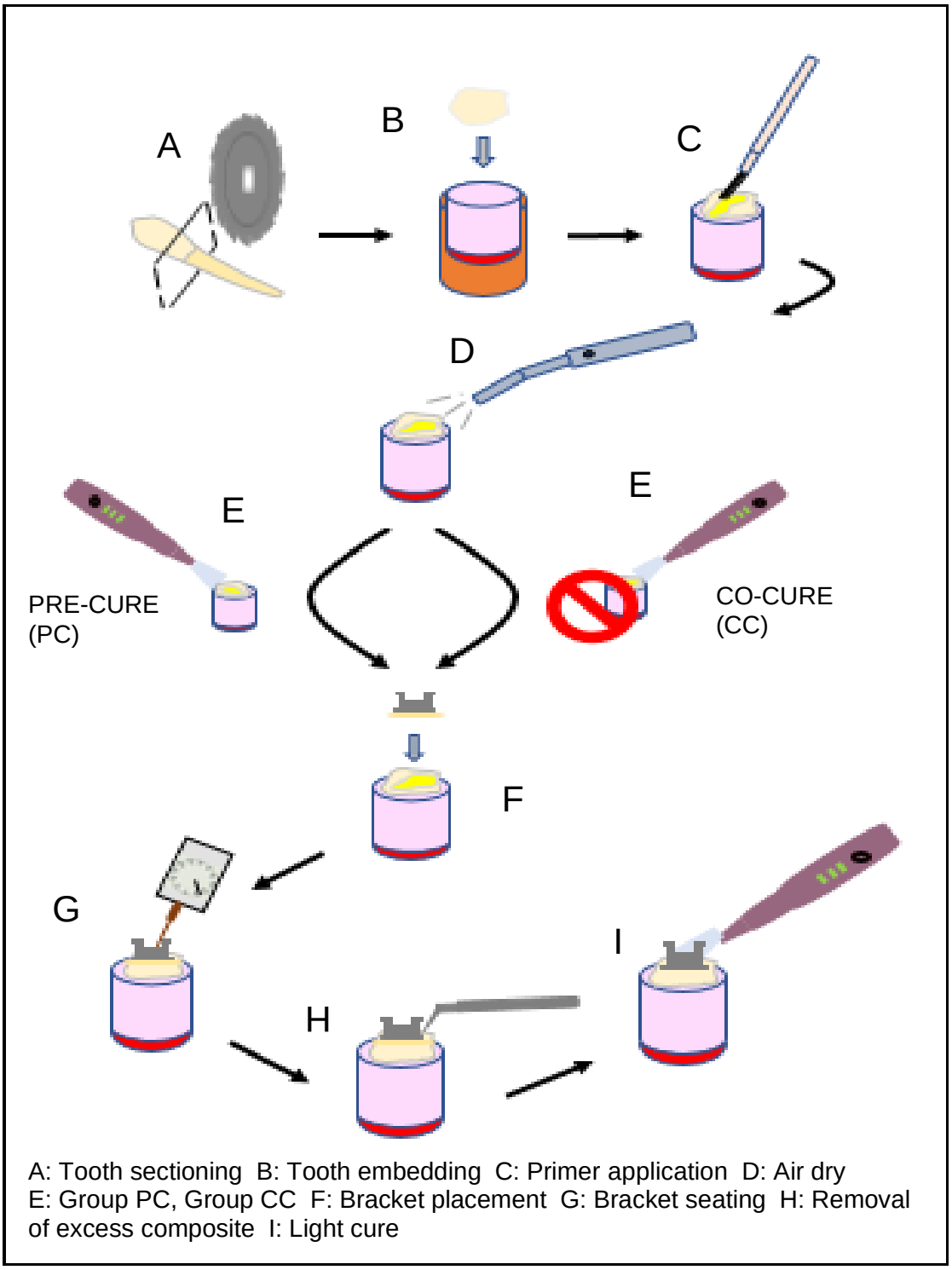


Fig. 2.1. Schematic diagram illustrating tooth specimen preparation, curing methods of self-etching primers in Pre-cure (PC) and Co-cure (CC) groups followed by placement, bonding and irradiation of a pre-coated orthodontic bracket.

2.3. Environmental Test Groups

Following placement and irradiation of the bracket on the tooth surface, the tooth-bracket specimens in each curing protocol group were randomly allocated to three subgroups (n=20) corresponding to three experimental conditions which were:

- Immediate debond (within 30 minutes from initial bonding)
- Thermal cycling between 5 ºC and 55 ºC for 500 cycles
- Water storage at 37 ºC for 7 days

A diagram of the experimental set-up and treatment of adhesives is illustrated in Fig 2.2.

2.3.1. Immediate Debonding (within 30 minutes from initial bonding)

Prepared bonded teeth specimens, assigned to the Immediate Debonding group, were reinserted into the cylindrical copper rings, as described, and fitted into a corresponding type "O" female test structure, which was mounted on a machine base adapter on the base plate of an Instron Universal Testing Machine (Series 5500/Model 5544 Instron UK, High Wycombe, UK). The positive fit of the copper cylinder within the Instron test structure was checked manually and visually by the operator to ensure non-axial forces would not be introduced during testing. A stainless steel clevis pin (6 mm diameter x 45 mm length) was inserted into an Instron Static 2 KN Load Cell Accessory. A prepared stainless steel wire loop (0.016 inch x 0.016 inch /0.406 mm x 0.406 mm wire dimension; 690 mm loop length; 4 mm diameter loop ends) was hung over the clevis pin and adjusted to be roughly parallel with the bracket on the tooth specimen below. Using the control panel, the load cell carrying the wire loop on the clevis pin, was lowered to position the wire loop beneath the gingival tie-wings of the bracket. A fine position thumbwheel allowed small, precise upward

adjustments to be made to the wire loop position to bring it as close as possible to the bracket stem, avoiding contact and pre-straining the wire, which would nullify the test.

The test was initiated with a crosshead speed of 0.5 mm/min, load cell 2 KN (200 kg, 450 lb), which engaged the wire loop with the bracket stem, and debonded the bracket with a shearing force. The mechanical stress generated by the contact between the wire loop and bracket stem produced electrical resistance change at the strain gauges within the load cell, changing the output signal. The peak force level at which the bracket became debonded from the tooth was recorded by the output signal, which was conditioned for the display readout in accordance with international force measurement standards including ASTM E4, ISO 7500-1 class 0.5, and JIS B7721, B7733 using integrated Merlin software. The peak force levels, automatically recorded on the testing machine, were converted to stress per unit area (Megapascals, MPa) by dividing the force (Newtons, N) by the mean unit area of the base of the bracket (10.60 mm^2). Retentive bracket base surface area was calculated by measuring length and width with digital Calipers (150 mm Venier Calipers, Mitutoyo, Kanagawa, Japan) and computing the area. The bracket surface area of 10.60 mm^2 (S.D. 0.04) was taken as a mean average calculated from 20 representative bracket specimens (Appendix 2). The shear bond testing in the Immediate Debonding group was carried out within half-an-hour from when brackets were bonded to the tooth samples.

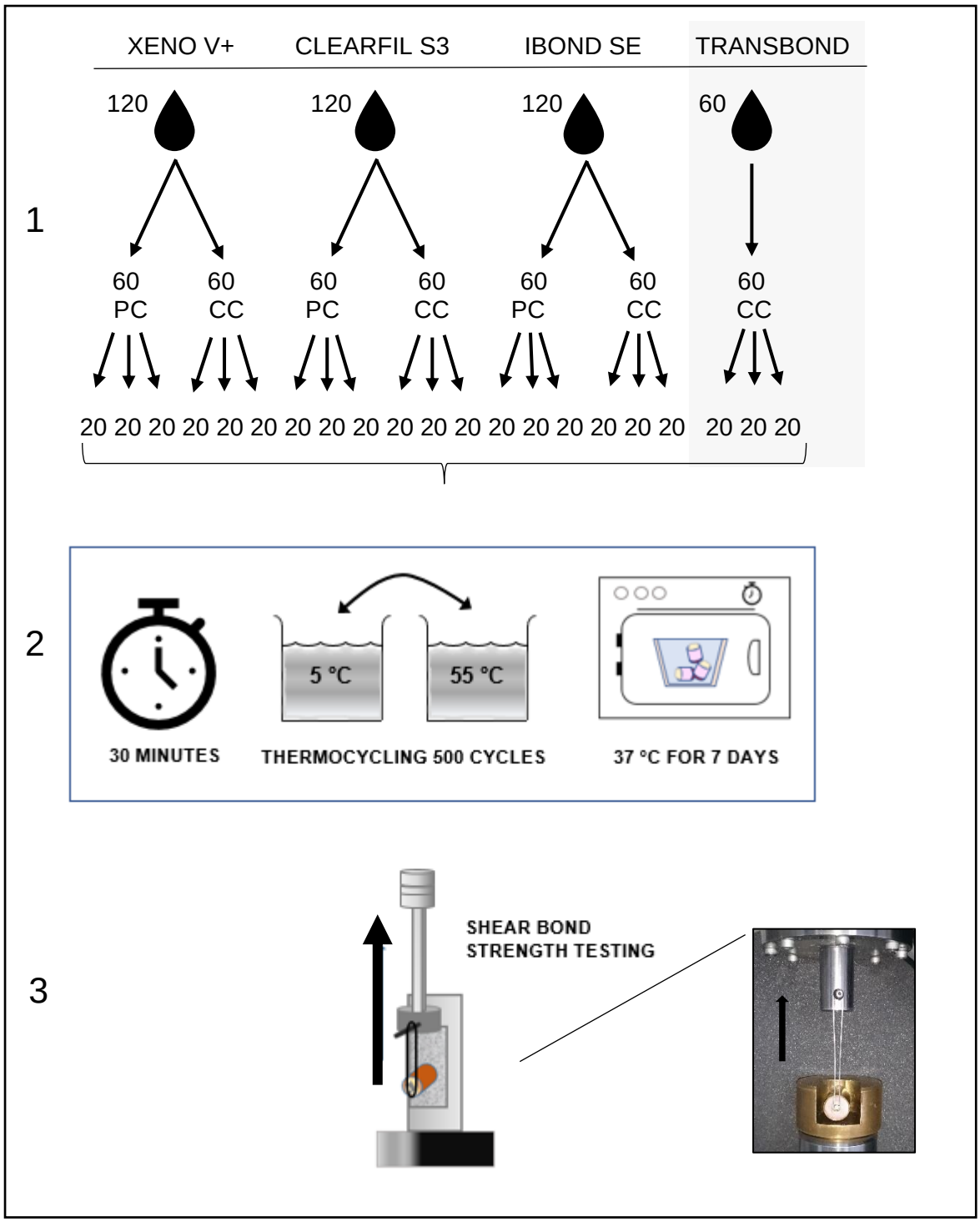


Fig. 2.2. A schematic diagram illustrating the experimental set-up including treatment of adhesives, environmental test groups and shear bond strength testing

2.3.2. Thermocycling between 5 ºC and 55 ºC for 500 Cycles

Twenty further bonded teeth specimens from each of the 7 curing protocol groups, underwent thermocycling between 5 ºC and 55 ºC for 500 cycles, to assess the effect of thermal stress on their shear bond strengths. In the clinical environment, orthodontic brackets in the oral cavity are surrounded by moisture from saliva and therefore all bonded teeth examples were thermocycled in a wet, rather than dry, condition. Twenty samples from each curing protocol were placed in bags made from 300 mm diameter circles of undyed, open weave cotton scrim fabric to allow water ingress. Each bag was gathered and tied with monofilament ethilon nylon suture thread, 3-0. A custom-built thermocycling machine (Proto-tech Portland Oregon USA) moved each bag containing 20 tooth-bracket samples between two thermostatically controlled water baths, in which the cotton bags were immersed for 30 seconds each time. The temperatures selected for each bath were 5 ºC and 55 ºC in accordance with ISO/TR 11405E recommendations (ISO, 1994) (340) with a transfer time of 15 seconds at room temperature (23 ºC +/- 1 ºC). Each sample underwent 500 cycles, after which samples were retrieved and the shear bond strengths were measured by the same technique as described previously in immediate debonding groups.

2.3.3. Water Storage at 37 ºC for 7 Days

Twenty bonded teeth samples from each of the 7 curing protocol groups were kept in deionised water at 37 ºC for 7 days in covered laboratory low-form glass beakers, 1000 ml (1 litre) capacity. The aim of storing prepared bonded teeth specimens in water was to examine the effect of oral-temperature moisture exposure on the shear bond strengths for each adhesive type. After 7 days, bonded teeth samples were subject to shear bond testing in the manner described for previous groups.

2.4. Evaluation of Failure Surfaces

After shear bond testing, tooth failure surfaces were examined at x 10 magnification with a binocular stereoscopic microscope (Wild, Herbrugg, Switzerland) to determine how each adhesive had failed. The failure sites were evaluated and scored by the same operator using the 4-point Adhesive Remnant Index (ARI) by Årtun and Bergland (358) where score 0 = no adhesive left on the tooth, score 1 = less than half of the adhesive left on the tooth, score 2 = more than half of the adhesive left on the tooth and score 3 = all adhesive left on the tooth with a distinct impression of the bracket mesh.

2.5. Statistical Analysis

The data were analysed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA).

2.5.1. Null Hypothesis 1

"There would be no statistically significant relationship between the type of adhesive used to bond orthodontic brackets to tooth enamel and mean shear bond strength, when the bracket is debonded either immediately, following thermocycling or following water storage."

The null hypothesis states that the mean values are equal to one another. This is written mathematically below. The alternative hypothesis (H_1) is that the mean shear bond strengths do not equal one another. The statistical test will look to either accept or reject the null hypothesis.

Pre-cure Group

$$H_0 = \mu_{ClearfilS3} = \mu_{IBond} = \mu_{XenoV+}$$

$$H_1 = \mu_{ClearfilS3} \neq \mu_{IBond} \neq \mu_{XenoV+}$$

Co-cure Group

$$H_0 = \mu_{ClearfilS3} = \mu_{IBond} = \mu_{Transbond} = \mu_{XenoV+}$$

$$H_1 = \mu_{ClearfilS3} \neq \mu_{IBond} \neq \mu_{Transbond} \neq \mu_{XenoV+}$$

ANOVA (Analysis of Variance) is a statistical test that compares the mean values of multiple groups. For this piece of analysis the independent variable is the Adhesive Type. The mean shear bond strength (dependant variable) will be compared to understand if the difference between them is significant. The pre-cured and co-cured data will be kept separate to ensure that only one variable differs between the groups, in order that the test is as robust as possible. Tukey's comparison of means test will then be used to understand where there is a significant difference between the mean shear bond strength from the ANOVA test and which group(s) has caused this difference.

2.5.2. Null Hypothesis 2

"Omitting the pre-cure step from the bonding protocol of 1-SEP adhesives Xeno V+, Clearfil S3 and iBond SE when bonding orthodontic brackets would have no statistically significant effect on the mean shear bond strength, when the bracket is debonded either immediately, following thermocycling or following water storage."

The null hypothesis (H_0) and alternative hypothesis (H_1) are stated below. The statistical test will look to either accept or reject the null hypothesis. This will be performed for each of the three treatments – 1) immediate debonding (within 30 mins) 2) thermocycling and 3) water storage to compare the pre-cured and co-cured mean shear bond strength values.

$$H_0: \mu_{Pre-Cured} = \mu_{NotPre-Cured}$$

$$H_1: \mu_{Pre-Cured} \neq \mu_{NotPre-Cured}$$

An unpaired t-test is used to understand if there are significant differences between mean values when there are only two groups to compare. An unpaired t-test will be used in this study as the tests were not repeated on the same sample, given that in this case, the bonded tooth specimen used was different. A t-test is appropriate, as only two variables are compared, and it is assumed that the data is normally distributed and have no significant differences between variance. An f-test is then used to confirm that there are no significant difference between variances.

2.5.3. Null Hypothesis 3

"There would be no statistically significant relationship between adhesive type and amount of adhesive remaining on the tooth surface, following the debonding of orthodontic brackets either immediately, following thermocycling or following water storage."

The null hypothesis (H_0) states that the variables are independent to one another, meaning that the adhesive type does not have an impact on the amount left on the tooth after debonding. The alternative hypothesis (H_1) states that the two variables are dependent on one another:

$$H_0: The\ row\ and\ column\ variables\ of\ the\ contingency\ table\ are\ independant$$

$$H_1: The\ row\ and\ column\ variables\ of\ the\ contingency\ table\ are\ dependant$$

This classification problem is set to understand if the two categorical variables - adhesive type and the amount of adhesive left on the tooth - are dependent on one another. The Chi-square would usually be the most appropriate test for this type of data and looks to see if there is any significant association between the variables. However, as the expected values for each variable are less than 5, the Chi-square test is not suitable in this case. Instead, Fisher's exact test can be used as an association test.

2.5.4. Null Hypothesis 4

"Omitting the pre-cure step from the bonding protocol of adhesives when bonding orthodontic brackets would have no statistically significant effect on the amount of adhesive remaining on the tooth surface, when brackets were debonded either immediately, following thermocycling or following water storage."

The null hypothesis (H_0) states that the two variables – curing protocol and adhesive remaining on the tooth - are independent to one another, meaning that co-curing the adhesive would have no effect on the amount of adhesive left on the tooth. The alternative hypothesis (H_1) states that the two variables are dependent on one another, meaning that

the amount of adhesive remaining on the tooth is affected by whether the adhesive underwent the pre-cure step or not:

$$H_0: The\ row\ and\ column\ variables\ of\ the\ contingency\ table\ are\ independant$$

$$H_1: The\ row\ and\ column\ variables\ of\ the\ contingency\ table\ are\ dependant$$

This is a classification problem to understand if the categorical variables - pre-cure or co-cure protocol and the amount of adhesive left on the tooth - are dependent on one another. Once more, Fisher's exact test is the more appropriate test, as the expected values for each variable are less than 5.

CHAPTER THREE: RESULTS

CHAPTER THREE: RESULTS

3.1. Bond Strength Test Results at Immediate Debonding

Immediate Debond (within 30 minutes)				
Adhesive	Curing Protocol	Shear Bond Strength (MPa)		
		Mean (S.D.)	Median	Range Min-Max
Xeno V+	PC	7.86 (2.27)	7.68	4.28- 12.89
Clearfil S3 Bond	PC	5.71 (2.43)	5.42	1.61 - 9.92
iBond Self Etch	PC	6.24 (1.96)	6.04	3.55 - 11.29
Xeno V+	CC	7.73 (3.41)	7.44	2.02 - 16.01
Clearfil S3 Bond	CC	5.46 (1.93)	5.34	2.67 - 10.36
iBond Self Etch	CC	4.38 (1.56)	4.31	1.60 - 8.20
Transbond Plus SEP (Control)	CC	7.15 (3.14)	7.02	0.01 - 13.84

Table 3.1. Mean (S.D.) and median shear bond strengths with range values for each adhesive at Immediate Debond (within 30 minutes) for Pre-cure (PC) and Co-cure (CC) groups.

3.1.1. Bond Strengths for Pre-cure Adhesive Types at Immediate Debonding

When looking at the suitability of 1-SEP adhesives for orthodontic bonding the shear bond strengths for three adhesive types, Xeno V+ PC, Clearfil S3 PC and iBond SE PC were compared immediately after bonding (Table 3.1.). Statistical comparison of pre-cured 1-SEP adhesives (PC group) to the control, which is co-cured (CC group), was omitted at this stage to avoid introducing a further variable. The results displayed above in Table 3.1 show that 1-SEP, which are pre-cured in accordance with recommendations by the manufacturer, may have sufficient bonding capability for orthodontic procedures according to the requirements suggested by Littlewood (6) (>5.4 MPa). Xeno V+ PC exhibited a

superior performance and obtained the highest bond strength of the group (7.86 +/- 2.27 MPa) which was significantly higher than the lowest value in the group given by Clearfil S3 PC (5.71 +/- 2.43 MPa) (P<.01). There were no other statistically significant differences between the mean bond strengths of adhesive types in the Pre-cured group (P<.01). The results of the ANOVA and Tukey's comparison of means test are detailed in Appendix 3. Normal distribution of data is one of the assumptions made to perform an ANOVA. A quantile-quantile plot (Q-Q plot) visually demonstrates that the data used in the statistical analysis is normally distributed since the points are close to the plotted line and is also copied in Appendix 3.

A box and whisker plot is shown below in Fig 3.1. to illustrate and compare the spread of data alongside median values for each of the pre-cured 1-SEP adhesives at immediate debonding. The SBS data for iBond SE PC were more concentrated around the median value

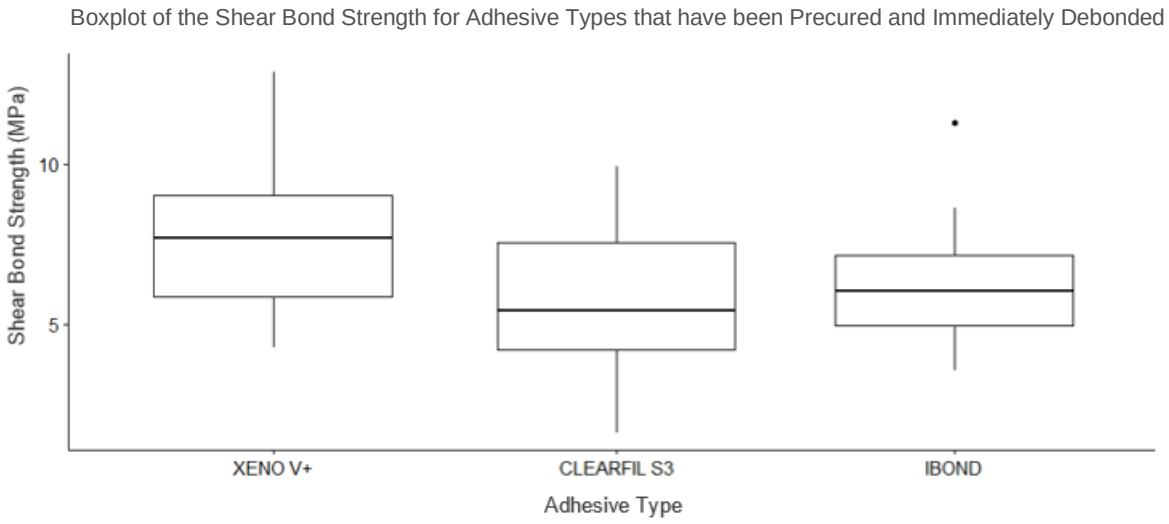


Fig. 3.1. Box and whisker plots (Tukey, 1977) showing frequency distribution of shear bond strengths for adhesives that have been pre-cured (PC) at immediate debonding. The "box" represents the interquartile range (IQR); the ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

and more evenly spread along the scale, than for either Xeno V+ PC or Clearfil S3 PC as indicated by the narrower interquartile range (IQR) for iBond SE PC and the more even sections in its boxplot respectively. Fig. 3.1. also shows that iBond SE PC adhesive had the smallest range of data (range 7.74 MPa) as compared with the other two adhesives, illustrated by its shorter "whiskers", after noting the single high outlying value within its data. Similar ranges between Xeno V+ PC and Clearfil S3 PC are illustrated in Fig. 3.1. (ranges 8.61 MPa and 8.31 MPa respectively) but with a higher median value for Xeno V+ PC clearly visualised by the graph. The bond values for Xeno V+ were also more variable in the higher parts of the scale, visualised by the longer upper "whisker", indicating that bond strengths for Xeno V+ CC may vary particularly at higher MPa values.

3.1.2. Bond Strengths for Co-cure Adhesive Types at Immediate Debonding

To determine the bonding efficacy of 1-SEP using a co-curing technique rather than two separate light cure applications, SBS values for Xeno V+ CC, Clearfil S3 CC and iBond SE CC were compared at immediate debonding and displayed in Table 3.1. The control, Transbond Plus SEP, was included in the analysis here because a co-cure bonding protocol is already the recognised procedure for this established SEP and would not introduce a further variable. The results in Table 3.1. show that Xeno V+ CC and Clearfil S3 CC were capable of clinically adequate bond strengths (>5.4 MPa) according to Littlewood (6). Xeno V+ CC had the highest bond strength of the group (7.73 +/-3.41 MPa) which was significantly higher than iBond SE CC (4.38 +/- 1.56 MPa) ($P<.01$). The mean bond strength value for iBond SE CC was also significantly lower than the control, Transbond Plus SEP (7.15 +/- 3.14 MPa) ($P<.01$), and the lower bond values obtained for iBond SE CC (4.38 +/- 1.56 MPa) were not clinically acceptable. The output for ANOVA and Tukey's test are

given in Appendix 4. A normal distribution to the data is illustrated by the Q-Q plot which is also referenced in Appendix 4.

A box and whisker plot, illustrated below in Fig. 3.2., displays the spread of data points for three 1-SEP adhesives types in Co-cure (CC) groups (Xeno V+ CC, Clearfil S3 CC and iBond SE CC) at immediate debonding alongside the control, Transbond Plus SEP CC, for comparison. IBond SE CC produced the smallest data range (range 6.6 MPa) shown in Fig. 3.2. by its narrower 'box' which represents a smaller interquartile range (IQR), a higher level of agreement between data points and a more consistent performance for iBond SE CC (except for one outlier). By contrast, the control, Transbond Plus SEP CC, presented a greater range to the data (range 13.83 MPa) with less consistency between SBS values.

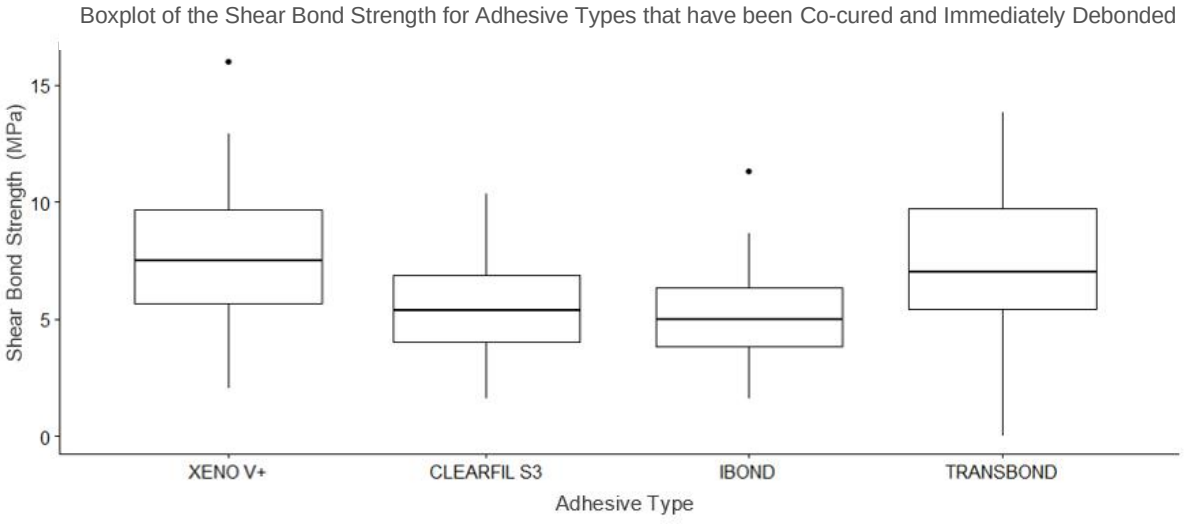


Fig. 3.2. Box and whisker plot (Tukey, 1977) showing frequency distribution of shear bond strengths for adhesives at immediate debonding for Co-cure Groups (CC) including the control. The "box" represents the interquartile range (IQR), the ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median, and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

3.1.3. Comparison of Pre-cure and Co-cure Groups at Immediate Debonding

Statistical analysis with an unpaired t-test was used to understand if there were significant differences between mean bond strengths obtained for 1-SEP at immediate debonding between Pre-cure (PC) and Co-cure (CC) groups which are displayed in Table 3.1. The output of the statistical tests are copied in Appendix 5. The findings indicate that there were no significant differences for either Xeno V+ or Clearfil S3 between PC and CC groups which suggests that bond strengths for these two adhesives were not significantly affected by omission of the pre-cure step when debonded immediately ($P<.05$) (Xeno PC 7.86 +/- 2.27 MPa vs CC 7.73 +/- 3.41 MPa; Clearfil S3 PC 5.71 +/- 2.43 MPa vs CC 5.46 +/- 1.93 MPa). There was, however, a statistically significant difference for iBond SE between mean bond strengths in PC and CC groups ($P=.002$) (IB PC 6.24 +/- 1.96 MPa vs CC 4.38 +/- 1.56 MPa) and is also supported by the boxplot in Fig. 3.3. which visualises the difference between the median values in PC and CC groups, depicted by the horizontal lines within the inner-quartile boxes. The results indicate that iBond SE was significantly affected by removing the pre-cure step, which lowered the mean bond strength value to a clinically ineffective level (>5.4 MPa, Littlewood 2000) (6) ($P=.002$).

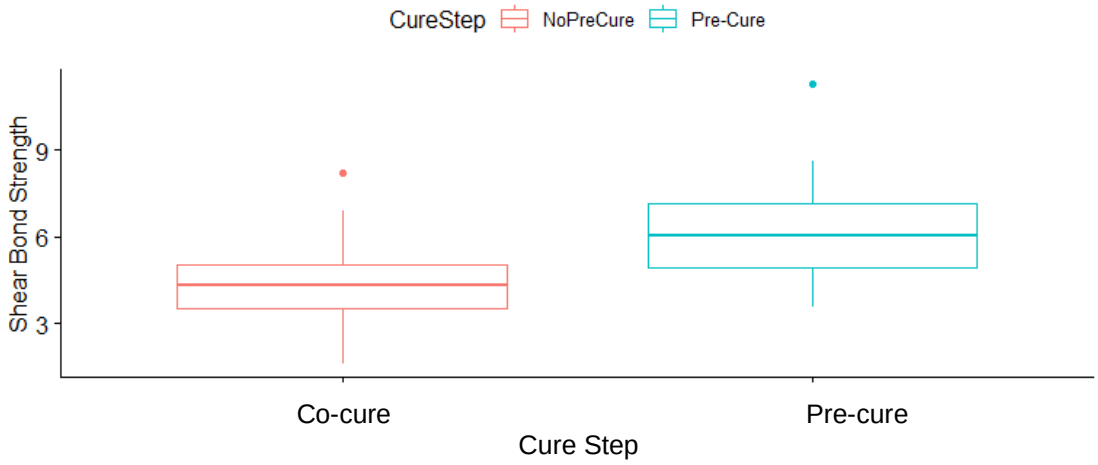


Fig. 3.3. Box and whisker plots (Tukey, 1977) showing the frequency distribution of shear bond strengths for iBond SE at immediate debonding comparing the co-cured adhesive with pre-cured adhesive. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values. Outliers are plotted with a small circle.

3.2. Bond Strength Test Results after Thermocycling

Thermocycling between 5 ºC and 55 ºC for 500 cycles				
Adhesive	Curing Protocol	Shear Bond Strength (MPa)		
		Mean (SD)	Median	Range Min-Max
Xeno V+	PC	2.11 (1.73)	1.67	0.04 - 5.06
Clearfil S3 Bond	PC	4.36 (3.31)	3.38	0.72 - 12.92
iBond Self Etch	PC	0.66 (0.62)	0.61	0.03- 2.05
Xeno V+	CC	8.04 (3.72)	8.16	0.49 - 14.95
Clearfil S3 Bond	CC	6.69 (5.13)	6.55	0.04 - 23.14
iBond Self Etch	CC	2.93 (3.09)	2.01	0.03 - 10.78
Transbond Plus SEP (Control)	CC	12.90 (6.38)	12.40	3.97 - 24.80

Table 3.2. Mean (S.D.) and median shear bond strengths with range values for each adhesive after thermocycling for Pre-cure (PC) and Co-cure (CC) groups.

3.2.1. Bond Strengths for Pre-cure Adhesive Types after Thermocycling

Adhesives that were pre-cured (PC) were subjected to a thermocycling regimen between 5 ºC and 55 ºC for 500 cycles to evaluate bond durability under thermal stress. The results after thermocycling are shown above in Table 3.2. None of the 1-SEP in Pre-cure groups were able to demonstrate mean bond strengths high enough for clinical use after thermocycling according to reference values suggested by Littlewood (6) (>5.4 MPa) (Xeno V+ PC 2.11 +/- 1.73 MPa, Clearfil S3 PC 4.36 +/- 3.31 MPa and iBond SE PC 0.66 +/- 0.62 MPa). The mean bond strength value for Clearfil S3 PC was statistically higher than for both Xeno V+ PC and iBond SE PC although all given values were low ($P<.01$). The statistical output from ANOVA and Tukey's comparison of means test are copied in Appendix 6 and the Q-Q plot validates that the data for PC adhesive groups after thermocycling is normally distributed (Appendix 6).

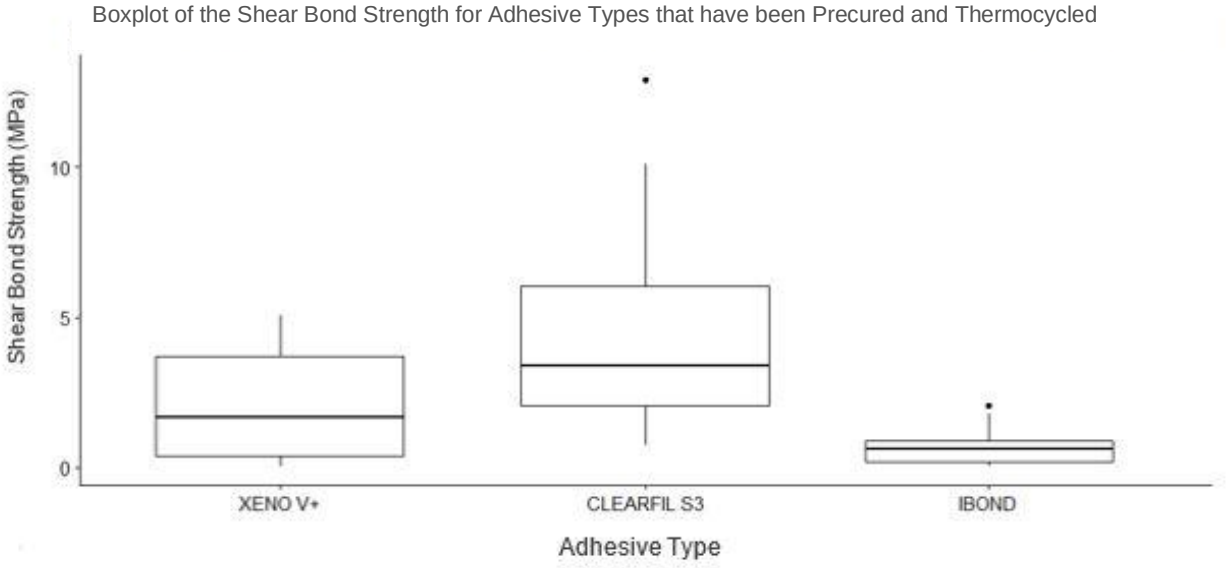


Fig. 3.4. Box and whisker plots (Tukey, 1977) showing frequency distribution of shear bond strengths for adhesives after thermocycling for Pre-Cure Groups (PC). The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

A box and whisker plot is shown above in Fig. 3.4. illustrating the spread of data and comparison of median values for three 1-SEP adhesive types, Xeno V+, Clearfil S3 and iBond SE in PC groups alongside the control, following thermocycling. This type of graph is also useful to detect outliers, where points may differ by a substantial amount from the rest of the data. Outliers are values that are beyond 1.5 times the interquartile range (IQR). IBond SE PC clearly had the lowest median value (0.61 MPa) of the group and the main bulk of its data was heavily concentrated around this low median value, as indicated by its short interquartile range or "box". The low median and narrow range (range 2.02 MPa) given for iBond SE PC suggests that this adhesive gave consistently poor bond strength values after thermocycling. For adhesives, Xeno V+ PC and Clearfil S3 PC the median values (1.67 MPa and 3.38 MPa respectively) are also low and below minimum clinical requirements. However, for Clearfil S3 PC, the data is more spread out in the upper parts of the scale seen by the larger upper section of the "box", the longer upper "whisker" and single

outlier at 23.14 MPa. The greater range for Clearfil S3 (range 12.20 MPa), and skew of data towards the upper spectrum, indicates that although the median value is low, the bond values vary from one another at higher MPa values suggesting there is opportunity for clinically acceptable bond values, but this opportunity is not consistent.

3.2.2. Bond Strengths for Co-cure Adhesive Types after Thermocycling

The bonding performance of 1-SEP adhesives using a single co-cure (CC) photopolymerisation method was evaluated after thermocycling between 5 ºC and 55 ºC for 500 cycles and the results are shown in Table 3.2. Both Xeno V+ CC and Clearfil S3 CC demonstrated clinically acceptable mean bond strengths (8.04 +/- 3.72 MPa and 6.69 +/- 5.13 MPa respectively) after thermocycling, which indicates these two 1-SEP adhesives may be able to perform reliably under these conditions even when the pre-cure was omitted (>5.4 MPa) (6). The mean bond strength for Xeno V+ CC was the highest value for the group (8.04 +/- 3.72 MPa) which was not significantly different from the control, Transbond Plus SEP (12.90 +/- 6.38 MPa) (P=.011). IBond SE CC produced the lowest mean bond strength in the group (2.93 +/- 3.09 MPa) which was below that which is considered clinically useful and was significantly lower than the control (P<.001). Details of the statistical output for ANOVA, Tukey's test and the Q-Q plot are copied in Appendix 7. The findings from the Tukey's test, which indicates significant differences between mean pairs iBond SE-Transbond Plus, iBond SE-Xeno V+ and Clearfil S3-Transbond Plus is also supported from observations of the boxplot graph in Fig. 3.5. as these pairs also have the biggest differences between their median values, illustrated by the horizontal lines within each "box".

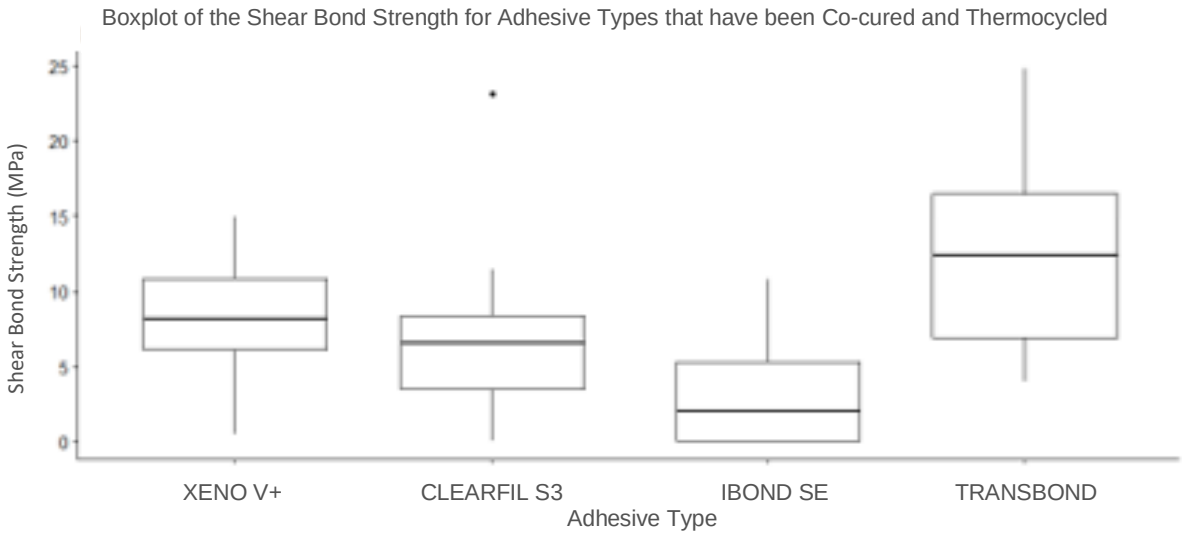


Fig. 3.5. Box and whisker plots (Tukey, 1977) showing frequency distribution of shear bond strengths for adhesives after thermocycling for Co-cure groups (CC) including the control. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

A box and whisker plot shown above in Fig. 3.5. is used to visualise the spread of data and range values for Co-cure (CC) adhesives after thermocycling. The interquartile range (IQR), indicated by the width of the "box", suggests a better relative concentration of data around the median for 1-SEP Xeno V+ CC, Clearfil S3 CC and iBond SE CC as compared with the control, Transbond Plus SEP, which produced a larger IQR, greater variability to the data (range 20.83 MPa) and a longer upper "whisker" extending to a maximum SBS value of 24.8 MPa without outliers. Comparison of median values, illustrated in Fig. 3.5. by the horizontal line in each "box", shows higher values for 1-SEP adhesives Xeno V+ and Clearfil S3 than for iBond SE, indicating that these two 1-SEP may be able to produce reliable, clinically acceptable bond strengths after thermocycling and without a pre-cure. However, observations of the large spread of data for Transbond Plus signifies a lower level of agreement between the bond strengths and greater variance between data points at higher

SBS values, indicating the possibility of very high bond strengths for Transbond Plus which can be less clinically desirable.

3.2.3. Comparison between Pre-cure and Co-cure Groups after Thermocycling

Statistical analysis with an unpaired t-test was used to identify any significant differences between mean bond strengths obtained for 1-SEP after thermocycling between Pre-cure (PC) and Co-cure (CC) groups, displayed in Table 3.2. All 1-SEP showed improvements in bond strengths in CC groups compared with corresponding PC groups after thermocycling, and the rise in values for Xeno V+ and iBond SE was statistically significant ($P<.05$) (Xeno V+ PC 2.11 +/- 1.73 MPa vs CC 8.04 +/- 3.72 MPa; Clearfil S3 PC 4.36 +/- 3.31 MPa vs CC 6.69 +/- 5.13 MPa; iBond SE PC 0.66 +/- 0.62 MPa vs iBond SE CC 2.93 +/- 3.09 MPa). The outcome from the t-test is supported by boxplots for Xeno V+ in Fig. 3.6. and iBond SE in Fig. 3.7., where Fig. 3.6. illustrates the entire IQR of the co-cured Xeno V+ adhesive above the upper quartile value of the pre-cured adhesive for Xeno V+. For iBond SE in Fig.3.7., the difference between the median values is easily visualised, in addition to the smaller range evident for iBond SE after thermocycling. Bond strengths for 1-SEP appear to benefit by the omission of a pre-cure step when thermocycled, and for Xeno V+ and Clearfil S3, this improvement raised mean bond strengths to a clinically acceptable standard (>5.4 MPa) (6). The output of the statistical tests has been copied in Appendix 8.

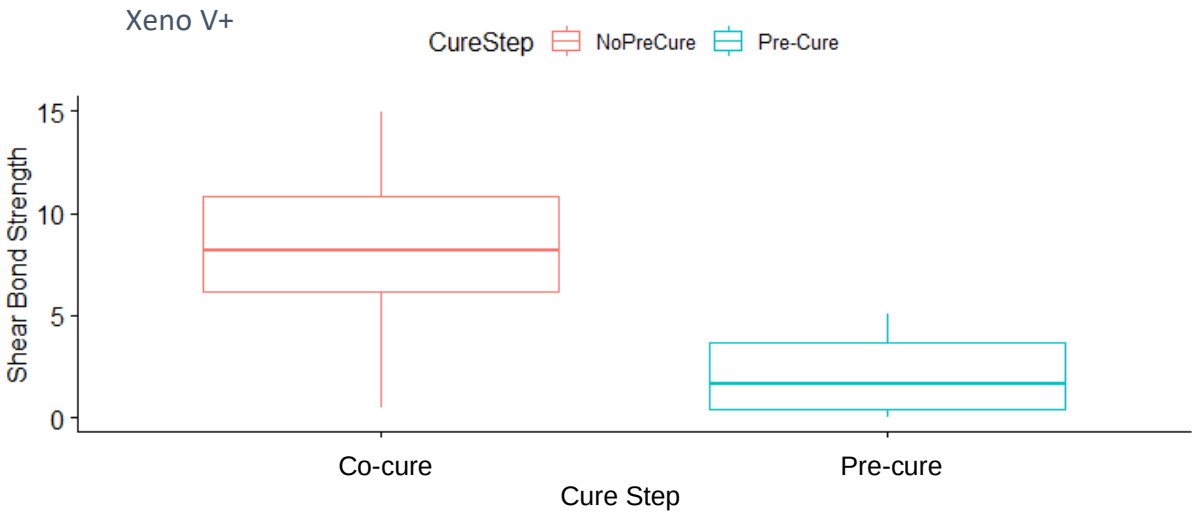


Fig. 3.6. Box and whisker plots (Tukey, 1977) showing the frequency distribution of shear bond strengths for Xeno V+ after thermocycling, comparing the Co-cure (CC) group with Pre-cure (PC) group. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

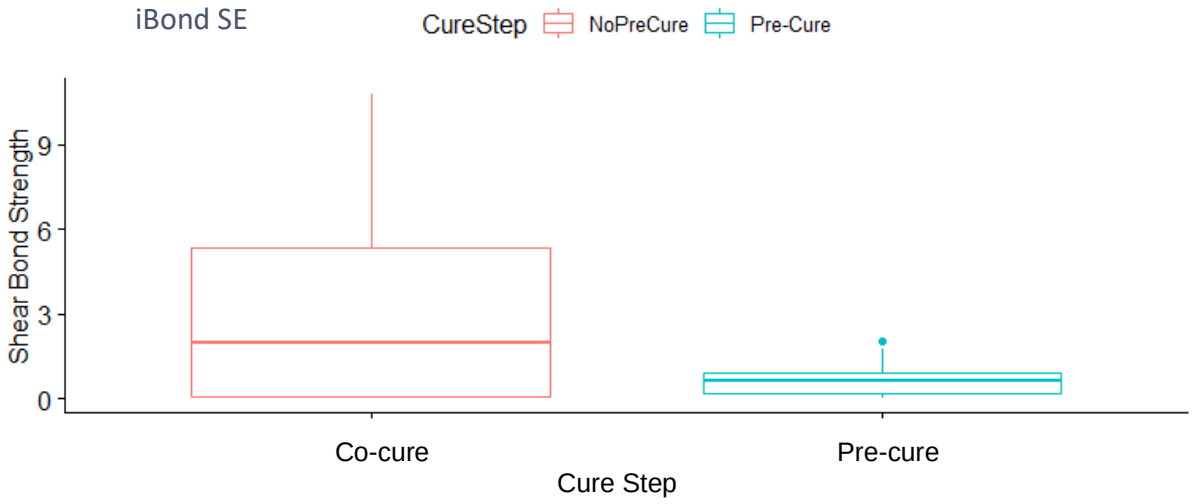


Fig. 3.7. Box and whisker plots (Tukey, 1977) showing the frequency distribution of shear bond strengths for iBond SE after thermocycling, comparing the Co-cure (CC) group with Pre-cure (PC) group. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

3.3. Bond Strength Test Results after Water Storage

Water Storage at 37°C for 7 days				
Adhesive	Curing Protocol	Shear Bond Strength (MPa)		
		Mean (SD)	Median	Range Min-Max
Xeno V+	PC	3.10 (3.08)	2.31	0.04 - 10.28
Clearfil S3 Bond	PC	5.16 (3.43)	4.20	0.03 - 11.99
iBond Self Etch	PC	4.48 (1.85)	4.43	1.75 - 7.34
Xeno V+	CC	13.09 (4.01)	12.69	7.43 - 22.99
Clearfil S3 Bond	CC	13.07 (4.03)	12.45	7.23 - 20.75
iBond Self Etch	CC	6.04 (2.37)	6.00	2.45 - 11.27
Transbond Plus SEP (Control)	CC	12.68 (4.74)	12.79	6.39 - 22.85

Table 3.3. Mean (S.D.) and median shear bond strengths with range values for each adhesive after water storage for Pre-cure (PC) and Co-cure (CC) groups.

3.3.1. Bond Strengths for Pre-cure Adhesive Types after Water Storage

It is important that any prospective orthodontic adhesive can maintain clinically effective bonds under wet conditions. The bonding effectiveness of 1-SEP adhesives following water storage was investigated by subjecting adhesives to a regimen of 7 days submersion in water at 37 °C, and results are displayed above in Table 3.3. All mean bond strength values for pre-cured (PC) 1-SEP adhesives were low after water storage and below recommended values for orthodontic clinical practice (>5.4 MPa) (6) (Xeno V+ PC 3.10 +/- 3.08 MPa; Clearfil S3 PC 5.16 +/- 3.43 MPa; iBond SE PC 4.48 +/- 1.85MPa). There was no significant difference between mean bond strength values of PC 1-SEP adhesive types ($P<.01$). The ANOVA test, Tukey's comparison of means test and Q-Q plot, which verifies normal distribution to the data, are copied in Appendix 9.

A box and whisker plot shown below in Fig. 3.8. is used to illustrate and compare the spread of data points and median values for PC 1-SEP adhesives after water storage. All median bond strengths given for adhesives Xeno V+ PC (2.31 MPa), Clearfil S3 PC (4.20 MPa) and iBond SE PC (4.43 MPa) were below minimum clinical requirements (>5.4 MPa) (6) and represented by the horizontal line in each "box". The boxplot graph in Fig. 3.8. supports the ANOVA finding that there was no statistically significant difference between the bond strengths in the group, since the median points are relatively close and the interquartile ranges are seen to overlap. Longer upper "whiskers" seen for Xeno V+ PC and Clearfil S3 PC indicate that, whilst the median value is closer to the lower end of the spectrum, the data for Xeno V+ PC and Clearfil S3 PC are more spread out at higher MPa values, extending to maximum values 10.28 MPa and 11.99 MPa respectively. It appears that higher bond strengths are possible for Xeno V+ PC and Clearfil S3 PC after water storage, whilst for iBond SE PC, clinically unsatisfactory bond strengths after water storage are more consistent.

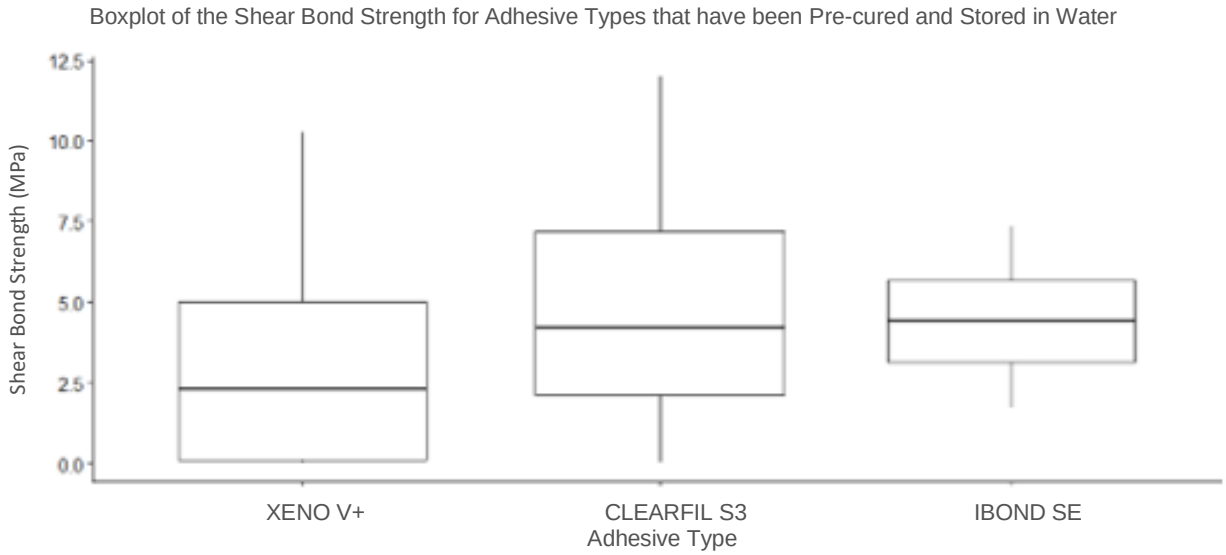


Fig. 3.8. Box and whisker plots (Tukey, 1977) showing frequency distribution of shear bond strengths for pre-cured (PC) 1-SEP adhesives following water storage. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

3.3.2. Bond Strengths for Co-cure Adhesive Types after Water Storage

Bond strength data for co-cured (CC) adhesives that underwent water submersion at 37 °C for 7 days are presented in Table 3.3. Xeno V+ CC and Clearfil S3 CC demonstrated mean bond strengths after water storage which were above minimum clinical reference values (>5.4 MPa) (6) and not statistically significantly different to the control, Transbond Plus SEP (*P*<.01) (Xeno V+ CC 13.09 +/- 4.01; Clearfil S3 CC 13.07 +/- 4.03 MPa; Transbond Plus SEP 12.68 +/- 4.74). The mean bond strength value for iBond SE CC was clinically acceptable (6.04 +/- 2.37 MPa) but significantly lower than the control, as well as to the other 1-SEP adhesives in the group (*P*<.01). The ANOVA test, Tukey's test and Q-Q plot are copied in Appendix 10. A box and whisker plot shown below in Fig.3.9. illustrates the data spread for co-cured adhesives after water storage. IBond SE clearly demonstrated the lowest median value for the group (6.0 MPa) as represented by the horizontal line within its "box".

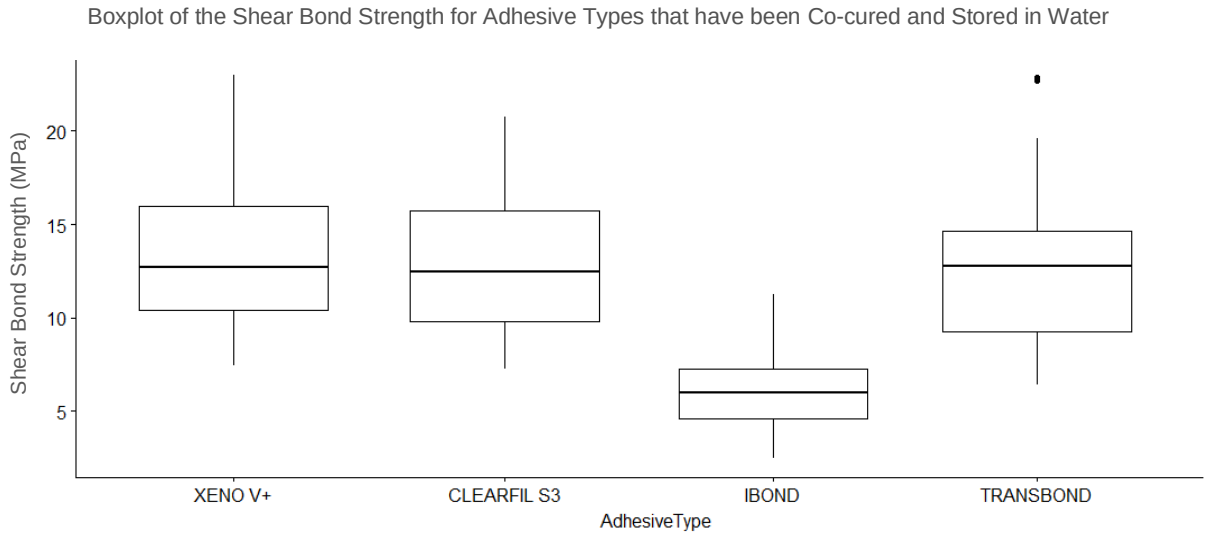


Fig. 3.9. Box and whisker plots (Tukey, 1977) showing frequency distribution of shear bond strengths for Co-cure (CC) adhesives following water storage. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

The boxplot in Fig. 3.9. also supports the ANOVA finding, that all statistical pairings with iBond SE CC adhesive type were significantly different from other adhesive type combinations. This is illustrated in Fig. 3.9. by the median line for iBond SE CC, which appears much lower than median values for the other adhesive types in the graph, and is below the lower quartile of other adhesives in the group. IBond SE CC also had the shortest interquartile range (IQR) represented by the smaller width of the "box" indicating a higher level of agreement between the data around this lower median value. It appears that the performance for iBond SE CC after water storage was the most consistent in the group, but at much lower bond strengths, comparatively. Higher median values were observed for Xeno V+ (12.69 MPa) and Clearfil S3 (12.45 MPa) which appear similar to the control, Transbond Plus SEP (12.79 MPa), and support the ANOVA findings. However, the plots for adhesives Xeno V+ CC and Clearfil S3 CC were characterised by larger ranges, as was the control, and notably there was a 15.56 MPa spread between the highest and lowest bond values for Xeno V+ CC, which was exceptional. The box plot in Fig. 3.9. highlights a good performance for average bond strengths produced for co-cured Xeno V+ and Clearfil S3 after water storage, which was similar to the control. However, once again, large data ranges for the best performing adhesives were observed.

3.3.3. Comparison between Pre-cure and Co-cure Groups after Water Storage

Statistical analysis with an unpaired t-test was used to understand if there were significant differences between mean bond strengths obtained for the adhesives after water storage between Pre-cure (PC) and Co-cure (CC) groups in Table 3.3. The statistical findings indicate that all 1-SEP mean bond strengths given for Co-cure groups were significantly higher than those given for corresponding Pre-cure groups ($P<.05$) (Xeno V+ PC: 3.10 +/-

3.08 MPa vs CC 13.09 +/- 4.01 MPa; Clearfil S3 PC 5.16 +/- 3.43 MPa vs CC 13.07 +/- 4.03 MPa; iBond SE PC 4.48 +/- 1.85 vs CC 6.04 +/- 2.37 MPa). These results suggests that clinically acceptable bond strengths (>5.4 MPa) (6) may be achievable for 1-SEP without a pre-cure after water storage, and that omitting the pre-cure step may be beneficial to the strength of the bond. The greatest rise in bond strength from PC to CC groups was detected for Xeno V+ and Clearfil S3, whereby omission of a pre-cure step elevated mean bond strengths to values which were not statistically different to the control (P<.05) (Transbond Plus SEP CC 12.68 +/- 4.74 MPa). The summary statistics are copied in Appendix 11. Output of the statistical tests are supported by the boxplots for each 1-SEP adhesive (Xeno V+ Fig. 3.10.; Clearfil S3 Fig. 3.11.; iBond SE Fig. 3.12.) which show clear differences between the median average values of PC and CC adhesives, and wherein the whole IQR range of pre-cured Xeno V+ and Clearfil S3 adhesive, is below that of the co-cured adhesive.

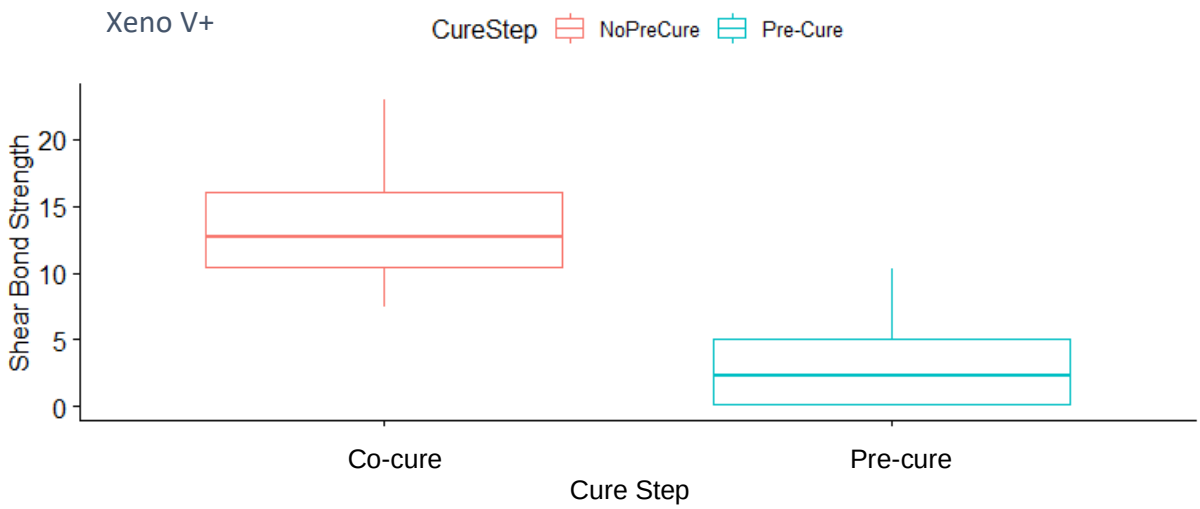


Fig. 3.10. Box and whisker plots (Tukey, 1977) showing the frequency distribution of shear bond strengths for Xeno V+ after water storage, comparing the Co-cure (CC) group with Pre-cure (PC) group. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

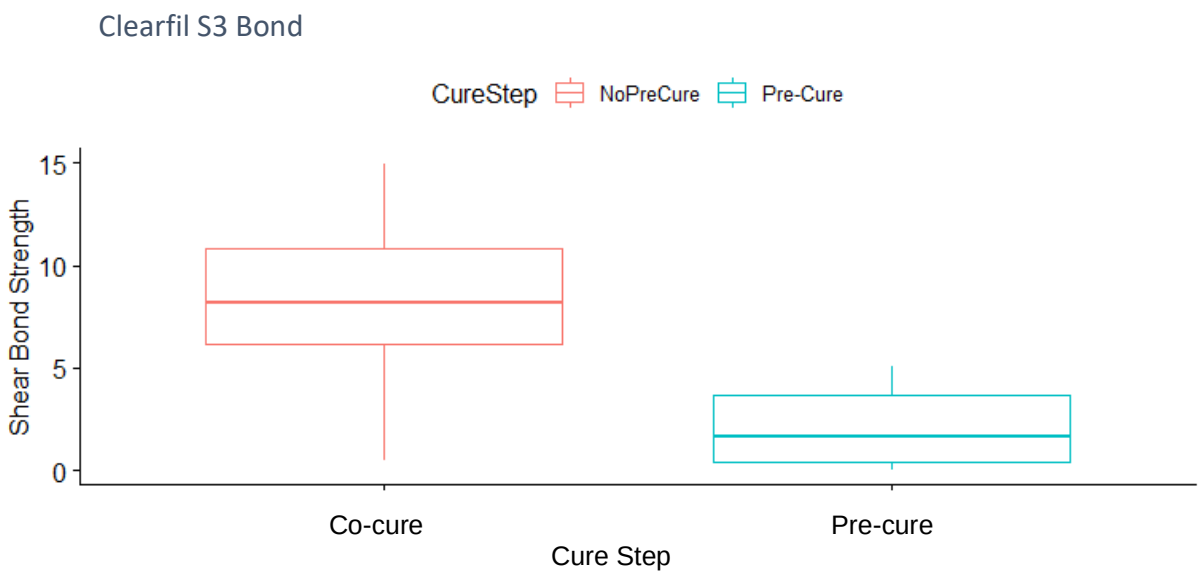


Fig. 3.11. Box and whisker plots (Tukey, 1977) showing the frequency distribution of shear bond strengths for Clearfil S3 after water storage, comparing the Co-cure (CC) group with Pre-cure (PC) group. The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR) of the corresponding quartile. Outlier values are plotted with a small circle.

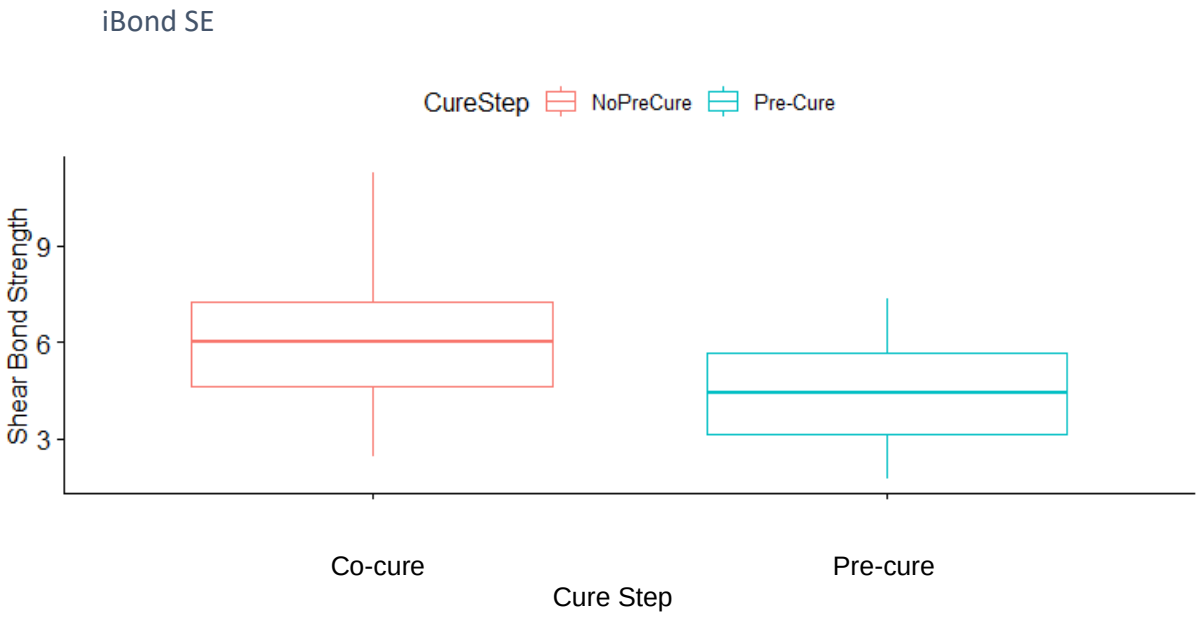


Fig. 3.12. Box and whisker plots (Tukey, 1977) showing the frequency distribution of shear bond strengths for iBond SE after water storage, comparing the Co-cure group (CC) with Pre-cure group (PC). The ends of the "box" represent the upper and lower quartiles, the horizontal line in the box is the median and the ends of the "whiskers" are minimum and maximum values still within 1.5 (IQR)of the corresponding quartile. Outlier values are plotted with a small circle.

3.4 Adhesive Remnant Index (ARI) Results at Immediate Debonding

Group (n=20)	ARI scores (%)			
	0	1	2	3
Xeno V+ (PC)	14 (70)	5 (25)	1 (5)	0
Clearfil S3 Bond (PC)	7 (35)	13 (65)	0	0
iBond Self Etch (PC)	14 (70)	6 (30)	0	0
Xeno V+ (CC)	18 (90)	2 (10)	0	0
Clearfil S3 Bond (CC)	7 (35)	11 (55)	1 (5)	1 (5)
iBond Self Etch (CC)	12 (60)	8 (40)	0	0
Transbond Plus SEP (Control)	0	6 (30)	3 (15)	11(55)

Table 3.4. Adhesive Remnant Index Scores (Årtun and Bergland, 1984) for each adhesive at Immediate Debond (after 30 minutes) for Pre-cure (PC) and Co-cure (CC) groups.

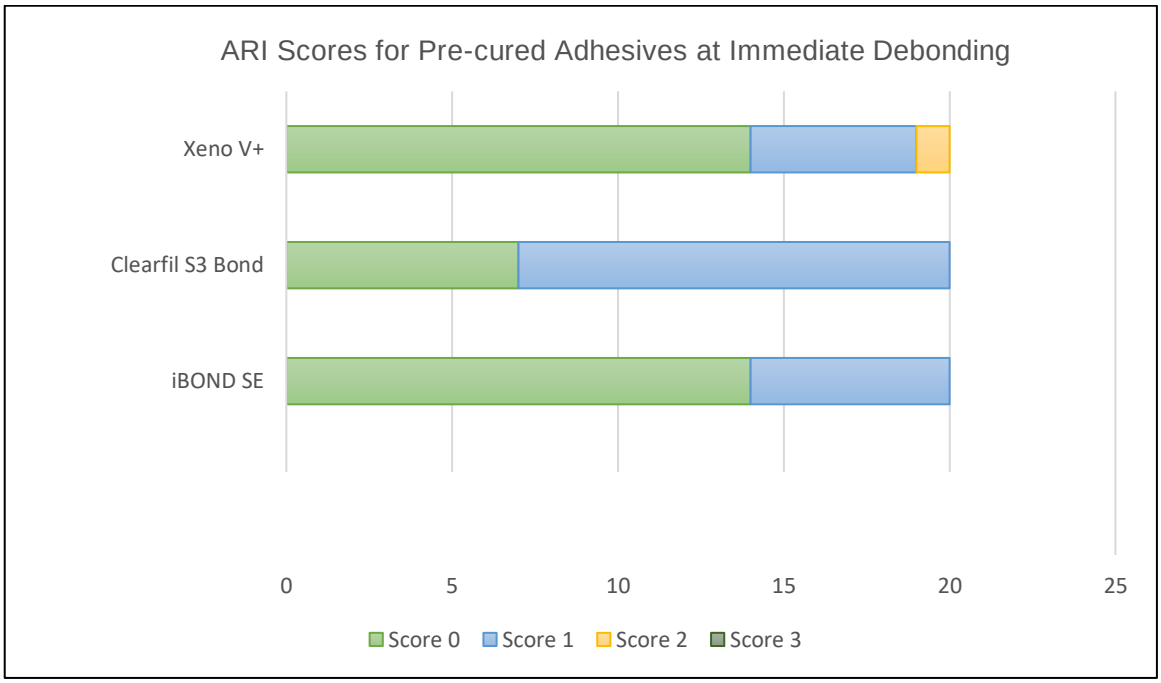
3.4.1. ARI Scores for Pre-cure Adhesive Types at Immediate Debonding

The failure characteristics of 1-SEP that had been pre-cured and debonded immediately, Xeno V+ PC, Clearfil S3 PC and iBond SE PC, were compared through examination of the Adhesive Remnant Index (ARI) by Årtun and Bergland, 1984 (358). The results of the ARI scores, indicating the amount of adhesive remaining on the tooth, are shown above in Table 3.4. and illustrated graphically in Fig 3.13. For Xeno V+ PC, Clearfil S3 PC and iBond SE PC, there was a high frequency (≥95 per cent) of ARI scores of 0 or 1, which indicated a tendency for less residual adhesive to remain on the enamel than on the bracket base after debonding, when the adhesive was pre-cured. The results of the ARI scores in Table 3.4. are supported by the horizontal bar chart in Fig. 3.13. which clearly visualises the greater frequency of scores 0 and 1 (green and blue respectively) for Xeno V+ PC,

Clearfil S3 PC and iBond SE PC. A Fisher's exact test was used as an association test to understand if the two categorical variables - adhesive type and the amount of adhesive left on the tooth - were dependent on one another. The results of the Fisher's test indicate that the type of pre-cured 1-SEP adhesive used does not impact on the amount of adhesive remaining on the tooth when debonded immediately (P=.03). The contingency table and output from the Fisher's exact test for Pre-cure groups (PC) are copied in Appendix 12.

3.4.2. ARI Scores for Co-cure Adhesive Types at Immediate Debonding

The ARI scores for adhesive types which had been co-cured (CC) and debonded within half-an-hour from initial bonding are displayed in Table 3.4. and depicted graphically in Fig. 3.13. For co-cured 1-SEP, there was a tendency to leave very little adhesive on the tooth surface, indicated by higher frequency (≥90 per cent) of ARI scores of 0 or 1. This was in contrast to the control, Transbond Plus SEP CC, which left significantly more residual adhesive on the enamel surface than 1-SEP (P<.001), demonstrated by a higher frequency (70 per cent) of ARI scores 2 and 3 for Transbond Plus SEP, and which is clearly visible from the graph in Fig. 3.13. The control was included in the analysis here, given that co-curing is the established protocol for Transbond Plus SEP, and would not introduce a further variable. A Fisher's exact test signified that the variables - adhesive type and the amount of adhesive left on the tooth - are dependent on one another, which indicates that the type of adhesive used does influence the amount of adhesive left on the tooth. The demonstration of a dependent relationship between variables is in accordance with the contingency table, which shows 1-SEP adhesives that are co-cured tend to leave less adhesive on the tooth surface, whereas the control SEP, Transbond Plus, is likely to leave much more adhesive on the enamel surface when debonded immediately (P<.001). Statistical summaries for the Fisher's exact test and contingency table are copied in Appendix 13.



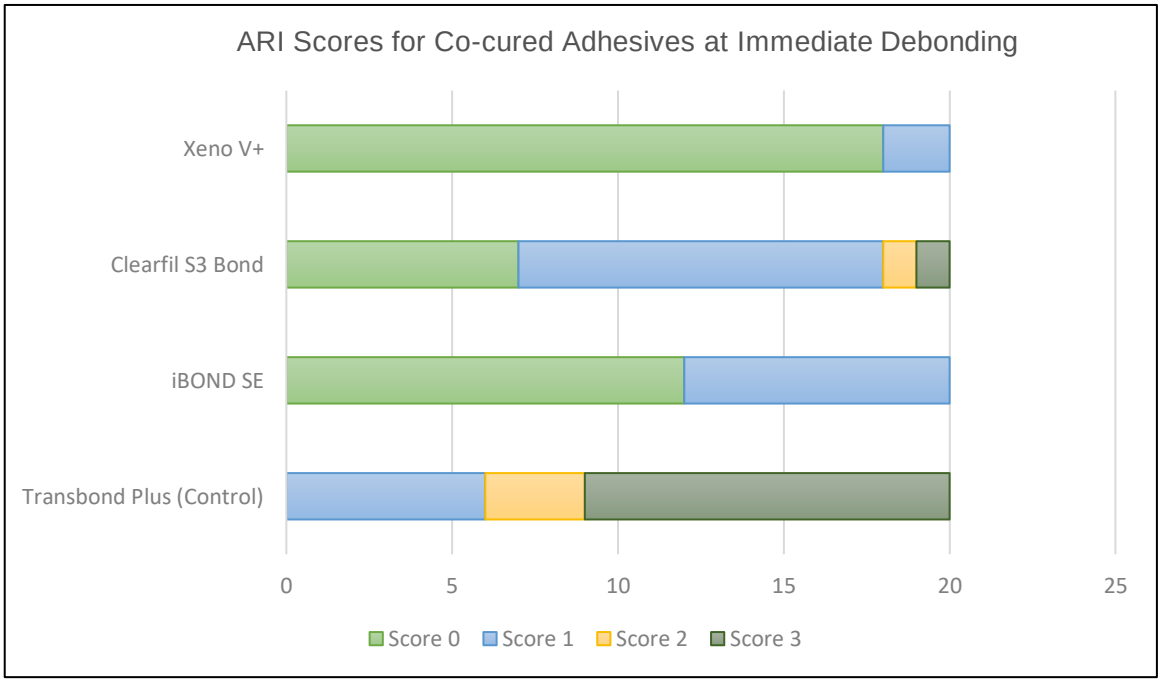


Fig. 3.13. Graphical illustration of ARI Scores for adhesives at immediate debond in Pre-cure (PC) groups (above) and Co-cure (CC) groups (below). Score 0 = No adhesive left of the tooth; Score 1 = Less than half of the adhesive left on the tooth; Score 2 = More than half of the adhesive left on the tooth; Score 3 = All the adhesive left on the tooth with distinct impression of the bracket mesh (Årtun and Bergland, 1984).

3.5. Adhesive Remnant Index (ARI) Results after Thermocycling

Group (n=20)	ARI scores (%)			
	0	1	2	3
Xeno V+ (PC)	15 (75)	5 (25)	0	0
Clearfil S3 Bond (PC)	7 (35)	13 (65)	0	0
iBond Self Etch (PC)	15 (75)	5 (25)	0	0
Xeno V+ (CC)	8 (40)	12 (60)	0	0
Clearfil S3 Bond (CC)	8 (40)	7 (35)	4 (20)	1 (5)
iBond Self Etch (CC)	17 (85)	3 (15)	0	0
Transbond Plus (Control)	3 (15)	17 (85)	0	0

Table 3.5. Adhesive Remnant Index Scores (Årtun and Bergland, 1984) for each adhesive after thermocycling for 500 cycles between 5°C and 55°C for Pre-cure (PC) and Co-cure (CC) groups.

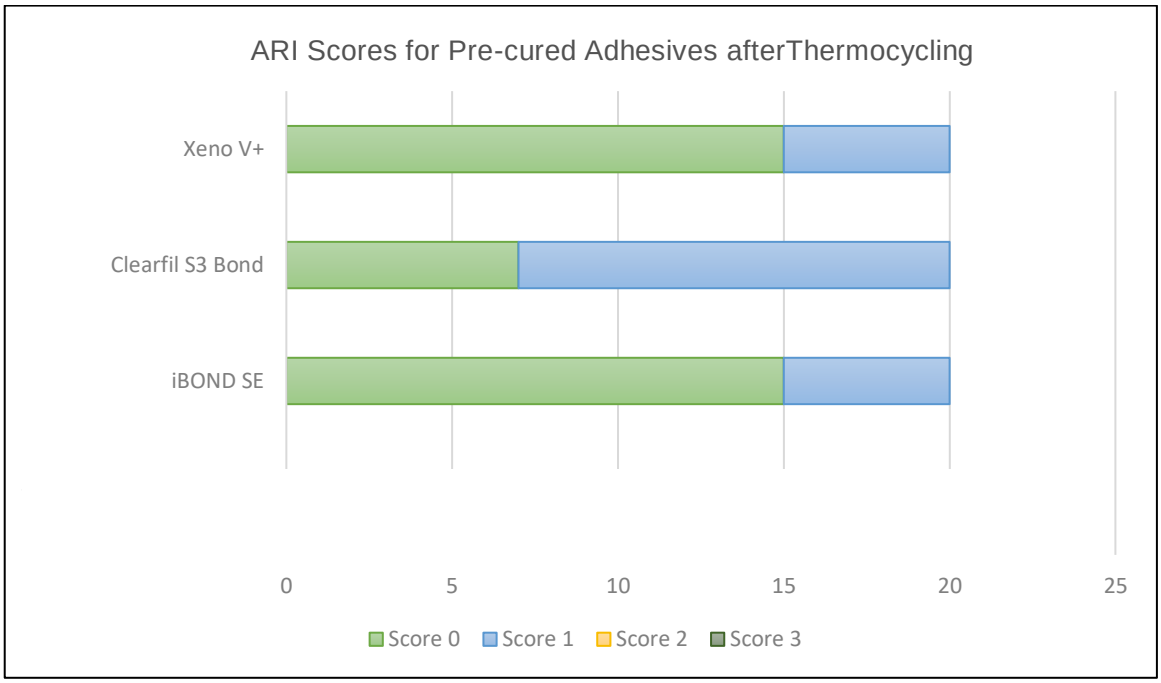
3.5.1. ARI Scores for Pre-cure Adhesive Types after Thermocycling

The ARI scores for pre-cured 1-SEP adhesive types, Xeno V+ PC, Clearfil S3 PC and iBond SE PC, were recorded after thermocycling for 500 cycles between 5 °C and 55 °C to investigate the impact of thermal stress on the failure mode of 1-SEP. The results of the ARI scores after thermocycling for PC groups are displayed above in Table 3.5. and presented graphically in Fig. 3.14. After thermocycling, all pre-cured 1-SEP PC adhesives recorded ARI scores of either 0 or 1, which indicated that the amount of residual adhesive on the tooth after debonding was less than 50 per cent. A Fisher's exact test and contingency table (Appendix 14) showed that adhesive type does not significantly impact on the amount of remnant adhesive on the tooth surface after thermocycling, when 1-SEP is pre-cured (P=.02).

Thus, you are just as likely to have an ARI score of 0 or 1 for all 1-SEP adhesives after thermocycling that are pre-cured as the variables - adhesive type and the amount of adhesive left on the tooth - are independent of one another. The graph in Fig. 3.14. supports the output of the Fisher's test by clearly displaying the greater frequency of scores 0 and 1 (green and blue respectively) for all three pre-cured 1-SEP materials.

3.5.2. ARI Scores for Co-cure Adhesive Types after Thermocycling

The ARI scores after thermocycling for co-cured 1-SEP, Xeno V+ CC, Clearfil S3 CC, iBond SE CC as well as the co-cured control SEP, Transbond Plus CC, are given in Table 3.5. and represented graphically in Fig. 3.14. There were significant differences in the failure mode between adhesive types after thermocycling - Clearfil S3 was statistically more likely to present a cohesive failure type at debond, leaving more adhesive on the tooth surface with higher incidences of scores 2 or 3, by comparison to the other adhesive types in the group, which presented with lower ARI scores after thermocycling (P <.001). Another significant difference was for iBond SE, which was more likely to leave no remnant on the tooth (ARI score 0) than the other adhesive types (P <.001). The control SEP, Transbond Plus, left less adhesive remnant on the tooth surface after thermocycling, compared to when debonded immediately, indicated by greater frequencies of lower ARI scores after thermocycling. The Fisher's exact test and contingency table are copied in Appendix 15.



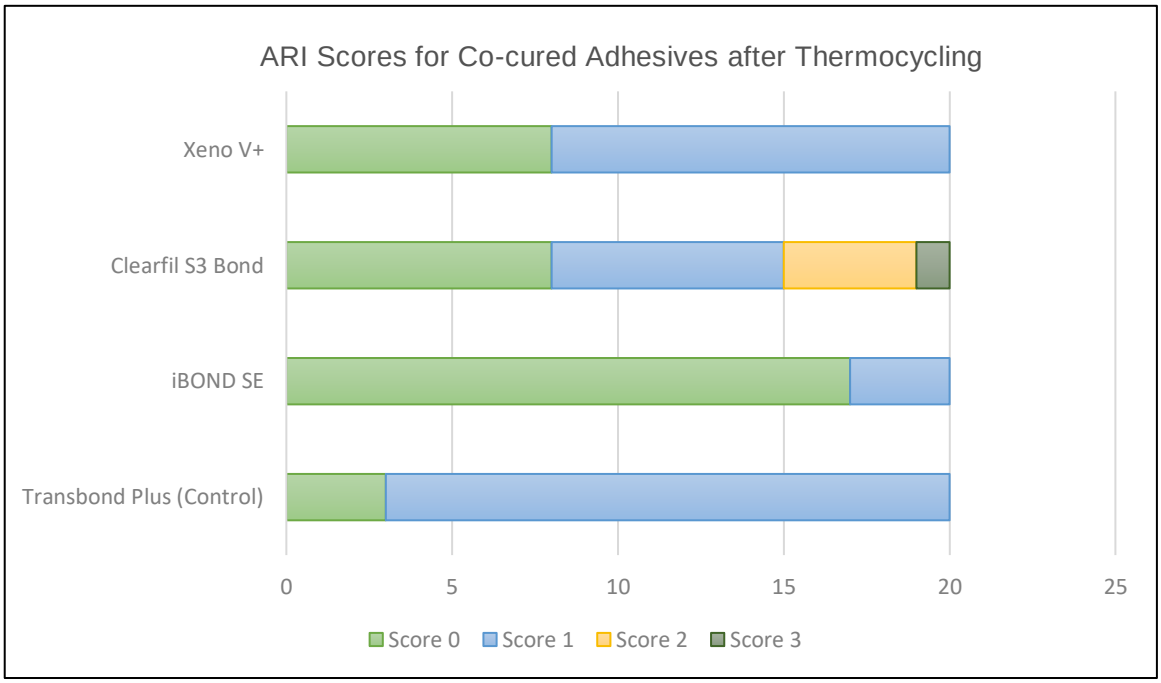


Fig. 3.14. Graphical illustration of ARI Scores for adhesives after thermocycling in Pre-cure (PC) groups (above) and Co-cure (CC) groups (below). Score 0 = No adhesive left of the tooth; Score 1 = Less than half of the adhesive left on the tooth; Score 2 = More than half of the adhesive left on the tooth; Score 3 = All the adhesive left on the tooth with distinct impression of the bracket mesh (Årtun and Bergland, 1984).

3.6. Adhesive Remnant Index (ARI) Results after Water Storage

Group (n=20)	ARI scores (%)			
	0	1	2	3
Xeno V+ (PC)	12 (60)	8 (40)	0	0
Clearfil S3 Bond (PC)	12 (60)	6 (30)	2 (10)	0
iBond Self Etch (PC)	20 (100)	0	0	0
Xeno V+ (CC)	4 (20)	15 (75)	1 (5)	0
Clearfil S3 Bond (CC)	1 (5)	12 (60)	3 (15)	4 (20)
iBond Self Etch (CC)	13 (65)	7 (35)	0	0
Transbond Plus SEP (Control)	1 (5)	9 (45)	5 (25)	5 (25)

Table 3.6. Adhesive Remnant Index Scores (Årtun and Bergland, 1984) for each adhesive after water storage for 7 days for Pre-cure (PC) and Co-cure (CC) groups.

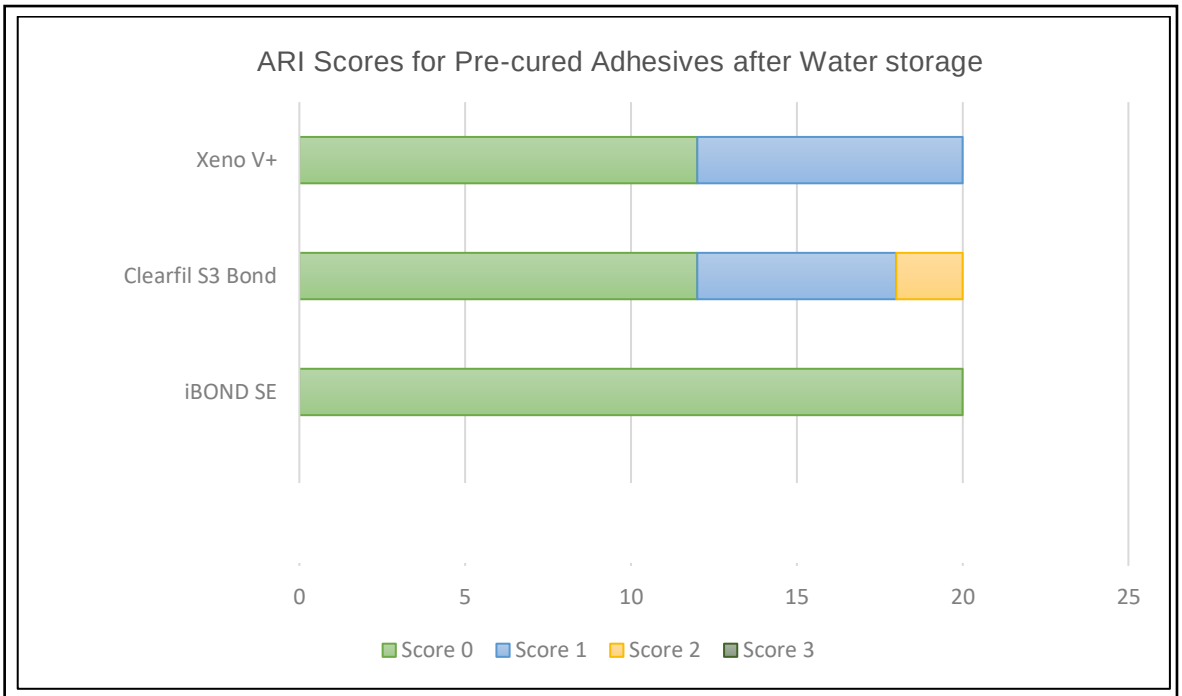
3.6.1. ARI Scores for Pre-cure Adhesive Types after Water Storage

To investigate the effect of prolonged submersion in water on the failure mechanism of pre-cured 1-SEP adhesives, the ARI scores were determined for adhesive types Xeno V+ PC, Clearfil S3 PC and iBond SE PC after water storage at 37 °C for 7 days. The results for adhesives in Pre-cure groups (PC) are shown above in Table 3.6. and illustrated in Fig. 3.15. The results in Table 3.6. show a higher frequency of ARI scores 0 and 1, representing adhesive remnants of ≤ 50 per cent on the tooth surface for 1-SEP in PC groups. There was a statistically significant difference in the failure mode for iBond SE PC, compared to other adhesive types in the group (P<.001), where iBond SE PC failed exclusively at the adhesive-enamel interface, which is clearly visualised by the graph in Fig. 3.15. indicating 100 per

cent of specimens with an ARI score of 0 (light green). The Fisher's exact test and contingency table are copied in Appendix 16.

3.6.2. ARI Scores for Co-cure Adhesive Types after Water Storage

Following water storage for 7 days, the ARI scores for co-cure 1-SEP, Xeno V+ CC, Clearfil S3 CC, iBond SE CC and the control, Transbond Plus SEP CC, are given in Table 3.6. and shown in Fig. 3.15. Clearfil S3 CC, Xeno V+ CC and Transbond Plus SEP CC had a higher proportion of cohesive or 'mixed' failures, by comparison to iBond SE CC, which left relatively less remnant on the tooth surface, and is illustrated by the graph in Fig. 3.15. showing the greater frequency of score 0 (light green) for iBond SE. The Fisher's exact test indicated that the null hypothesis (3) is rejected, so that adhesive type does impact on the amount of adhesive left on the tooth surface when adhesives are co-cured and stored in water, and significant differences highlighted in the contingency table for Clearfil S3 CC and Transbond Plus SEP CC ($P<.001$). Thus, an ARI score of 2 or 3 is significantly more likely for Clearfil S3 and Transbond Plus SEP adhesives types, compared to the other adhesives in the group ($P<.001$). The statistical summaries for the Fisher's exact test and contingency table are copied in Appendix 17.



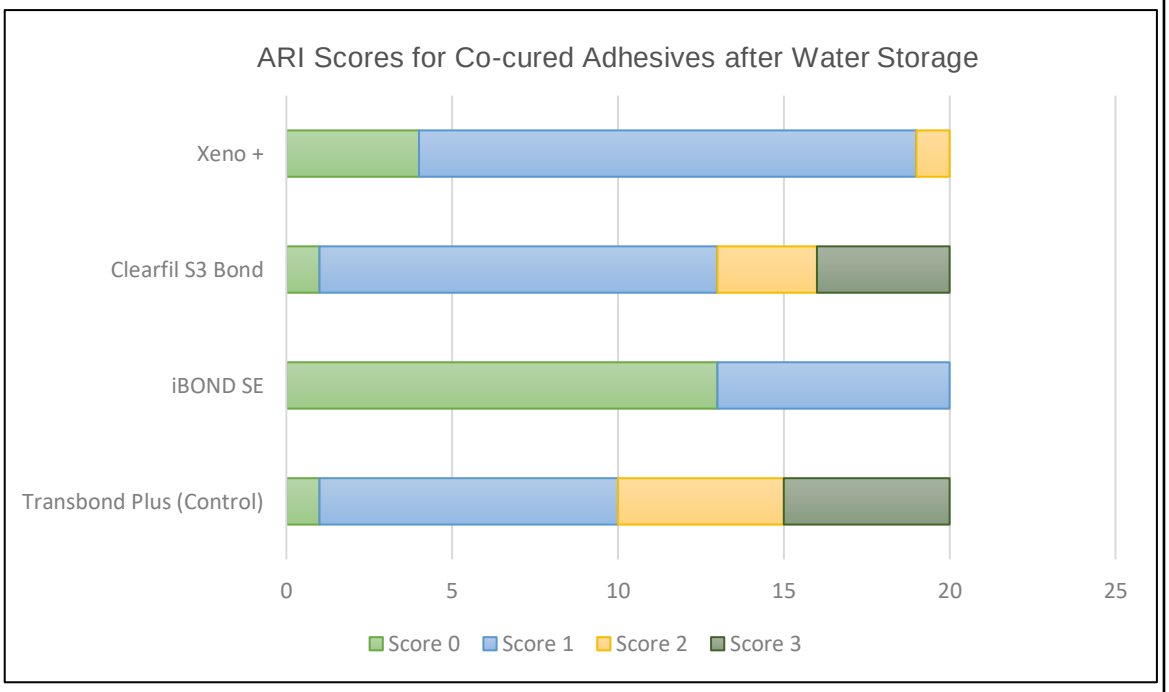


Fig. 3.15. Graphical illustration of ARI Scores for adhesives after water storage in Pre-cure (PC) groups (above) and Co-cure (CC) groups (below). Score 0 = No adhesive left of the tooth; Score 1 = Less than half of the adhesive left on the tooth; Score 2 = More than half of the adhesive left on the tooth; Score 3 = All the adhesive left on the tooth with distinct impression of the bracket mesh.

3.7. Comparison of ARI Scores between Pre-cure and Co-cure Groups

To investigate the impact of different curing protocols on the failure characteristics of 1-SEP adhesives, ARI scores were compared for Pre-cure (PC) and Co-cure (CC) groups for three 1-SEP adhesive types Xeno V+, Clearfil S3 and iBond SE. A Fisher's exact test was used to understand if the categorical variables – pre-cure/co-cure protocol and the amount of adhesive left on the tooth – are dependent on one another. The Chi-square test would have been the most appropriate test for this type of data, and seeks to determine if there is any significant association between the variables, however, the expected values for each variable are less than 5 and, therefore, the Chi-square test was not suitable in this case (Appendix 18). The Fisher's exact test can be used, instead, as an association test beside the contingency tables, to compare each adhesive type. The data for each adhesive represents a grouping of results across all three conditions (immediate debonding, thermocycling and water storage) as it was deemed appropriate to combine these results for the purpose of this analysis.

The results of the Fisher's exact test showed that the curing protocol, either pre-cured or co-cured, had no impact on the failure mechanism of Xeno V+ (P=.07) or iBond SE (P=.20). However, Clearfil S3 that was co-cured, left more adhesive remnant on the tooth surface than pre-cured adhesive, a significant number of times (P=.006). The Fisher's exact test and contingency tables for each adhesive type are displayed in Appendix 18.

CHAPTER FOUR: DISCUSSION

CHAPTER FOUR: DISCUSSION

The development of self-etching primers marks an important step in the advancement of dental adhesive technologies away from traditional products and multi-step procedures towards an evolution of complex, combined formulations which offer simplified techniques, faster bonding times and more conservative etching (15, 18, 23, 26). Successful performance of the orthodontic self-etching primer, Transbond Plus, has seen the adoption of this adhesive product into the mainstream armamentarium of clinical practice where it continues to provide durable bonds to enamel as an alternative to conventional total-etch techniques (53). While undoubtedly more convenient than its predecessors, Transbond Plus SEP still requires a mix prior to use which interrupts the efficiency of bonding procedures. To overcome a final impediment, the latest generation of self-etching adhesive products are pre-mixed solutions of activated, acidic monomers which encompass various proprietary blends of esters and solvents into a single bottle, ready to use and designed for ultimate convenience (15, 17).

Many studies have scrutinised the performance of the new single-bottle adhesives in operative dentistry, however limited information exists to examine their suitability for orthodontic bonding. Therefore, this investigation sets out a series of laboratory experiments to test the new 1-SEP adhesives in a manner which aptly reflects typical orthodontic conditions. For example, brackets were debonded after just 30 minutes, rather than the usual 24 hours, to replicate the early challenge to the bond when orthodontic wires are first ligated to the teeth. Thermocycling effects were also studied using protocols in accordance with ISO/TR 11405E recommendations (340) to reflect the high importance of understanding the impact of thermal stress on the bond strengths of new adhesives, as recommended by Bishara

(77). Thirdly, prolonged water storage over 7 days was used to evaluate the durability of the bond in resisting hydrolytic attack.

In addition to bond strength testing, a further aim of this investigation was to assess the impact of co-curing on the performance of 1-SEP. Co-cure polymerisation is a technique which describes the light-cure of both adhesive and composite simultaneously after bracket placement, by comparison to a conventional two-stage process in which adhesive and composite are cured separately, before and after bracket placement. Co-curing is already the established practice for the orthodontic SEP, Transbond Plus, and clinicians have become accustomed to the time-saving benefits of this technique (54). Thus, this investigation was extended to include an exploration of bond strength values as a function of the curing protocol, ascribing each adhesive to either Pre-cure (PC) or Co-cure (CC) protocol groups in order that a comprehensive view of adhesive performance could be sought, in an endeavour to reflect the expectation of the market.

4.1. Bond Strengths and ARI Scores at Immediate Debonding

In this study, within 30 minutes from bonding, all of the three 1-SEP adhesives showed SBS values greater than those minimally required for orthodontic procedures (>5.4 MPa) (6). This encouraging finding for 1-SEP performance after 30 minutes is in agreement with a number of previous investigations which also described clinically acceptable bond strengths for 1-SEP in similar in vitro trials (7, 43, 73, 75). In most studies, effectiveness of adhesives is assessed after 24 hours once the bond has gained some benefit from the enhanced polymerisation and improved physical properties seen for methacrylate resins during this period. Instead, the bond strengths for this investigation were reported after 30 minutes to reflect the high importance of describing early bond strengths in orthodontic studies, true to

the pattern of clinical work, and within this context the SBS values produced in this study were expectedly lower than in the aforementioned studies which were recorded after 24 hours. However, most relevantly, this study is able to provide useful information on the efficacy of 1-SEP in the immediate time period after bonding and it appears that SBS values for 1-SEP may be robust enough to withstand the force of initial ligation.

A superior performance was given by the adhesive Xeno V+ with a SBS value of 7.86 +/- 2.27 MPa after 30 minutes which was significantly higher than that achieved by Clearfil S3 (5.71 +/- 2.43 MPa) ($P<.01$). The excellent early bond strength for Xeno V+ may be attributed to its chemical composition. Tertiary-butanol is used as a solvent for Xeno V+ which provides an effective, balanced polarity to encourage stability in the combined solution which may enhance bond strengths (17). Also, the bifunctional acrylic amides used as crosslinkers for Xeno V+ promote a very good penetration into enamel of some larger crosslinking monomer groups to establish a compact resin network after polymerisation which serves to fortify the bond (15). Although lower than Xeno V+, the adhesive strength produced by Clearfil S3 after 30 minutes (5.71 +/- 2.43 MPa) was also clinically acceptable which is in agreement with the findings of similar previous orthodontic bonding studies (7, 75). A number of reasons, different to those described for Xeno V+, may account for the successful early bond strengths for Clearfil S3, reflecting the proprietary individuality of 1-SEP products. In the first instance, the chemical bonding capabilities of the 10-MDP monomer may have provided Clearfil S3 with a particular advantage in resisting early debonding forces (72, 373). A strong ionic bond forms between the 10-MDP monomer and hydroxyapatite of enamel, to create water insoluble MDP-Ca salts that promote superior bond strength and durability to the tooth surface (71). Secondly, the “ultra-mild” pH of the Clearfil S3 formulation (pH ~2.7) leaves hydroxyapatite crystals available and relatively more intact to maximise chemical bonding opportunities and heighten the bond (184).

Thirdly, a fine homogenisation of the Clearfil S3 solution through proprietary "molecular dispersion technology" may reportedly reduce incidence of phase separation for Clearfil S3 and could provide another explanation for the good early bond strengths reported for Clearfil S3 after 30 minutes. Phase separation is a phenomenon characterised by microscopic water droplets which remain in the 1-SEP adhesive layer after evaporation, arising from the inadvertent separation of water molecules from other solvent molecules in combined 1-SEP adhesive solutions after evaporation (68). Less residual water molecules at the cured interface for Clearfil S3, has been shown to provide the adhesive layer with improved structural integrity (180) which may enable a more robust, resilient performance under early mechanical testing. Shear bond strengths for iBond SE after 30 minutes were also clinically acceptable with a mean value of 6.24 +/-1.96 MPa which was not significantly different to either Xeno V+ (7.86 +/- 2.27 MPa) or Clearfil S3 (5.71 +/- 2.43 MPa) ($P<.01$). Similarly to Clearfil S3, the effective early enamel adhesion demonstrated by iBond SE may have been facilitated by additional chemical bonding to calcium hydroxyapatite by the 4-MET monomer in its formulation. The functional 4-MET monomer is known to interact chemically with tooth substance to enhance the bond and although considered less potent than 10-MDP, it is likely that the ionic bond of 4-MET to calcium hydroxyapatite contributed a positive early impact to bond strengths. Comparable orthodontic data is scarce, but the good bonding efficiency of iBond SE reported here after 30 minutes is in agreement with the results of a similar in vitro orthodontic bonding study by Holzmeier (7). However, a wider account of the bonding capabilities of iBond SE from operative research papers are controversial, where reported incidences of phase separation, lower bond strengths and compromised clinical outcomes have been described (180, 374, 375). The particular problems documented by some previous researchers for iBond SE did not appear to be borne out in the early debonding data in this study where given mean SBS values after 30 minutes in dry conditions were achieved within the range modestly required for orthodontic

treatment. The control SEP, Transbond Plus, also demonstrated clinically acceptable bond strengths after 30 minutes (7.15 +/- 3.14 MPa) which is in agreement with a wide assembly of previously published clinical and laboratory tests (20, 22, 23, 51, 53). Without chemical bonding capabilities, the success of Transbond Plus SEP relies on an aggressive etch (pH ~1.0) which provides a deepened penetration of resin to capitalise on mechanical retention in an approach not dissimilar to conventional total-etch techniques and which proved effective at bonding brackets during this investigation under an early environment.

Examination of ARI scores showed that brackets bonded with 1-SEP had a tendency to leave low amounts of residual adhesive on the enamel surface within 30 minutes from initial bonding, indicated by high frequencies of ARI scores 0 and 1. Comparison of the three 1-SEP adhesives in the Immediate Debonding group found no significant differences between ARI scores (P=.032). There were no ARI scores of 3 for any 1-SEP in the Immediate Debonding group and only a single score of 2, which suggests a stronger union at the bracket-adhesive interface than at the enamel-adhesive interface thus leaving a relatively cleaner enamel surface at debond. The present findings also indicated that the brackets bonded using 1-SEP adhesives failed in a different manner to that of the control, Transbond Plus SEP, which instead typically failed at the bracket-adhesive interface or within the adhesive and left comparatively more adhesive material on the tooth surface after debonding. The present findings are in agreement with previous studies that, likewise, describe the tendency for single-component self-etching primers to leave less residual adhesive on enamel after debonding compared to either Transbond Plus SEP or traditional AE techniques (7, 43).

The failure mechanism of an adhesive provides valuable information as to where the majority of adhesive remains following the debonding of brackets and this evidence, in

conjunction with bond strength data, enables clinicians to assess the risk of damage to the enamel surface at debond. The different failure modes of adhesives present with their relative advantages and disadvantages. For example, an adhesive-enamel failure that leaves less remnant adhesive on the tooth surface, will require little time for clean-up and reduces the risk of iatrogenic damage from cleaning burs. However, this type of adhesive-enamel failure instead exposes the surface to an increased risk of enamel fracture when the bracket is debonded, depending upon how elevated the bond strengths present. Alternatively, a bracket-adhesive failure mode, which leaves relatively more adhesive on the tooth after debonding, allows the enamel surface to remain intact and concealed beneath the adhesive covering, although requires much more time for clean-up and, accordingly, the iatrogenic risks to cleaning enamel with burs are increased. Clinicians will tend to take an individualised view on preferred failure modes although debate may be prompted by new materials or advances in either the latest debonding methods or cleaning technologies.

4.2. Bond Strengths and ARI Scores after Thermocycling

It has been proven in this investigation, and in previous studies, that 1-SEP may be used reliably to bond brackets in a dry environment (7, 37, 58). However, the question remains as to whether 1-SEP can withstand the same challenges after thermocycling. Thermal cycling is an artificial ageing process which simulates the conditions of the oral cavity by utilising repeated, abrupt changes in temperature to stress the bond. The regimen used for this study is compliant with ISO/TR 11405E recommendations (ISO, 1994) (340) using the approved programme of 500 cycles between water baths of 5 °C and 55 °C in order to align with other standardised protocols and facilitate ready comparison across the literature (77). The artificial ageing effect induced by thermal cycling is generated through two principal

mechanisms - firstly, known differences between the thermal coefficients of expansion for composite, adhesive and tooth substrate are exploited to stress the bond (342). Fluctuating temperatures induce repeated cycles of contraction and expansion stress at the adhesive interface brought about by the higher co-efficient of expansion for restorative materials relative to tooth structure. Continued cycling can overcome marginal bond integrity leading to crack propagation along the bonded surface, and the subsequent development of larger gaps facilitates the "in-out" percolation of water between the dislocated layers, causing additional deterioration to the bond (81, 344). Further effects of thermocycling in water are inextricably linked to hydrolysis effects, and it is likely that the swell and degradation of polymer material that characterises hydrolytic assault, is accelerated by the higher temperature of the water used in thermocycling protocols.

Following the standardised protocol of thermocycling, the results of this study showed that mean shear bond strengths for all 1-SEP adhesives after thermocycling were inadequate for clinical use (>5.4 MPa) (6) (Xeno V+ 2.11 +/- 1.73 MPa; Clearfil S3 4.36 +/- 3.31 MPa; iBond SE 0.66 +/- 0.62 MPa). There is a scarcity of evidence reporting on the effects of thermocycling for brackets bonded with 1-SEP and, therefore, useful comparison with other studies is limited. However, a study in 2010 by Pithon (58) reported results which contradict the findings of this study showing a high mean SBS value of 20.74 MPa after thermocycling for the 1-SEP adhesive, Xeno IV. Clearly, the high bond strengths results reported by Pithon are in contrast to the low bond strengths reported in this study after utilising the same standardised thermocycling regimen. The discrepancy between the results of these two studies may be attributed to variances in adhesive composition, where different types of functional monomers, solvents and other constituent elements present in 1-SEP, will significantly alter the character and functionality of the adhesive. Also, there are distinctions between the methodologies of the two experiments. Bovine central incisor teeth were

selected as the bonding surface for the study by Pithon, and the maxillary central incisor brackets chosen for the study have a flatter base profile and larger mean unit base area of 14.2 mm^2. In contrast, premolar brackets were used in the present study which have a smaller, curved base with a mean base surface area of 10.6 mm^2 and which are designed to fit onto the convex premolar tooth surface. The variation in bracket profiles and greater surface area for the central incisor brackets may have contributed to the higher bond strengths reported by Pithon (314). Also, Pithon conducted shear bond testing using a chisel-shaped rod which has been associated with relatively higher bond strengths as compared to systems using wire loops, such as was used in the present study (335).

The control adhesive, Transbond Plus SEP, produced satisfactory mean bond strengths following thermocycling (12.90 +/- 6.38 MPa), which is in accordance with the findings of previous in vitro experiments (367), as well as many other studies which document a similar resilience to thermal stress for the control in clinical trials (23, 50, 51). The effective bond produced after thermocycling for Transbond Plus SEP may be accounted for by an entrenched resin lamination facilitated through the aggressive etching capabilities of this traditional SEP product which secured and maintained a substantial mechanical retention under harsh thermocycling conditions. On the contrary, the deficient bonds produced by 1-SEP after thermocycling may be reasoned upon and speculated in turn since proprietary differences in monomer and solvent components are likely to have significantly altered behaviour in responding to thermal stress. The poorest performance following thermocycling was given by iBond SE and Xeno V+ which both produced very low mean bond strength values of 0.66 +/- 0.62 MPa and 2.11 +/- 1.73 MPa respectively. Particularly, the small range to the iBond SE data (0.03-2.05 MPa) indicated a consistency for mean bond strengths at a very low level. One reason to account for the poor performance of iBond SE after thermocycling could be the milder etching capabilities for iBond SE (pH ~2) which,

contrary to the "strong" acidity of Transbond Plus SEP (pH 1.0), forgoes a deeper mechanical retention with the greater intention of leaving hydroxyapatite crystals available for secondary chemical bonding to enhance the bond. However, it could be speculated that whilst a lighter resin lamination and chemical bond were proven sufficient for early orthodontic requirements after 30 minutes, these attributes may not have been robust enough to withstand the interface stress induced by larger variations in temperature during thermocycling. Moreover, the Ca-4MET salt is highly soluble and it is possible that some of the advantage afforded to the interface by chemical bonding during dry tests may have been mitigated by the solubility of Ca-4MET under wet, thermocycling conditions. Both iBond SE and Xeno V+ adhesives are HEMA-free formulas which have been associated with phase separation (220), and this problematic experience could have played a detrimental role in weakening the bond after thermocycling in this study. The porosities and blisters in the cured adhesive which characterise the phase separation event, may have substantively weakened the integrity of the material such that repetitive expansion and contraction stresses generated by thermal cycling were able to accelerate crack propagation and percolation of fluids in an expedient manner compared to dry field testing. Although Xeno V+ and iBond SE were shown capable of generating acceptable bond strengths in dry conditions within 30 minutes, with regard to the poor outcomes achieved after thermocycling, it highlights a concern that these adhesives may not be as effective when it comes to bonding to enamel in authentic conditions in vivo. The inadequate bond strengths for iBond SE after thermocycling included a number of pretesting failures (Appendix 21) which posed the question as to how far these very ineffective bonds should be acknowledged statistically without distorting the veracity of the result. However, a similar pattern of elevated pretesting failures has been documented previously for iBond SE (174) and consequently, a decision was taken to include spontaneous failures in any analyses as a more correct depiction of material behaviour.

The mean bond strength for Clearfil S3 adhesive after thermocycling was also inadequate for orthodontic brackets (4.36 +/- 3.31 MPa) and could similarly be attributed to an ineffective enamel adhesion. Inadequate etching of enamel for Clearfil S3 and an over-reliance on additional chemical bonding by the 10-MDP monomer, may be reasons as to why Clearfil S3 struggled to resist the weakening effects of thermocycling. Some former operative studies have shown a much more resilient performance for Clearfil S3 after thermocycling events when bonding restorations to dentine (69, 78, 79), which are in contrast to the results of this study. However, the present research provides new information describing an impaired performance for Clearfil S3 after thermocycling when bonding orthodontic brackets to unground enamel. The failure type of 1-SEP adhesives after thermocycling was similar to that shown when brackets were debonded immediately, which remains a proclivity for less remnant on the tooth surface.

4.3. Bond Strengths and ARI Scores after Water Storage

Brackets bonded with 1-SEP adhesive types Xeno V+, Clearfil S3 and iBond SE were submersed in water at 37 °C for 7 days to investigate the durability of the bond in a prolonged wet environment. The storage of adhesives in water is an important ageing method which supervises a progressive degradation of the polymer substance and corruption of material properties over time (223). The uptake of water into self-etching primer adhesives is governed by two principal mechanisms – firstly, water sorption that occurs through passive diffusion of water molecules into the nano-spaces between polymer molecules is termed ‘unbound’ water; a secondary mechanism, the uptake of ‘bound’ water, is a more dynamic process by which water molecules are ionically attracted to hydrophilic or ionic functional groups in the polymer and attach via hydrogen bonding. Thus, ‘bound’ water absorption is

influenced by resin polarity, and the hydrophilicity of an adhesive can have a significant impact on the quantity of water absorbed into the material. Whereas, the amount of 'unbound' water sorption may be shaped by the chain topography of the various polymers (346, 352). The ensuing uptake of 'bound' and 'unbound' water induces a softening and swelling of the polymer material body, separating filler particles and encouraging further moisture to encroach into the system. Friction between polymer chains is reduced which produces a tangible impairment to the properties of the adhesive in a process known as 'plasticisation' (355). Sustained submersion will eventually saturate the material and water sorption stabilises, although the components of resin destruction are long-leached into the surrounding water as the adhesive deteriorates (223).

After 7 days of water storage, the results of this investigation showed that mean bond strengths for 1-SEP were below the minimum requirements for orthodontic bonding (Xeno V+ 3.10 +/- 3.08 MPa; Clearfil S3 5.16 +/- 3.43 MPa; iBond SE 4.48 +/- 1.85 MPa) and there were no significant differences between the mean bond strength values of each 1-SEP adhesive type (P=.076). Strikingly, 1-SEP adhesives performed poorly compared to the control, Transbond Plus, which showed great durability under the same water storage process producing a mean bond strength of 12.68 +/- 4.74 MPa. There is a lack of similar evidence investigating the performance of 1-SEP orthodontic bonds following prolonged water submersion which restricts the useful comparison of the results of this study to analogous data. However, some former operative research is available which implicates an excessive and persistent hydrophilicity as a cause to compromise bond durability for 1-SEP which could be a contributing factor to explain the impaired performance for 1-SEP in this study (66). The juxtaposition of antagonistic hydrophilic and hydrophobic elements in aqueous solution is a defining character of single-component self-etch adhesives and the balance of polarity greatly influences the functioning of the adhesive. Broadly, hydrophilic

monomers promote “wetting” of moist tooth tissue to optimise the depth of penetration whereas hydrophobic monomers encourage hydrolytic stability and shelf-life in the product, as well as interacting with restorative methacrylate composite materials to accomplish a bond for restoration. Some reports of residual polarity in the cured adhesive suggest that 1-SEP may continue to attract moisture after polymerisation, accelerating the hydrolysis and breakdown of the bond (67). The majority of published literature providing evidence of enduring hydrophilicity for 1-SEP relate to bonds to dentine and, accordingly, describe water being drawn from the inherently moist dentine substrate beneath, into the polymer to impair the bond. However, this investigation offers new insight into the performance of 1-SEP bonds to anhydrous enamel surfaces after storage in water, and reports minimal and inadequate orthodontic bond strengths for brackets after 7 days submersion which may, in part, be explained by a prolonged affinity for water in the cured adhesive. Further issues that may play a role in deteriorating 1-SEP bonds in water, are phase separation events. Integrated water is a requisite ingredient in combined self-etching primers necessary for the ionisation of functional monomers and promotion of demineralising properties (376). Evaporation of the water molecules from solution prior to polymerisation will accomplish a structural integrity in the cured material. Yet, this vital step is wholly challenging to complete due to the relatively low vapour pressure of water. The onset of evaporation during the air-drying step also increases the monomer to water ratio, which reduces the vapour pressure of water further and makes it even more difficult to evaporate the last quantities. Assorted co-solvents, such as ethanol or acetone, are useful to rapidly accelerate the evaporation of water from SEP by forming hydrogen bonds with water molecules which create positive azeotropes to increase the vapour pressure and facilitate water loss. However, residual water droplets have still been reported for 1-SEP as they separate from monomer components during air-drying, in some cases extensively, which may generate voids in the cured adhesive and increase the vulnerability of 1-SEP to hydrolytic degradation (68). It is

possible that an expedient diffusion of moisture into a permeable, hydrophilic bond may have accelerated the swelling and degradation of the adhesive layer for the 1-SEP in this investigation which could have contributed to the poor bonding performance of 1-SEP after 7 days water storage described in this report.

Variations to proprietary formulations for 1-SEP are also likely to influence the behaviour of adhesives in water. Although there was no significant difference between mean bond strengths of 1-SEP adhesive types after water storage (P=.076) and all 1-SEP performed below clinical requirements, the data range for iBond SE after water storage was much smaller than for the other adhesive types. In addition to the aforementioned problems of hydrophilicity and phase separation, iBond SE may also have deteriorated inexorably in water due to its lighter lamination and solubility of its Ca-4MET salt which could have played a role in the consistency of its poor performance (70). To the contrary, the hydrophilic terminal-ending phosphate group of the 10-MDP monomer in Clearfil S3 becomes more hydrophobic once chemically reacted and polymerised, plus the Ca-10-MDP salt is water insoluble which may account for why Clearfil S3 produced the highest mean bond strength in the group (72, 373).

Evaluation of the Adhesive Remnant Index scores of 1-SEP brackets showed that prolonged submersion in water did not change the typical pattern of failure for 1-SEP seen in previous groups which remains to be a preference for adhesive-enamel failure, leaving less adhesive behind on the enamel surface after debonding represented by higher frequencies of ARI scores 0 and 1. To mirror the consistency in bond strength performance for iBond SE, albeit at a ineffective clinical value, the failure mode for iBond SE was also the most consistent in the group whereby all debonded brackets left a clean enamel surface, leaving no trace of remnant on the tooth (100% score 0). By comparison, the control Transbond Plus, whose

scores were more broadly spread over the index, tended to fail cohesively, leaving rather more adhesive on the tooth surface (5% score 0).

In addition to bond strength testing, this investigation sought to examine the impact of co-curing on the performance of 1-SEP adhesives. Co-curing describes a technique where both adhesive and composite are cured simultaneously in one light application after the bracket is seated, as compared with a conventional process where adhesive and composite are cured in two separate steps. Co-curing saves chair-time, and it also reduces the opportunity for contamination with saliva, thereby lowering the sensitivity of the procedure. Light curing both the self-etch primer and composite in one step is already the established protocol for most standard orthodontic bonding procedures, including for bonding brackets using the control SEP Transbond Plus (50). However, for the single-component self-etching primers intended for operative dentistry, the manufacturer's instructions state a required separate cure of adhesive and composite, which adheres to the long-established bonding practice for composite restorations. Previous in vitro studies have demonstrated that only one light-cure application may be necessary for 1-SEP to achieve satisfactory bond strengths, however no ageing methods were conducted (82, 377). Therefore, for the purposes of this investigation, to comprehensively examine the effectiveness of 1-SEP adhesives as an orthodontic bonding agent, all preceding tests including ageing procedures were repeated using a co-cure bonding technique in addition to the manufacturer's recommended separate cure of adhesive and composite.

4.4. Comparison between Pre-cure and Co-cure Results at Immediate Debonding

Despite omission of a pre-cure, there was no significant deterioration in mean bond strength for two of the three 1-SEP adhesives, Xeno V+ or Clearfil S3 (*P*=.88 and *P*=.72 respectively) (Xeno V+ PC 7.86 +/- 2.27 MPa vs CC 7.73 +/- 3.41 MPa; Clearfil S3 PC 5.71 +/- 2.43 MPa vs CC 5.46 +/- 1.93 MPa). Accordingly, the protocol of co-curing produced clinically acceptable mean bond strengths for Xeno V+ and Clearfil S3 which were not significantly different to the control, Transbond Plus, which is also co-cured (*P*<.01). The findings of this study are consistent with the results of a previous report which also showed 1-SEP could produce adequate orthodontic bonding capabilities using a single light cure application when debonded within half-an-hour from initial bonding (82). The reason for why orthodontic bonds may be clinically successful using just one light cure application rather than two, may be the very thin film of composite required beneath brackets which differentiates orthodontic bonding procedures as one which is particularly amenable to a simultaneous cure in a way that that is unsuitable for deeper composite restorations. Also, orthodontic bonds are temporary and the minimum clinical requirements for brackets (>5.4 MPa, Littlewood 2000) is below that of permanent operative bonds, which demand stronger, more durable bonds to enamel.

The iBond SE adhesive, however, still produced greater bond strengths when the manufacturer's recommended two-cure protocol was followed (iBond SE PC 6.24 +/- MPa vs CC 4.38 +/- 1.56 MPa) and the explanation as to why modifying the curing technique should discriminate between adhesive types is unclear. It was also noted that co-curing reduced the mean bond strengths for iBond SE to an ineffective clinical level which were significantly lower than the control (7.15 +/- 3.14 MPa) (*P*<.01). One may consider that a lowering of the bonding proficiency for iBond SE when co-cured, is likely to be attributed

to a less effective interaction between the adhesive and the tooth surface given that the site of failure remains at the adhesive-enamel interface for both Pre-cure and Co-cure groups. The iBond SE formula has a milder etching capability and modest secondary bonding ability by comparison to the stronger etch of Xeno V+ (pH ~1.3) and potent ionic bond of Clearfil S3. The restrained combination of both attributes of acidity and chemical bonding in the iBond SE formulation, in preference to more singularly potent versions of either, appears to have responded less favourably to the omission of a pre-cure than the other two adhesive types but further work would be needed to clarify the cause of this particular variance.

4.5. Comparison between Pre-cure and Co-cure Results after Thermocycling

The performance of 1-SEP brackets with a modified, co-curing bonding protocol was roundly investigated to include thermocycling tests. The findings demonstrated that after thermocycling, 1-SEP showed improvements to bond strengths when utilising a co-cure technique rather than the manufacturer's recommended two-step bonding procedure and the rise was statistically significant for Xeno V+ and iBond SE ($P<.05$) (Xeno V+ PC 2.11 +/- 1.73 MPa vs CC 8.04 +/- 3.72 MPa; Clearfil S3 PC 4.36 +/- 3.31 MPa vs CC 6.69 +/- 5.13 MPa; iBond SE PC 0.66 +/- 0.62 MPa vs CC 2.93 +/- 3.09 MPa). In particular, for adhesive types Xeno V+ and Clearfil S3 the benefit to the bond derived from a simultaneous cure was sufficient to elevate mean values to a clinically acceptable level (> 5.4 MPa) (6) and for Xeno V+, co-cured bond strengths were not significantly different to the control (12.90 +/- 6.38 MPa) ($P<.01$). Some corroboration for the results in this study may be found in a paper written by Bishara (82) in 2007 which also documented a rise in bond strengths by co-curing

Clearfil S3 instead of following the manufacturer's recommended two light applications, although the increase was smaller and statistically insignificant.

The failure mode is able to inform the route by which co-curing provided enhancement to 1-SEP bond strengths after thermocycling. Observations suggest that co-curing reinforces bond unions between the adhesive and tooth surface since adhesive-enamel failure types present predominantly in both Pre-Cure and Co-Cure groups. It is possible, of course, that modifying the curing protocol may have also altered the bond strength at the bracket-adhesive border but this information remains unidentified whilst failure at the enamel-adhesive interface prevails in both curing groups. The mechanism by which a co-curing technique might increase the strength of the bond between adhesive and enamel during thermocycling may be speculated upon since the precise reason is not understood. Hypothetically, the pressure of seating the bracket atop uncured 1-SEP may have enhanced the interaction between 1-SEP and the enamel surface by increasing the infiltration and penetration of resin, thereby boosting mechanical retention and/or intensifying secondary bonding capabilities. With specific consideration to thermocycling, the indication that 1-SEP bond strengths may derive some benefit from co-curing the brackets was unexpected given that in earlier tests where brackets were debonded immediately, co-curing appeared to provide no clear advantage over separate stage curing. It seems that a stronger adhesive interlocking retention and optimised secondary bonding for co-cured brackets may have provided the co-cured bonded interface the greater ability to withstand repeated cycles of contraction and expansion stress, causing divergence between the results of Pre-cure and Co-cure groups after thermocycling.

4.6. Comparison between Pre-cure and Co-cure Results after Water storage

Brackets bonded with 1-SEP adhesives were co-cured and subjected to 7 days water storage at 37 °C. Bond strength data and ARI scores of 1-SEP in Pre-cure (PC) and Co-cure (CC) groups were compared to analyse the impact of modifying the bonding protocol on adhesive performance following prolonged submersion. The results indicated that mean SBS values responded positively to a co-cure where brackets that were co-cured achieved significantly higher mean bond strengths compared to brackets which were cured separately across all 1-SEP adhesive types ($P<.05$) (Xeno V+ PC 3.10 +/- 3.08 MPa vs CC 13.09 +/- 4.01 MPa; Clearfil S3 PC 5.16 +/- 3.43 MPa vs CC 13.07 +/- 4.03 MPa; iBond SE PC 4.48 +/- 1.85 MPa vs CC 6.04 +/-2.37 MPa). For Xeno V+ and Clearfil S3 elevated co-cured values were not significantly different to the co-cured control adhesive, Transbond Plus after submersion in water ($P<.01$)(TP 12.68 +/- 4.74 MPa).

After water storage the enhancement to bond strengths in Co-cured groups, by comparison to Pre-cure groups, was pronounced and these findings appear to suggest that co-curing fortifies the bond, improving resistance to hydrolysis. The positive effect that co-curing may have provided the bond could be explained by a forcibly heightened interaction of 1-SEP components with the enamel surface ministered by the firm seating of brackets atop of uncured adhesive. The advancement of the concentrated acidic formulation of uncured Xeno V+ into enamel substrate may have deepened and consolidated resin lamination to increase bond strengths. For Clearfil S3 the same intensified interaction with enamel, may have also boosted secondary bonding capabilities through the recruitment of additional 10-MDP bonding sites and/or enhanced nano-layering structures. The more singularly potent features which characterise the Xeno V+ and Clearfil S3 formulations appear to have particularly benefitted from a co-cured bond, given the sizable differentiation between

pre- and co-cured data. By comparison, it appears that after water storage iBond SE adhesive acquired less benefit to bond strengths through a co-curing procedure than either Xeno V+ or Clearfil S3, and mean values for iBond SE were significantly lower than the control ($P<.01$) which could possibly be attributed to differences in proprietary formulation. IBond SE has a milder acidic profile and less compelling chemical bond, which did not appear to amplify bond strengths to the same degree by comparison to the adhesive formulations with stronger acids or more potent chemical bonding, when brackets were co-cured. Also, it has been shown by previous researchers that iBond SE may suffer ostensibly in water (70) and it is possible that following prolonged submersion, a propensity for phase separation, persistent hydrophilicity and solubility of the 4-METCa salt may have overwhelmed the majority of any advantage which may have been proffered to iBond SE by co-curing.

Interestingly, the co-curing of 1-SEP adhesives, which produced a rise in mean bond strengths, also produced a smaller shift in the failure profile of adhesive specimens from a predominantly adhesive-enamel failure (score 0) in Pre-cure groups to more mixed failure types (ARI scores 1 and 2) when co-curing was employed although this was only statistically significant for Clearfil S3 over the three test conditions ($P<.01$). The change in failure mode for co-cured Clearfil S3 indicates that co-curing significantly reduced the difference between the bond strengths of the enamel-adhesive and adhesive-bracket unions and one might speculate that effective ionic bonding to calcium hydroxyapatite by the 10-MDP monomer may have played a role, however more information would be required about the bracket-adhesive bond for this to be more than speculation, which is difficult under the limitations of this experiment, where the bracket-adhesive bond prevails and remains unexamined. The finding that co-cured Clearfil S3 adhesive may leave more remnant on the tooth surface than the pre-cured adhesive, could be of interest to clinicians who prefer adhesive to leave more remnant on the tooth surface at debond as a way of mitigating the risk of enamel fracture.

It is necessary to acknowledge some of the challenges surrounding laboratory bond strength testing in order to appreciate outcomes within context. Much criticism of various bond testing methodologies underlines the continued problems experienced by researchers attempting to obtain clinically relevant information from simulated laboratory experiments and, as yet, no study design is without its limitations (378). The protocols of orthodontic bonding studies usually aspire to shear-peel rather than tensile debonding methods as a more accurate reproduction of clinical loads. Although, in reality, an unknown combination of shear peel, tensile and torsional force may be the more authentic debonding experience of brackets in treatment, particularly due to the engagement with active wires to teeth in displaced positions. Wire loop debonding methods might be considered the more appropriate laboratory technique to represent in vivo conditions given that the shear-peel forces generated by this technique also inevitably comprise some components of tensile stress (332). However, difficulties in controlling wire loop angulation and an inability to standardise the direction of debonding force remain inherent shortcomings of wire loop debonding methods and have been suggested to be partly responsible for the higher dispersions to datasets produced often in this type of experiment (337). Indeed, greater variability was a noted feature of the bond strength data produced particularly by Xeno V+ and Clearfil S3 in this study which could be attributed, in some measure, to a lack of standardisation in wire loop angulation as well as possibly to a number of other unknown mechanical and surface variables. A more consistent performance was often presented by iBond SE, by comparison with Xeno V+ and Clearfil S3, although it is conceivable that the lower bond strengths frequently reported for iBond SE (including a greater number of pretesting failures) may have reduced the opportunity for method inconsistencies to be borne out in the data variability.

For other researchers, a shear blade debonding method may be preferred in order to usefully isolate a precise shearing force to the interface and eradicate tensile stress components (378). Although, shear blade methods may not represent true clinical forces and descriptions of erroneous wedge-shaped opening forces, along with inaccurate depictions of material behaviour, have been reported using shear blades (332). Neither a wire loop nor shear blade debonding technique is ideal and improvements have been suggested (333, 337). For the wire loop method used in this study, a rectangular wire rather than a round wire was chosen to provide a more stable point of application following the recommendations suggested by Fox (333) and care was taken to align the wire loop parallel to the bracket base to mitigate against the inherent shortcomings of this technique.

Previous research has shown that crosshead speed is able to significantly affect the results of in vitro bond strength testing whereby an increase of 1.0 mm per minute (mm/min) in crosshead speed can produce an increase in average bond strength of 1.3 MPa (334) although a few studies have reported the opposite, describing an inverse relationship between velocity and bond strength (379, 380). Crosshead speed variation between 0.1-5.0 mm/min is found commonly in orthodontic bonding studies (334). A systematic review by Finnema (334) reported that a crosshead speed of 0.5 mm/min was most frequently chosen by the 28 studies included in the review and was also selected as the velocity for this study in order to contain as many variables across the literature as possible.

Considerable heterogeneity in the test protocols of in vitro bonding studies makes the interpretation and comparison of bond strength data across the published literature especially difficult. Also, reservations about the validity of extrapolated data and the merits of comparing bond strength values to “clinically acceptable” norms have led to suggestions that it may be more important to view the performance of new adhesives relative to the

effectiveness of other adhesives in a study (334). The minimum clinically acceptable reference value used in this study was selected from papers written in 2000 and 2001 by Littlewood (5, 6) which states that a 5% chance of failure tolerated during treatment equates to a minimum bond strength of 5.4 MPa. The analytical approach used by Littlewood to define clinically acceptable bond strength values, is a more contemporary consideration of the topic than the oft cited review article from 1975 which recommends reference values of 6-8 MPa (4). However, there is no universally accepted method for evaluating bond strength data or for determining clinically relevant values and the diverse manner in which bond strength data is analysed and interpreted remains a challenge for comparing the effectiveness of new adhesives in bonding experiments of this type.

CHAPTER FIVE: CONCLUSION

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The development of one-bottle self-etching primer solutions (1-SEP) realised the pre-mixing of solutions containing aqueous methacrylated phosphoric acid esters, thereby refining operating techniques to just a single step for the greatest bonding convenience (17). Although previous research had investigated 1-SEP performance for a variety of restorative procedures, less information was available to determine the appropriateness of 1-SEP as a bonding adhesive for orthodontic brackets.

5.1. Review of Research Aims and Objectives

The overall intention of this research was to investigate the suitability of 1-SEP adhesives as orthodontic bonding agents. In order to achieve the aims of the study, the research was designed as an in vitro bonding experiment wherein the performance of three 1-SEP adhesives, Xeno V+, Clearfil S3 and iBond SE, were examined. Bond strength data was gathered following the debonding of brackets after three environmental conditions – 1) within 30 minutes from initial bonding 2) thermocycling between 5 °C and 55 °C for 500 cycles and 3) water storage at 37 °C for 7 days. The failure mode of adhesives was also determined using the Adhesive Remnant Index by Årtun and Bergland (358). A secondary aim of the research was to explore whether 1-SEP could be clinically effective for orthodontic bonding using just one light cure application rather than two, similar to the practice of some established orthodontic self-etching primers. This thesis sought to explore this additional question by repeating all previous bonding tests following omission of the

pre-cure step from the bonding protocols of 1-SEP. Bond strength data and failure mode for adhesives that had been co-cured were then collected and analysed.

5.2. Conclusion

Within half-an-hour from initial bonding, 1- SEP can produce clinically useful mean bond strengths according to minimum reference values set out by Littlewood (>5.4 MPa) (6). Yet, when ageing techniques are introduced to simulate oral environments using regimens of thermocycling or water storage, mean bond strengths are inadequate. Poor bonding performance, following simulated ageing, suggests that Xeno V+, Clearfil S3 and iBond SE, may be ineffective in vivo.

This study supports the use of a co-cure technique when using Xeno V+ or Clearfil S3 to bond orthodontic brackets. For Xeno V+ and Clearfil S3, the use of a co-cure technique can greatly enhance bond strengths for water- and thermally-aged specimens and can elevate mean values to a clinically acceptable level. Co-curing may improve bonding performance of 1-SEP by way of a heightened interaction between adhesive and enamel. Using one light cure application is less influential to bond strengths produced by iBond SE.

Failure modes for 1-SEP demonstrate a tendency for less residual adhesive to remain on the tooth surface at debond. The predominant enamel-adhesive failure type remains largely unchanged by either ageing tests or by modifying the curing protocol for Xeno V+ and iBond SE. The case exception is for Clearfil S3, which leaves significantly more adhesive on the tooth surface when the adhesive is co-cured.

Based on the data obtained from this study and within the limitations of each experiment, it can be concluded:

1. Bond strengths for 1-SEP were clinically useful in the immediate time period after bonding.
2. Bond strengths for 1-SEP after thermocycling and water storage were unsatisfactory.
3. Co-curing improved bond strengths of aged specimens, in some cases, substantially.
4. Improvements in bond strength of 1-SEP adhesives by using a co-curing technique were adhesive dependent.
5. The predominant failure type for 1-SEP was at the enamel-adhesive interface.
6. Clearfil S3 left more remnant on the tooth surface when co-cured.

5.3. Recommendations for Future Work

The limitations and weaknesses of the study have indicated the following areas as recommendations for future work:

1. Further research may include in vitro orthodontic bond testing of different 1-SEP adhesive types. In light of the results of this investigation, it is recommended that future bonding studies for 1-SEP should include ageing techniques, such as thermocycling or water storage, in order to comprehensively contribute to knowledge regarding the usefulness of these materials for clinical treatment.

2. There is an increasing prevalence of adult orthodontic treatment and a rising demand for aesthetic systems. This suggests that an interesting focus for future research may be to investigate the performance of 1-SEP for bonding aesthetic ceramic or polycarbonate brackets, as an alternative to the metal brackets used in this study. Extending the bonding experiments described in this study, to include aesthetic brackets, would be a valuable future avenue for in vitro research, so as to broaden the understanding of 1-SEP bonding capabilities with a variety of popular and contemporary bracket materials.

LIST OF REFERENCES

1. Eliades T, Brantley W. Orthodontic Applications of Biomaterials: A Clinical Guide: Elsevier Science & Technology; 2016 p.191-201.
2. Mitchell L. An introduction to orthodontics. 3rd ed. ed. Oxford: Oxford University Press; 2007 p.189-200.
3. House K, Ireland AJ, Sherriff M. An investigation into the use of a single component self-etching primer adhesive system for orthodontic bonding: a randomized controlled clinical trial. J Orthod. 2006;33(1):38-44; discussion 28.
4. Reynolds IR. A Review of Direct Orthodontic Bonding. British Journal of Orthodontics. 1975;2(3):171-8.
5. Littlewood SJ, Mitchell L, Greenwood DC, Bubb NL, Wood DJ. Investigation of a hydrophilic primer for orthodontic bonding: an in vitro study. J Orthod. 2000;27(2):181-6.
6. Littlewood SJ, Mitchell L, Greenwood DC. A randomized controlled trial to investigate brackets bonded with a hydrophilic primer. J Orthod. 2001;28(4):301-5.
7. Holzmeier M, Schaubmayr M, Dasch W, Hirschfelder U. A new generation of self-etching adhesives: comparison with traditional acid etch technique. J Orofac Orthop. 2008;69(2):78-93.
8. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955;34(6):849-53.
9. Newman GV, Snyder WH, Wilson CE. Acrylic adhesives for bonding attachments to tooth surfaces. Angle Orthod. 1968;38(1):12-8.
10. Shinohara MS, de Oliveira MT, Di Hipólito V, Giannini M, de Goes MF. SEM analysis of the acid-etched enamel patterns promoted by acidic monomers and phosphoric acids. J Appl Oral Sci. 2006;14(6):427-35.
11. Retief DH. Effect of conditioning the enamel surface with phosphoric acid. J Dent Res. 1973;52(2):333-41.
12. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res. 1975;9(5):373-87.
13. Prévost AP, Fuller JL, Peterson LC. Composite and intermediate resin tag formation in acid-etched enamel: a scanning electron microscopy evaluation. J Prosthet Dent. 1984;52(2):204-7.
14. Giannini M, Makishi P, Ayres AP, Vermelho PM, Fronza BM, Nikaido T, et al. Self-etch adhesive systems: a literature review. Braz Dent J. 2015;26(1):3-10.
15. Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. Dent Mater. 2005;21(10):895-910.
16. Hannig M, Bock H, Bott B, Hoth-Hannig W. Inter-crystallite nanoretention of self-etching adhesives at enamel imaged by transmission electron microscopy. Eur J Oral Sci. 2002;110(6):464-70.
17. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007;28(26):3757-85.
18. Cal-Neto JP, Miguel JA. Scanning electron microscopy evaluation of the bonding mechanism of a self-etching primer on enamel. Angle Orthod. 2006;76(1):132-6.
19. Fjeld M, Øgaard B. Scanning electron microscopic evaluation of enamel surfaces exposed to 3 orthodontic bonding systems. Am J Orthod Dentofacial Orthop. 2006;130(5):575-81.

20. Pandis N, Polychronopoulou A, Eliades T. Failure rate of self-ligating and edgewise brackets bonded with conventional acid etching and a self-etching primer: a prospective in vivo study. Angle Orthod. 2006;76(1):119-22.
21. Grubisa HS, Heo G, Raboud D, Glover KE, Major PW. An evaluation and comparison of orthodontic bracket bond strengths achieved with self-etching primer. Am J Orthod Dentofacial Orthop. 2004;126(2):213-9; quiz 55.
22. Aljubouri YD, Millett DT, Gilmour WH. Six and 12 months' evaluation of a self-etching primer versus two-stage etch and prime for orthodontic bonding: a randomized clinical trial. Eur J Orthod. 2004;26(6):565-71.
23. Banks P, Thiruvenkatachari B. Long-term clinical evaluation of bracket failure with a self-etching primer: a randomized controlled trial. J Orthod. 2007;34(4):243-51.
24. Burgess AM, Sherriff M, Ireland AJ. Self-etching primers: is prophylactic pumicing necessary? A randomized clinical trial. Angle Orthod. 2006;76(1):114-8.
25. Ireland AJ, Sherriff M. The effect of pumicing on the in vivo use of a resin modified glass poly(alkenoate) cement and a conventional no-mix composite for bonding orthodontic brackets. J Orthod. 2002;29(3):217-20; discussion 196.
26. Vilchis RJ, Hotta Y, Yamamoto K. Examination of enamel-adhesive interface with focused ion beam and scanning electron microscopy. Am J Orthod Dentofacial Orthop. 2007;131(5):646-50.
27. Hosein I, Sherriff M, Ireland AJ. Enamel loss during bonding, debonding, and cleanup with use of a self-etching primer. Am J Orthod Dentofacial Orthop. 2004;126(6):717-24.
28. Kim MJ, Lim BS, Chang WG, Lee YK, Rhee SH, Yang HC. Phosphoric acid incorporated with acidulated phosphate fluoride gel etchant effects on bracket bonding. Angle Orthod. 2005;75(4):678-84.
29. Chen CS, Hsu ML, Chang KD, Kuang SH, Chen PT, Gung YW. Failure analysis: enamel fracture after debonding orthodontic brackets. Angle Orthod. 2008;78(6):1071-7.
30. Janiszewska-Olszowska J, Tomkowski R, Tandecka K, Stepien P, Szatkiewicz T, Sporniak-Tutak K, et al. Effect of orthodontic debonding and residual adhesive removal on 3D enamel microroughness. PeerJ. 2016;4:e2558.
31. Sifakakis I, Zinelis S, Eliades G, Koletsi D, Eliades T. Enamel gloss changes induced by orthodontic bonding. J Orthod. 2018;45(4):269-74.
32. Tay FR, Pashley DH, King NM, Carvalho RM, Tsai J, Lai SCN, et al. Aggressiveness of self-etch adhesives on unground enamel. Operative dentistry. 2004;29(3):309-16.
33. Santos BM, Pithon MM, Ruellas AC, Sant'Anna EF. Shear bond strength of brackets bonded with hydrophilic and hydrophobic bond systems under contamination. Angle Orthod. 2010;80(5):963-7.
34. Cacciafesta V, Sfondrini MF, De Angelis M, Scribante A, Klersy C. Effect of water and saliva contamination on shear bond strength of brackets bonded with conventional, hydrophilic, and self-etching primers. Am J Orthod Dentofacial Orthop. 2003;123(6):633-40.
35. Jain P, Stewart GP. Effect of dentin primer on shear bond strength of composite resin to moist and dry enamel. Oper Dent. 2000;25(1):51-8.
36. Zeppieri IL, Chung CH, Mante FK. Effect of saliva on shear bond strength of an orthodontic adhesive used with moisture-insensitive and self-etching primers. Am J Orthod Dentofacial Orthop. 2003;124(4):414-9.
37. House K, Ireland AJ, Sherriff M. An in-vitro investigation into the use of a single component self-etching primer adhesive system for orthodontic bonding: a pilot study. J Orthod. 2006;33(2):116-24.
38. dos Santos JE, Quioca J, Loguercio AD, Reis A. Six-month bracket survival with a self-etch adhesive. Angle Orthod. 2006;76(5):863-8.

39. Buyukyilmaz T, Usumez S, Karaman AI. Effect of self-etching primers on bond strength--are they reliable? Angle Orthod. 2003;73(1):64-70.
40. Vicente A, Bravo LA, Romero M. Influence of a nonrinse conditioner on the bond strength of brackets bonded with a resin adhesive system. The Angle Orthodontist. 2005;75(3):400-5.
41. Elekdag-Turk S, Turk T, Isci D, Ozkalayci N. Thermocycling effects on shear bond strength of a self-etching primer. Angle Orthod. 2008;78(2):351-6.
42. Murfitt PG, Quick AN, Swain MV, Herbison GP. A randomised clinical trial to investigate bond failure rates using a self-etching primer. Eur J Orthod. 2006;28(5):444-9.
43. Zope A, Zope-Khalekar Y, Chitko SS, Kerudi VV, Patil HA, Bonde PV, et al. Comparison of Self-Etch Primers with Conventional Acid Etching System on Orthodontic Brackets. J Clin Diagn Res. 2016;10(12):ZC19-ZC22.
44. David VA, Staley RN, Bigelow HF, Jakobsen JR. Remnant amount and cleanup for 3 adhesives after debracketing. Am J Orthod Dentofacial Orthop. 2002;121(3):291-6.
45. Janiszewska-Olszowska J, Tandecka K, Szatkiewicz T, Stępień P, Sporniak-Tutak K, Grocholewicz K. Three-dimensional analysis of enamel surface alteration resulting from orthodontic clean-up -comparison of three different tools. BMC Oral Health. 2015;15(1):146.
46. Degrazia FW, Genari B, Ferrazzo VA, Santos-Pinto AD, Grehs RA. Enamel Roughness Changes after Removal of Orthodontic Adhesive. Dent J (Basel). 2018;6(3).
47. Retief DH. Failure at the dental adhesive-etched enamel interface. J Oral Rehabil. 1974;1(3):265-84.
48. Bowen R, Rodriguez MS. Tensile strength and modulus of elasticity of tooth structure and several restorative materials. The Journal of the American Dental Association. 1962;64(3):378-87.
49. Bishara SE, Oonsombat C, Ajlouni R, Laffoon JF. Comparison of the shear bond strength of 2 self-etch primer/adhesive systems. Am J Orthod Dentofacial Orthop. 2004;125(3):348-50.
50. Reis A, dos Santos JE, Loguercio AD, de Oliveira Bauer JR. Eighteen-month bracket survival rate: conventional versus self-etch adhesive. Eur J Orthod. 2008;30(1):94-9.
51. Cal-Neto JP, Quintão CA, Almeida MA, Miguel JA. Bond failure rates with a self-etching primer: a randomized controlled trial. Am J Orthod Dentofacial Orthop. 2009;135(6):782-6.
52. Fleming PS, Johal A, Pandis N. Self-etch primers and conventional acid-etch technique for orthodontic bonding: a systematic review and meta-analysis. American journal of orthodontics and dentofacial orthopedics. 2012;142(1):83-94.
53. Hu H, Li C, Li F, Chen J, Sun J, Zou S, et al. Enamel etching for bonding fixed orthodontic braces. Cochrane Database Syst Rev. 2013(11):CD005516.
54. Manning N, Chadwick SM, Plunkett D, Macfarlane TV. A randomized clinical trial comparing 'one-step' and 'two-step' orthodontic bonding systems. J Orthod. 2006;33(4):276-83; discussion 56-7.
55. Elekdag-Turk S, Cakmak F, Isci D, Turk T. 12-month self-ligating bracket failure rate with a self-etching primer. The Angle Orthodontist. 2008;78(6):1095-100.
56. Elekdag-Turk S, Isci D, Turk T, Cakmak F. Six-month bracket failure rate evaluation of a self-etching primer. The European Journal of Orthodontics. 2008;30(2):211-6.
57. Scougall-Vilchis RJ, Ohashi S, Yamamoto K. Effects of 6 self-etching primers on shear bond strength of orthodontic brackets. Am J Orthod Dentofacial Orthop. 2009;135(4):424.e1-7; discussion -5.
58. Pithon MM, dos Santos RL, Ruellas AC, Sant'Anna EF. One-component self-etching primer: a seventh generation of orthodontic bonding system? Eur J Orthod. 2010;32(5):567-70.
59. Yaseen SM, Reddy VS. Comparative evaluation of shear bond strength of two self-etching adhesives (sixth and seventh generation) on dentin of primary and permanent teeth: An in

vitro study. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2009;27(1):33.
60. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater. 2011;27(1):17-28.
61. De Munck J, Vargas M, Iracki J, Van Landuyt K, Poitevin A, Lambrechts P, et al. One-day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. Oper Dent. 2005;30(1):39-49.
62. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater. 2001;17(4):296-308.
63. Tani C, Finger WJ. Effect of smear layer thickness on bond strength mediated by three all-in-one self-etching priming adhesives. J Adhes Dent. 2002;4(4):283-9.
64. Koshiro K, Sidhu SK, Inoue S, Ikeda T, Sano H. New concept of resin-dentin interfacial adhesion: the nanointeraction zone. J Biomed Mater Res B Appl Biomater. 2006;77(2):401-8.
65. Tanaka J, Ishikawa K, Yatani H, Yamashita A, Suzuki K. Correlation of dentin bond durability with water absorption of bonding layer. Dent Mater J. 1999;18(1):11-8.
66. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent. 2002;30(7-8):371-82.
67. Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? J Can Dent Assoc. 2003;69(11):726-31.
68. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, et al. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res. 2005;84(2):183-8.
69. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P, et al. Adhesives and cements to promote preservation dentistry. Operative Dentistry. 2001;26:119-44.
70. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res. 2004;83(6):454-8.
71. Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Irie M, Ogawa T, et al. Nanolayering of phosphoric acid ester monomer on enamel and dentin. Acta Biomater. 2011;7(8):3187-95.
72. Fujita K, Nikaido T, Burrow MF, Iwasaki T, Tanimoto Y, Hirayama S, et al. Effect of the demineralisation efficacy of MDP utilized on the bonding performance of MDP-based all-in-one adhesives. Journal of dentistry. 2018;77:59-65.
73. Sharma S, Tandon P, Nagar A, Singh GP, Singh A, Chugh VK. A comparison of shear bond strength of orthodontic brackets bonded with four different orthodontic adhesives. J Orthod Sci. 2014;3(2):29-33.
74. Hellak A, Ebeling J, Schauseil M, Stein S, Roggendorf M, Korbmacher-Steiner H. Shear Bond Strength of Three Orthodontic Bonding Systems on Enamel and Restorative Materials. Biomed Res Int. 2016;2016:6307107.
75. Ostby AW, Bishara SE, Denehy GE, Laffoon JF, Warren JJ. Effect of self-etchant pH on the shear bond strength of orthodontic brackets. Am J Orthod Dentofacial Orthop. 2008;134(2):203-8.
76. Bishara SE, Otsby AW, Ajlouni R, Laffoon J, Warren JJ. A new premixed self-etch adhesive for bonding orthodontic brackets. Angle Orthod. 2008;78(6):1101-4.
77. Bishara SE, Ajlouni R, Laffoon JF. Effect of thermocycling on the shear bond strength of a cyanoacrylate orthodontic adhesive. Am J Orthod Dentofacial Orthop. 2003;123(1):21-4.
78. Poptani B, Gohil KS, Ganjiwale J, Shukla M. Microtensile dentin bond strength of fifth with five seventh-generation dentin bonding agents after thermocycling: An in vitro study. Contemp Clin Dent. 2012;3(Suppl 2):S167-71.

79. El-Damanhoury HM, Gaintantzopoulou M. Effect of Thermocycling, Degree of Conversion, and Cavity Configuration on the Bonding Effectiveness of All-in-One Adhesives. Oper Dent. 2015;40(5):480-91.
80. Tay FR, King NM, Suh BI, Pashley DH. Effect of delayed activation of light-cured resin composites on bonding of all-in-one adhesives. J Adhes Dent. 2001;3(3):207-25.
81. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005;84(2):118-32.
82. Bishara SE, Ostby AW, Laffoon JF, Warren JJ. The effect of modifying the self-etchant bonding protocol on the shear bond strength of orthodontic brackets. Angle Orthod. 2007;77(3):504-8.
83. Yu B, Ahn JS, Lee YK. Measurement of translucency of tooth enamel and dentin. Acta Odontol Scand. 2009;67(1):57-64.
84. Joiner A. Tooth colour: a review of the literature. J Dent. 2004;32 Suppl 1:3-12.
85. ten Bosch JJ, Coops JC. Tooth color and reflectance as related to light scattering and enamel hardness. J Dent Res. 1995;74(1):374-80.
86. Lee YK, Yu B. Measurement of opalescence of tooth enamel. J Dent. 2007;35(8):690-4.
87. Eimar H, Marelli B, Nazhat SN, Abi Nader S, Amin WM, Torres J, et al. The role of enamel crystallography on tooth shade. J Dent. 2011;39 Suppl 3:e3-10.
88. Grine FE. Scaling of tooth enamel thickness and molar crown size reduction in modern humans: research letter. South African Journal of Science. 2002;98(9-10):503-9.
89. Grine FE. Enamel thickness of deciduous and permanent molars in modern Homo sapiens. Am J Phys Anthropol. 2005;126(1):14-31.
90. Harris EF, Hicks JD, Barcroft BD. Tissue contributions to sex and race: differences in tooth crown size of deciduous molars. Am J Phys Anthropol. 2001;115(3):223-37.
91. Schwartz GT, Dean MC. Sexual dimorphism in modern human permanent teeth. Am J Phys Anthropol. 2005;128(2):312-7.
92. Berkovitz BKB, Moxham BJ, Linden RWA, Sloan AJ. Master Dentistry Volume 3: Oral Biology: Churchill Livingstone; 2010 p. 142-161 29/10/2010.
93. Gutiérrez-Salazar MdP, Reyes-Gasga J. Microhardness and chemical composition of human tooth. Materials Research. 2003;6:367-73.
94. Gale WF, Totemeir TC. Smithells Metals Reference Book. In: Gale WF, Totemeir TC, editors. Smithells Metals Reference Book. 8th ed: Butterworth-Heinemann; 2003 p.22-141.
95. Habelitz S, Marshall SJ, Marshall GW, Jr., Balooch M. Mechanical properties of human dental enamel on the nanometre scale. Arch Oral Biol. 2001;46(2):173-83.
96. Chun K, Choi H, Lee J. Comparison of mechanical property and role between enamel and dentin in the human teeth. J Dent Biomech. 2014;5:1758736014520809.
97. Bajaj D, Arola DD. On the R-curve behavior of human tooth enamel. Biomaterials. 2009;30(23-24):4037-46.
98. Myoung S, Lee J, Constantino P, Lucas P, Chai H, Lawn B. Morphology and fracture of enamel. J Biomech. 2009;42(12):1947-51.
99. Barani A, Keown AJ, Bush MB, Lee JJ, Chai H, Lawn BR. Mechanics of longitudinal cracks in tooth enamel. Acta Biomater. 2011;7(5):2285-92.
100. Bajaj D, Arola D. Role of prism decussation on fatigue crack growth and fracture of human enamel. Acta Biomater. 2009;5(8):3045-56.
101. Yahyazadehfar M, Bajaj D, Arola DD. Hidden contributions of the enamel rods on the fracture resistance of human teeth. Acta Biomater. 2013;9(1):4806-14.
102. Atlas of Stress-Strain Curves. Atlas of Stress-Strain Curves. 2nd ed: ASM International; 2002. p. 18.
103. Ikeda T, Uno S, Tanaka T, Kawakami S, Komatsu H, Sano H. Relation of enamel prism orientation to microtensile bond strength. Am J Dent. 2002;15(2):109-13.

104. Carvalho RM, Santiago SL, Fernandes CA, Suh BI, Pashley DH. Effects of prism orientation on tensile strength of enamel. J Adhes Dent. 2000;2(4):251-7.
105. Giannini M, Soares CJ, de Carvalho RM. Ultimate tensile strength of tooth structures. Dent Mater. 2004;20(4):322-9.
106. Gwinnett AJ. Structure and composition of enamel. Oper Dent. 1992;Suppl 5:10-7.
107. Chen H, Tang Z, Liu J, Sun K, Chang SR, Peters MC, et al. Acellular Synthesis of a Human Enamel-like Microstructure. Advanced Materials. 2006;18(14):1846-51.
108. Daculsi G, Kerebel B. High-resolution electron microscope study of human enamel crystallites: size, shape, and growth. J Ultrastruct Res. 1978;65(2):163-72.
109. Kerebel B, Daculsi G, Kerebel LM. Ultrastructural studies of enamel crystallites. J Dent Res. 1979;58(Spec Issue B):844-51.
110. Daculsi G, Menanteau J, Kerebel LM, Mitre D. Length and shape of enamel crystals. Calcif Tissue Int. 1984;36(5):550-5.
111. Robinson C, Kirkham J, Brookes SJ, Shore R. Dental enamel: formation to destruction. CRC Press; 1995. p. 175-6.
112. Ge J, Cui FZ, Wang XM, Feng HL. Property variations in the prism and the organic sheath within enamel by nanoindentation. Biomaterials. 2005;26(16):3333-9.
113. La Fontaine A, Zavgorodniy A, Liu H, Zheng R, Swain M, Cairney J. Atomic-scale compositional mapping reveals Mg-rich amorphous calcium phosphate in human dental enamel. Sci Adv. 2016;2(9):e1601145.
114. Ding H, Pan H, Xu X, Tang R. Toward a Detailed Understanding of Magnesium Ions on Hydroxyapatite Crystallization Inhibition. Crystal Growth & Design. 2014;14(2):763-9.
115. Jiang Y, Spears IR, Macho GA. An investigation into fractured surfaces of enamel of modern human teeth: a combined SEM and computer visualisation study. Arch Oral Biol. 2003;48(6):449-57.
116. Dusevich V, Xu C, Wang Y, Walker MP, Gorski JP. Identification of a protein-containing enamel matrix layer which bridges with the dentine-enamel junction of adult human teeth. Arch Oral Biol. 2012;57(12):1585-94.
117. Moradian-Oldak J. Amelogenins: assembly, processing and control of crystal morphology. Matrix Biol. 2001;20(5-6):293-305.
118. Hatakeyama J, Fukumoto S, Nakamura T, Haruyama N, Suzuki S, Hatakeyama Y, et al. Synergistic roles of amelogenin and ameloblastin. J Dent Res. 2009;88(4):318-22.
119. Nakamura T, Lu C, Korach CS. Mechanical properties of tooth enamel: microstructural modelling and characterisation. In: Proulx T, editor. Mechanics of Biological Systems and Materials. Conference Proceedings of the Society for Experimental Mechanics Series. 22011. p. 171-9.
120. Fernandes CP, Chevitarese O. The orientation and direction of rods in dental enamel. J Prosthet Dent. 1991;65(6):793-800.
121. Lee JJ, Kwon JY, Chai H, Lucas PW, Thompson VP, Lawn BR. Fracture modes in human teeth. J Dent Res. 2009;88(3):224-8.
122. Hannig M, Fiebiger M, Guntzer M, Dobert A, Zimehl R, Nekrashevych Y. Protective effect of the in situ formed short-term salivary pellicle. Arch Oral Biol. 2004;49(11):903-10.
123. Ventura TM, Cassiano LP, Souza ESCM, Taira EA, Leite AL, Rios D, et al. The proteomic profile of the acquired enamel pellicle according to its location in the dental arches. Arch Oral Biol. 2017;79:20-9.
124. Zhang YF, Li DY, Yu JX, He HT. On the thickness and nanomechanical properties of salivary pellicle formed on tooth enamel. J Dent. 2016;55:99-104.
125. Lendenmann U, Grogan J, Oppenheim FG. Saliva and dental pellicle--a review. Adv Dent Res. 2000;14:22-8.
126. Hannig M, Joiner A. The structure, function and properties of the acquired pellicle. Monogr Oral Sci. 2006;19:29-64.

127. Rudiger SG, Dahlen G, Carlen A. Protein and bacteria binding to exposed root surfaces and the adjacent enamel surfaces in vivo. Swed Dent J. 2015;39(1):11-22.
128. Kajisa L, Prakobphol A, Schiodt M, Fisher SJ. Effect of plasma on composition of human enamel and cementum pellicle. Scand J Dent Res. 1990;98(6):461-71.
129. Siqueira WL, Custodio W, McDonald EE. New insights into the composition and functions of the acquired enamel pellicle. J Dent Res. 2012;91(12):1110-8.
130. Cheaib Z, Lussi A. Impact of acquired enamel pellicle modification on initial dental erosion. Caries Res. 2011;45(2):107-12.
131. Hara AT, Zero DT. The potential of saliva in protecting against dental erosion. Monogr Oral Sci. 2014;25:197-205.
132. Hannig M, Hannig C. The pellicle and erosion. Monogr Oral Sci. 2014;25:206-14.
133. Nekrashevych Y, Hannig M, Stosser L. Assessment of enamel erosion and protective effect of salivary pellicle by surface roughness analysis and scanning electron microscopy. Oral Health Prev Dent. 2004;2(1):5-11.
134. Siqueira WL, Margolis HC, Helmerhorst EJ, Mendes FM, Oppenheim FG. Evidence of intact histatins in the in vivo acquired enamel pellicle. J Dent Res. 2010;89(6):626-30.
135. Diaz PI, Chalmers NI, Rickard AH, Kong C, Milburn CL, Palmer RJ, Jr., et al. Molecular characterization of subject-specific oral microflora during initial colonization of enamel. Appl Environ Microbiol. 2006;72(4):2837-48.
136. Guo L, He X, Shi W. Intercellular communications in multispecies oral microbial communities. Front Microbiol. 2014;5:328.
137. Marsh PD. Contemporary perspective on plaque control. Br Dent J. 2012;212(12):601-6.
138. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. J Clin Periodontol. 2017;44 Suppl 18:S12-S22.
139. Heller D, Helmerhorst EJ, Gower AC, Siqueira WL, Paster BJ, Oppenheim FG. Microbial Diversity in the Early In Vivo-Formed Dental Biofilm. Appl Environ Microbiol. 2016;82(6):1881-8.
140. Peterson SN, Meissner T, Su AI, Snesrud E, Ong AC, Schork NJ, et al. Functional expression of dental plaque microbiota. Front Cell Infect Microbiol. 2014;4:108.
141. Hojo K, Nagaoka S, Ohshima T, Maeda N. Bacterial interactions in dental biofilm development. J Dent Res. 2009;88(11):982-90.
142. Adshead VM, Parke JM, Chambers PJ, Davies RM, Cole JA. An in-vitro study of the role of sucrose and interactions between oral bacteria in possible mechanisms of dental plaque formation. Arch Oral Biol. 1983;28(8):723-7.
143. Koo H, Falsetta ML, Klein MI. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. J Dent Res. 2013;92(12):1065-73.
144. Forssten SD, Bjorklund M, Ouwehand AC. Streptococcus mutans, caries and simulation models. Nutrients. 2010;2(3):290-8.
145. Nishimura J, Saito T, Yoneyama H, Bai LL, Okumura K, Isogai E. Biofilm formation by *Streptococcus mutans* and related bacteria. Advances in Microbiology. 2012;2(2):208-15.
146. Zhao W, Li W, Lin J, Chen Z, Yu D. Effect of sucrose concentration on sucrose-dependent adhesion and glucosyltransferase expression of S. mutans in children with severe early-childhood caries (S-ECC). Nutrients. 2014;6(9):3572-86.
147. Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS. Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends Microbiol. 2003;11(2):94-100.
148. Bowen WH, Koo H. Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res. 2011;45(1):69-86.
149. Mashima I, Nakazawa F. The interaction between Streptococcus spp. and Veillonella tobetsuensis in the early stages of oral biofilm formation. J Bacteriol. 2015.

150. Senadheera D, Cvitkovitch DG. Quorum sensing and biofilm formation by Streptococcus mutans. Adv Exp Med Biol. 2008;631:178-88.
151. Rickard AH, Palmer RJ, Jr., Blehert DS, Campagna SR, Semmelhack MF, Egland PG, et al. Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. Mol Microbiol. 2006;60(6):1446-56.
152. Roberts AP, Kreth J. The impact of horizontal gene transfer on the adaptive ability of the human oral microbiome. Front Cell Infect Microbiol. 2014;4:124.
153. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet. 2007;369(9555):51-9.
154. Salli KM, Ouwehand AC. The use of in vitro model systems to study dental biofilms associated with caries: a short review. J Oral Microbiol. 2015;7:26149.
155. Nascimento MM, Gordan VV, Garvan CW, Browngardt CM, Burne RA. Correlations of oral bacterial arginine and urea catabolism with caries experience. Oral Microbiol Immunol. 2009;24(2):89-95.
156. Bauer W. Methacrylic Acid and Derivatives. In: Wiley-VCH, editor. Ullmann's Encylopaedia of Industrial Chemistry: Wiley-VCH; 2011.
157. Nakamura M, Oshima H, Hashimoto Y. Monomer permeability of disposable dental gloves. J Prosthet Dent. 2003;90(1):81-5.
158. Salz U, Zimmermann J, Zeuner F, Moszner N. Hydrolytic stability of self-etching adhesive systems. J Adhes Dent. 2005;7(2):107-16.
159. Andreasson H, Boman A, Johnsson S, Karlsson S, Barregard L. On permeability of methyl methacrylate, 2-hydroxyethyl methacrylate and triethyleneglycol dimethacrylate through protective gloves in dentistry. Eur J Oral Sci. 2003;111(6):529-35.
160. Nakabayashi N, Saimi Y. Bonding to intact dentin. J Dent Res. 1996;75(9):1706-15.
161. Nakabayashi N, Takarada K. Effect of HEMA on bonding to dentin. Dent Mater. 1992;8(2):125-30.
162. Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, Pashley DH. Effects of HEMA on water evaporation from water-HEMA mixtures. Dent Mater. 1998;14(1):6-10.
163. Nakabayashi N, Watanabe A, Gendusa NJ. Dentin adhesion of "modified" 4-META/MMA-TBB resin: function of HEMA. Dent Mater. 1992;8(4):259-64.
164. Jacobsen T, Söderholm KJ. Some effects of water on dentin bonding. Dent Mater. 1995;11(2):132-6.
165. Burrow MF, Inokoshi S, Tagami J. Water sorption of several bonding resins. Am J Dent. 1999;12(6):295-8.
166. Geurtsen W. Biocompatibility of resin-modified filling materials. Crit Rev Oral Biol Med. 2000;11(3):333-55.
167. Nakabayashi N, Hiranuma K. Effect of etchant variation on wet and dry dentin bonding primed with 4-META/acetone. Dent Mater. 2000;16(4):274-9.
168. Chang JC, Hurst TL, Hart DA, Estey AW. 4-META use in dentistry: a literature review. J Prosthet Dent. 2002;87(2):216-24.
169. Nakabayashi N, Pashley DH. Hybridization of Dental Hard Tissues: Quintessence Publishing Company; 1998 p.48-68.
170. Unemori M, Matsuya Y, Matsuya S, Akashi A, Akamine A. Water absorption of poly(methyl methacrylate) containing 4-methacryloxyethyl trimellitic anhydride. Biomaterials. 2003;24(8):1381-7.
171. Yoshioka M, Yoshida Y, Inoue S, Lambrechts P, Vanherle G, Nomura Y, et al. Adhesion/decalcification mechanisms of acid interactions with human hard tissues. J Biomed Mater Res. 2002;59(1):56-62.
172. Yoshida Y, Inoue S. Chemical analyses in dental adhesive technology. Japanese Dental Science Review. 2012;48(2):141-52.

173. Baldissara P, Querze M, Monaco C, Scotti R, Fonseca RG. Efficacy of surface treatments on the bond strength of resin cements to two brands of zirconia ceramic. J Adhes Dent. 2013;15(3):259-67.
174. Perdigao J, Gomes G, Gondo R, Fundingsland JW. In vitro bonding performance of all-in-one adhesives. Part I--microtensile bond strengths. J Adhes Dent. 2006;8(6):367-73.
175. Peumans M, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Five-year clinical effectiveness of a two-step self-etching adhesive. J Adhes Dent. 2007;9(1):7-10.
176. Ozcan M, Bernasconi M. Adhesion to zirconia used for dental restorations: a systematic review and meta-analysis. J Adhes Dent. 2015;17(1):7-26.
177. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, et al. Self-assembled Nano-layering at the Adhesive interface. J Dent Res. 2012;91(4):376-81.
178. Yoshihara K, Yoshida Y, Nagaoka N, Fukegawa D, Hayakawa S, Mine A, et al. Nano-controlled molecular interaction at adhesive interfaces for hard tissue reconstruction. Acta Biomater. 2010;6(9):3573-82.
179. Van Meerbeek B, Yoshida Y, Suzuki K. The AD-concept revisited as basis for durable tooth bonding. IADR; July 14-17, 2010; Barcelona, Spain2010.
180. Van Landuyt KL, Mine A, De Munck J, Jaecques S, Peumans M, Lambrechts P, et al. Are one-step adhesives easier to use and better performing? Multifactorial assessment of contemporary one-step self-etching adhesives. J Adhes Dent. 2009;11(3):175-90.
181. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008;24(1):90-101.
182. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res. 2003;82(2):136-40.
183. Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, et al. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res. 2004;83(11):843-8.
184. Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dent Mater. 2001;17(5):430-44.
185. Peutzfeldt A. Resin composites in dentistry: the monomer systems. Eur J Oral Sci. 1997;105(2):97-116.
186. Labella R, Davy KW, Lambrechts P, Van Meerbeek B, Vanherle G. Monomethacrylate co-monomers for dental resins. Eur J Oral Sci. 1998;106(3):816-24.
187. Asmussen E, Peutzfeldt A. Influence of UEDMA BisGMA and TEGDMA on selected mechanical properties of experimental resin composites. Dent Mater. 1998;14(1):51-6.
188. Sideridou I, Tserki V, Papanastasiou G. Study of water sorption, solubility and modulus of elasticity of light-cured dimethacrylate-based dental resins. Biomaterials. 2003;24(4):655-65.
189. Schafer TE, Lapp CA, Hanes CM, Lewis JB, Wataha JC, Schuster GS. Estrogenicity of bisphenol A and bisphenol A dimethacrylate in vitro. J Biomed Mater Res. 1999;45(3):192-7.
190. Odian G. Principles of Polymerization: Wiley; 2004 p.198-349.
191. Cook WD, Standish PM. Polymerization kinetics of resin-based restorative materials. J Biomed Mater Res. 1983;17(2):275-82.
192. Sideridou ID, Achilias DS, Karava O. Reactivity of Benzoyl Peroxide/Amine System as an Initiator for the Free Radical Polymerization of Dental and Orthopaedic Dimethacrylate Monomers: Effect of the Amine and Monomer Chemical Structure. Macromolecules. 2006;39(6):2072-80.
193. Arrais CA, Rueggeberg FA, Waller JL, de Goes MF, Giannini M. Effect of curing mode on the polymerization characteristics of dual-cured resin cement systems. J Dent. 2008;36(6):418-26.
194. Hiemenz PC, Lodge TP. Polymer Chemistry, Second Edition: Taylor & Francis; 2007.

195. Park YJ, Chae KH, Rawls HR. Development of a new photoinitiation system for dental light-cure composite resins. Dent Mater. 1999;15(2):120-7.
196. Leprince JG, Palin WM, Hadis MA, Devaux J, Leloup G. Progress in dimethacrylate-based dental composite technology and curing efficiency. Dent Mater. 2013;29(2):139-56.
197. Taira M, Urabe H, Hirose T, Wakasa K, Yamaki M. Analysis of photo-initiators in visible-light-cured dental composite resins. J Dent Res. 1988;67(1):24-8.
198. Moss L, Rueggeberg FA, Stansbury JW. Effect of solvent type on absorption profile of camphorquinone. Journal of Dental Research. 2002;81(1_suppl):A225.
199. Janda R, Roulet JF, Kaminsky M, Steffin G, Latta M. Color stability of resin matrix restorative materials as a function of the method of light activation. Eur J Oral Sci. 2004;112(3):280-5.
200. Fujimori Y, Kaneko T, Kaku T, Yoshioka N, Nishide H, Tsuchida E. Polymerization and photoinitiation behavior in the light-cured dental composite resins. Polymers for Advanced Technologies. 1992;3(8):437-41.
201. Jakubiak J, Allonas X, Fouassier JP, Sionkowska A, Andrzejewska E, Linden LÅ, et al. Camphorquinone–amines photoinitating systems for the initiation of free radical polymerization. Polymer. 2003;44(18):5219-26.
202. Stansbury JW. Curing dental resins and composites by photopolymerization. J Esthet Dent. 2000;12(6):300-8.
203. Sanares AM, Itthagarun A, King NM, Tay FR, Pashley DH. Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites. Dent Mater. 2001;17(6):542-56.
204. Finger WJ, Osada T, Tani C, Endo T. Compatibility between self-etching adhesive and self-curing resin by addition of anion exchange resin. Dent Mater. 2005;21(11):1044-50.
205. Kamoun EA, Winkel A, Eisenburger M, Menzel H. Carboxylated camphorquinone as visible-light photoinitiator for biomedical application: Synthesis, characterization, and application. Arabian Journal of Chemistry. 2016;9(5):745-54.
206. Nakayama Y, Ji-Youn K, Nishi S, Ueno H, Matsuda T. Development of high-performance stent: gelatinous photogel-coated stent that permits drug delivery and gene transfer. J Biomed Mater Res. 2001;57(4):559-66.
207. Sun GJ, Park YJ, Chae KH. New photosensitizers for a visible light-cured urethane dimethacrylate dental resin composite. Polymer Korea. 1999;23(1):113-21.
208. Fujisawa S, Kadoma Y, Yokoe I. Radical-scavenging activity of butylated hydroxytoluene (BHT) and its metabolites. Chem Phys Lipids. 2004;130(2):189-95.
209. Yehye WA, Rahman NA, Ariffin A, Abd Hamid SB, Alhadi AA, Kadir FA, et al. Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): a review. Eur J Med Chem. 2015;101:295-312.
210. Nassar H, Chu TM, Platt J. Optimizing light-cured composite through variations in camphorquinone and butylhydroxytoluene concentrations. Braz Oral Res. 2016;30(1).
211. Michelsen VB, Moe G, Skalevik R, Jensen E, Lygre H. Quantification of organic eluates from polymerized resin-based dental restorative materials by use of GC/MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;850(1-2):83-91.
212. Reed M, Fujiwara H, Thompson DC. Comparative metabolism, covalent binding and toxicity of BHT congeners in rat liver slices. Chem Biol Interact. 2001;138(2):155-70.
213. Lapp CA, Schuster GS. Effects of DMAEMA and 4-methoxyphenol on gingival fibroblast growth, metabolism, and response to interleukin-1. J Biomed Mater Res. 2002;60(1):30-5.
214. Morrison RT, Boyd RN. Organic chemistry: Allyn and Bacon; 1987.
215. Hansen SACM. Hansen Solubility Parameters - A User's Handbook: 2007 2nd Edition.
216. Asmussen E, Uno S. Solubility parameters, fractional polarities, and bond strengths of some intermediary resins used in dentin bonding. J Dent Res. 1993;72(3):558-65.

217. Hiraishi N, Nishiyama N, Ikemura K, Yau JY, King NM, Tagami J, et al. Water concentration in self-etching primers affects their aggressiveness and bonding efficacy to dentin. J Dent Res. 2005;84(7):653-8.
218. Pashley DH, Agee KA, Nakajima M, Tay FR, Carvalho RM, Terada RS, et al. Solvent-induced dimensional changes in EDTA-demineralized dentin matrix. J Biomed Mater Res. 2001;56(2):273-81.
219. Ikeda T, De Munck J, Shirai K, Hikita K, Inoue S, Sano H, et al. Effect of evaporation of primer components on ultimate tensile strengths of primer-adhesive mixture. Dent Mater. 2005;21(11):1051-8.
220. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, et al. Technique-sensitivity of contemporary adhesives. Dent Mater J. 2005;24(1):1-13.
221. Hotta M, Kondoh K, Kamemizu H. Effect of primers on bonding agent polymerization. J Oral Rehabil. 1998;25(10):792-9.
222. Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR, Pashley DH. Adhesive permeability affects coupling of resin cements that utilise self-etching primers to dentine. J Dent. 2004;32(1):55-65.
223. Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, et al. Effect of resin hydrophilicity and water storage on resin strength. Biomaterials. 2004;25(26):5789-96.
224. Tay FR, Frankenberger R, Krejci I, Bouillaguet S, Pashley DH, Carvalho RM, et al. Single-bottle adhesives behave as permeable membranes after polymerization. I. In vivo evidence. J Dent. 2004;32(8):611-21.
225. Robbins J, Summitt J. Fundamentals of operative dentistry: a contemporary approach. 2001.
226. Miyazaki M, Ando S, Hinoura K, Onose H, Moore BK. Influence of filler addition to bonding agents on shear bond strength to bovine dentin. Dent Mater. 1995;11(4):234-8.
227. Di Hipólito V, Reis AF, Mitra SB, de Goes MF. Interaction morphology and bond strength of nanofilled simplified-step adhesives to acid etched dentin. Eur J Dent. 2012;6(4):349-60.
228. Lohbauer U, Wagner A, Belli R, Stoetzel C, Hilpert A, Kurland HD, et al. Zirconia nanoparticles prepared by laser vaporization as fillers for dental adhesives. Acta Biomater. 2010;6(12):4539-46.
229. Wagner A, Belli R, Stötzel C, Hilpert A, Müller FA, Lohbauer U. Biomimetically- and hydrothermally-grown HAp nanoparticles as reinforcing fillers for dental adhesives. J Adhes Dent. 2013;15(5):413-22.
230. Collares FM, Ogliari FA, Lima GS, Fontanella VR, Piva E, Samuel SM. Ytterbium trifluoride as a radiopaque agent for dental cements. Int Endod J. 2010;43(9):792-7.
231. Schulz H, Schimmoeller B, Pratsinis SE, Salz U, Bock T. Radiopaque dental adhesives: dispersion of flame-made Ta2O5/SiO2 nanoparticles in methacrylic matrices. J Dent. 2008;36(8):579-87.
232. Gauthier MA, Stangel I, Ellis TH, Zhu XX. Oxygen inhibition in dental resins. J Dent Res. 2005;84(8):725-9.
233. Kemp-Scholte CM, Davidson CL. Complete marginal seal of Class V resin composite restorations effected by increased flexibility. J Dent Res. 1990;69(6):1240-3.
234. Martins GC, Reis A, Loguercio AD, Zander-Grande C, Meier M, Mazur RF, et al. Does Making An Adhesive System Radiopaque by Filler Addition Affect Its Bonding Properties? J Adhes Dent. 2015;17(6):513-9.
235. Mjör IA, Moorhead JE, Dahl JE. Reasons for replacement of restorations in permanent teeth in general dental practice. Int Dent J. 2000;50(6):361-6.
236. Collares FM, Klein M, Santos PD, Portella FF, Ogliari F, Leitune VC, et al. Influence of radiopaque fillers on physicochemical properties of a model epoxy resin-based root canal sealer. J Appl Oral Sci. 2013;21(6):533-9.
237. Young A, von der Fehr FR, Sønju T, Nordbø H. Fluoride release and uptake in vitro from a composite resin and two orthodontic adhesives. Acta Odontol Scand. 1996;54(4):223-8.

238. Tay FR, Moulding KM, Pashley DH. Distribution of nanofillers from a simplified-step adhesive in acid-conditioned dentin. J Adhes Dent. 1999;1(2):103-17.
239. Yoshida Y, Shirai K, Nakayama Y, Itoh M, Okazaki M, Shintani H, et al. Improved filler-matrix coupling in resin composites. J Dent Res. 2002;81(4):270-3.
240. Meiers JC, Miller GA. Antibacterial activity of dentin bonding systems, resin-modified glass ionomers, and polyacid-modified composite resins. Oper Dent. 1996;21(6):257-64.
241. Schüpbach P, Lutz F, Finger WJ. Closing of dentinal tubules by Gluma desensitizer. Eur J Oral Sci. 1997;105(5 Pt 1):414-21.
242. Felton DA, Bergenholtz G, Kanoy BE. Evaluation of the desensitizing effect of Gluma Dentin Bond on teeth prepared for complete-coverage restorations. Int J Prosthodont. 1991;4(3):292-8.
243. Ritter AV, Swift EJ, Yamauchi M. Effects of phosphoric acid and glutaraldehyde-HEMA on dentin collagen. Eur J Oral Sci. 2001;109(5):348-53.
244. Hansen EK, Asmussen E. Improved efficacy of dentin-bonding agents. Eur J Oral Sci. 1997;105(5 Pt 1):434-9.
245. Hartshorn SR. Structural Adhesives: Chemistry and Technology: Springer US; 2012.
246. Galvão MR, Caldas SG, Bagnato VS, de Souza Rastelli AN, de Andrade MF. Evaluation of degree of conversion and hardness of dental composites photo-activated with different light guide tips. Eur J Dent. 2013;7(1):86-93.
247. Emami N, Söderholm KJ. How light irradiance and curing time affect monomer conversion in light-cured resin composites. Eur J Oral Sci. 2003;111(6):536-42.
248. Ogunyinka A, Palin W, Shortall A, Marquis P. Photoinitiation chemistry affects light transmission and degree of conversion of curing experimental dental resin composites. Dental Materials. 2007;23(7):807-13.
249. de Lange C, Bausch JR, Davidson CL. The curing pattern of photo-initiated dental composites. J Oral Rehabil. 1980;7(5):369-77.
250. Mohamad D, Young RJ, Mann AB, Watts DC. Post-polymerization of dental resin composite evaluated with nanoindentation and micro-Raman spectroscopy. Archives of Orofacial Sciences. 2007;2:26-31.
251. Ferracane JL, Greener EH. Fourier transform infrared analysis of degree of polymerization in unfilled resins--methods comparison. J Dent Res. 1984;63(8):1093-5.
252. Ferracane JL, Greener EH. The effect of resin formulation on the degree of conversion and mechanical properties of dental restorative resins. J Biomed Mater Res. 1986;20(1):121-31.
253. Eliades GC, Vougiouklakis GJ, Caputo AA. Degree of double bond conversion in light-cured composites. Dent Mater. 1987;3(1):19-25.
254. Ruyter IE, Oysaed H. Analysis and characterization of dental polymers. CRC Critical Reviews in biocompatibility. 1988;4(3):247-79.
255. Watts D, Silikas N. In situ photo-polymerisation and polymerisation-shrinkage phenomena. Dental hard tissues and bonding: Springer; 2005. p. 123-54.
256. Watts DC. Orthodontic adhesive resins and composites: principles of adhesion. Orthodontic materials Scientific and clinical aspects New York, USA: Thieme Stuttgart. 2001:190-200.
257. Dall'Igna CM, Marchioro EM, Spohr AM, Mota EG. Effect of curing time on the bond strength of a bracket-bonding system cured with a light-emitting diode or plasma arc light. Eur J Orthod. 2011;33(1):55-9.
258. Bang HC, Lim BS, Yoon TH, Lee YK, Kim CW. Effect of plasma arc curing on polymerization shrinkage of orthodontic adhesive resins. J Oral Rehabil. 2004;31(8):803-10.
259. Carvalho RM, Pereira JC, Yoshiyama M, Pashley DH. A review of polymerization contraction: the influence of stress development versus stress relief. Oper Dent. 1996;21(1):17-24.

260. Labella R, Lambrechts P, Van Meerbeek B, Vanherle G. Polymerization shrinkage and elasticity of flowable composites and filled adhesives. Dent Mater. 1999;15(2):128-37.
261. Braem M, Lambrechts P, Vanherle G, Davidson CL. Stiffness increase during the setting of dental composite resins. J Dent Res. 1987;66(12):1713-6.
262. Davidson CL, Feilzer AJ. Polymerization shrinkage and polymerization shrinkage stress in polymer-based restoratives. J Dent. 1997;25(6):435-40.
263. Bouschlicher MR, Vargas MA, Boyer DB. Effect of composite type, light intensity, configuration factor and laser polymerization on polymerization contraction forces. Am J Dent. 1997;10(2):88-96.
264. Uysal T, Ulker M, Ramoglu SI, Ertas H. Microleakage under metallic and ceramic brackets bonded with orthodontic self-etching primer systems. Angle Orthod. 2008;78(6):1089-94.
265. Arhun N, Arman A, Cehreli SB, Arikan S, Karabulut E, Gülşahi K. Microleakage beneath ceramic and metal brackets bonded with a conventional and an antibacterial adhesive system. Angle Orthod. 2006;76(6):1028-34.
266. Hofmann N, Markert T, Hugo B, Klaiber B. Effect of high intensity vs. soft-start halogen irradiation on light-cured resin-based composites. Part II: Hardness and solubility. Am J Dent. 2004;17(1):38-42.
267. Boaro LC, Gonçalves F, Guimarães TC, Ferracane JL, Pfeifer CS, Braga RR. Sorption, solubility, shrinkage and mechanical properties of "low-shrinkage" commercial resin composites. Dent Mater. 2013;29(4):398-404.
268. Truffier-Boutry D, Demoustier-Champagne S, Devaux J, Biebuyck JJ, Mestdagh M, Larbanois P, et al. A physico-chemical explanation of the post-polymerization shrinkage in dental resins. Dent Mater. 2006;22(5):405-12.
269. Bishara SE, VonWald L, Olsen ME, Laffoon JF. Effect of time on the shear bond strength of glass ionomer and composite orthodontic adhesives. Am J Orthod Dentofacial Orthop. 1999;116(6):616-20.
270. Stansbury JW, Dickens SH. Determination of double bond conversion in dental resins by near infrared spectroscopy. Dent Mater. 2001;17(1):71-9.
271. Truffier-Boutry D, Gallez XA, Demoustier-Champagne S, Devaux J, Mestdagh M, Champagne B, et al. Identification of free radicals trapped in solid methacrylated resins. Journal of Polymer Science Part A: Polymer Chemistry. 2003;41(11):1691-9.
272. Kanehira M, Finger WJ, Hoffmann M, Endo T, Komatsu M. Relationship between degree of polymerization and enamel bonding strength with self-etching adhesives. J Adhes Dent. 2006;8(4):211-6.
273. Abdelnaby YL, Al-Wakeel ElS. Effect of early orthodontic force on shear bond strength of orthodontic brackets bonded with different adhesive systems. Am J Orthod Dentofacial Orthop. 2010;138(2):208-14.
274. Miles PG, Weyant RJ, Rustveld L. A clinical trial of Damon 2 vs conventional twin brackets during initial alignment. Angle Orthod. 2006;76(3):480-5.
275. McLaughlin RP, Bennett JC, Trevisi HJ. Systemized orthodontic treatment mechanics: Elsevier Health Sciences; 2001.
276. Naceur IB, Charfi A, Bouraoui T, Elleuch K. Finite element modeling of superelastic nickel-titanium orthodontic wires. J Biomech. 2014;47(15):3630-8.
277. Ong E, Ho C, Miles P. Alignment efficiency and discomfort of three orthodontic archwire sequences: a randomized clinical trial. J Orthod. 2011;38(1):32-9.
278. Proffit WR, Fields Jr HW, Sarver DM. Contemporary orthodontics: Elsevier Health Sciences; 2006.
279. Harzer W, Bourauel C, Gmyrek H. Torque capacity of metal and polycarbonate brackets with and without a metal slot. Eur J Orthod. 2004;26(4):435-41.

280. Morina E, Eliades T, Pandis N, Jäger A, Bourauel C. Torque expression of self-ligating brackets compared with conventional metallic, ceramic, and plastic brackets. Eur J Orthod. 2008;30(3):233-8.
281. Matarese G, Nucera R, Militi A, Mazza M, Portelli M, Festa F, et al. Evaluation of frictional forces during dental alignment: an experimental model with 3 nonleveled brackets. Am J Orthod Dentofacial Orthop. 2008;133(5):708-15.
282. Loftus BP, Artun J, Nicholls JI, Alonzo TA, Stoner JA. Evaluation of friction during sliding tooth movement in various bracket-arch wire combinations. Am J Orthod Dentofacial Orthop. 1999;116(3):336-45.
283. Eliades T, Athanasiou AE. In vivo aging of orthodontic alloys: implications for corrosion potential, nickel release, and biocompatibility. Angle Orthod. 2002;72(3):222-37.
284. Oh KT, Choo SU, Kim KM, Kim KN. A stainless steel bracket for orthodontic application. Eur J Orthod. 2005;27(3):237-44.
285. Archambault A, Lacoursiere R, Badawi H, Major PW, Carey J, Flores-Mir C. Torque expression in stainless steel orthodontic brackets. A systematic review. Angle Orthod. 2010;80(1):201-10.
286. Tselepis M, Brockhurst P, West VC. The dynamic frictional resistance between orthodontic brackets and arch wires. Am J Orthod Dentofacial Orthop. 1994;106(2):131-8.
287. Chakravarthi S, Padmanabhan S, Chitharanjan AB. Allergy and orthodontics. J Orthod Sci. 2012;1(4):83-7.
288. Rahilly G, Price N. Nickel allergy and orthodontics. J Orthod. 2003;30(2):171-4.
289. Birnie D. Ceramic brackets. Br J Orthod. 1990;17(1):71-4.
290. Swartz ML. Ceramic brackets. J Clin Orthod. 1988;22(2):82-8.
291. Russell JS. Aesthetic orthodontic brackets. J Orthod. 2005;32(2):146-63.
292. Lee YK. Colour and translucency of tooth-coloured orthodontic brackets. Eur J Orthod. 2008;30(2):205-10.
293. Yu B, Lee YK. Aesthetic colour performance of plastic and ceramic brackets -- an in vitro study. J Orthod. 2011;38(3):167-74.
294. Ghafari J. Problems associated with ceramic brackets suggest limiting use to selected teeth. Angle Orthod. 1992;62(2):145-52.
295. Fairhurst CW. Dental ceramics: the state of the science. Adv Dent Res. 1992;6:78-81.
296. Nishio C, Mendes AeM, Almeida MA, Tanaka E, Tanne K, Elias CN. Evaluation of esthetic brackets' resistance to torsional forces from the archwire. Am J Orthod Dentofacial Orthop. 2009;135(1):42-8.
297. Suliman SN, Trojan TM, Tantbirojn D, Versluis A. Enamel loss following ceramic bracket debonding: A quantitative analysis in vitro. Angle Orthod. 2015;85(4):651-6.
298. Holberg C, Winterhalder P, Holberg N, Wichelhaus A, Rudzki-Janson I. Orthodontic bracket debonding: risk of enamel fracture. Clin Oral Investig. 2014;18(1):327-34.
299. Theodorakopoulou LP, Sadowsky PL, Jacobson A, Lacefield W. Evaluation of the debonding characteristics of 2 ceramic brackets: an in vitro study. Am J Orthod Dentofacial Orthop. 2004;125(3):329-36.
300. Wang WN, Meng CL, Tarng TH. Bond strength: a comparison between chemical coated and mechanical interlock bases of ceramic and metal brackets. Am J Orthod Dentofacial Orthop. 1997;111(4):374-81.
301. Zinelis S, Eliades T, Eliades G, Makou M, Silikas N. Comparative assessment of the roughness, hardness, and wear resistance of aesthetic bracket materials. Dent Mater. 2005;21(9):890-4.
302. Eliades T. Orthodontic materials research and applications: part 2. Current status and projected future developments in materials and biocompatibility. Am J Orthod Dentofacial Orthop. 2007;131(2):253-62.

303. Feldner JC, Sarkar NK, Sheridan JJ, Lancaster DM. In vitro torque-deformation characteristics of orthodontic polycarbonate brackets. Am J Orthod Dentofacial Orthop. 1994;106(3):265-72.
304. Alkire RG, Bagby MD, Gladwin MA, Kim H. Torsional creep of polycarbonate orthodontic brackets. Dent Mater. 1997;13(1):2-6.
305. Ali O, Makou M, Papadopoulos T, Eliades G. Laboratory evaluation of modern plastic brackets. Eur J Orthod. 2012;34(5):595-602.
306. Sharma-Sayal SK, Rossouw PE, Kulkarni GV, Titley KC. The influence of orthodontic bracket base design on shear bond strength. Am J Orthod Dentofacial Orthop. 2003;124(1):74-82.
307. Bishara SE, Soliman MM, Oonsombat C, Laffoon JF, Ajlouni R. The effect of variation in mesh-base design on the shear bond strength of orthodontic brackets. Angle Orthod. 2004;74(3):400-4.
308. Wang WN, Li CH, Chou TH, Wang DD, Lin LH, Lin CT. Bond strength of various bracket base designs. Am J Orthod Dentofacial Orthop. 2004;125(1):65-70.
309. Lo Giudice G, Lo Giudice A, Isola G, Fabiano F, Artemisia A, Fabiano V, et al. Evaluation of bond strength and detachment interface distribution of different bracket base designs. Acta Medica Mediterranea. 2015;31:585-90.
310. Knox J, Kralj B, Hubsch P, Middleton J, Jones ML. An evaluation of the quality of orthodontic attachment offered by single- and double-mesh bracket bases using the finite element method of stress analysis. Angle Orthod. 2001;71(2):149-55.
311. Chung CH, Friedman SD, Mante FK. Shear bond strength of rebonded mechanically retentive ceramic brackets. Am J Orthod Dentofacial Orthop. 2002;122(3):282-7.
312. Sorel O, El Alam R, Chagneau F, Cathelineau G. Comparison of bond strength between simple foil mesh and laser-structured base retention brackets. Am J Orthod Dentofacial Orthop. 2002;122(3):260-6.
313. Brantley WA, Eliades T. Orthodontic materials: scientific and clinical aspects: Thieme Stuttgart; 2001.
314. 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(3):276-81.
315. Smith DC, Maijer R. Improvements in bracket base design. Am J Orthod. 1983;83(4):277-81.
316. Matasa CG. Direct bonding metallic brackets: where are they heading? Am J Orthod Dentofacial Orthop. 1992;102(6):552-60.
317. Droese V, Diedrich P. [The tensile bonding strength of metal plasma-coated bracket bases]. Fortschr Kieferorthop. 1992;53(3):142-52.
318. Atsü SS, Gelgör IE, Sahin V. Effects of silica coating and silane surface conditioning on the bond strength of metal and ceramic brackets to enamel. Angle Orthod. 2006;76(5):857-62.
319. Hanson GH, Gibbon WM, Shimizu H. Bonding bases coated with porous metal powder: a comparison with foil mesh. Am J Orthod. 1983;83(1):1-4.
320. Nihei T. Dental applications for silane coupling agents. J Oral Sci. 2016;58(2):151-5.
321. Toroglu MS, Yaylali S. Effects of sandblasting and silica coating on the bond strength of rebonded mechanically retentive ceramic brackets. Am J Orthod Dentofacial Orthop. 2008;134(2):181e1-7.
322. Viazis AD, Cavanaugh G, Bevis RR. Bond strength of ceramic brackets under shear stress: an in vitro report. Am J Orthod Dentofacial Orthop. 1990;98(3):214-21.
323. Eliades T, Viazis AD, Lekka M. Failure mode analysis of ceramic brackets bonded to enamel. Am J Orthod Dentofacial Orthop. 1993;104(1):21-6.
324. Bishara SE, Fehr DE. Ceramic brackets: something old, something new, a review. Semin Orthod. 1997;3(3):178-88.

325. Kang DY, Choi SH, Cha JY, Hwang CJ. Quantitative analysis of mechanically retentive ceramic bracket base surfaces with a three-dimensional imaging system. Angle Orthod. 2013;83(4):705-11.
326. Pickett KL, Sadowsky PL, Jacobson A, Lacefield W. Orthodontic in vivo bond strength: comparison with in vitro results. Angle Orthod. 2001;71(2):141-8.
327. Hajrassie MK, Khier SE. In-vivo and in-vitro comparison of bond strengths of orthodontic brackets bonded to enamel and debonded at various times. Am J Orthod Dentofacial Orthop. 2007;131(3):384-90.
328. Britton JC, McInnes P, Weinberg R, Ledoux WR, Retief DH. Shear bond strength of ceramic orthodontic brackets to enamel. Am J Orthod Dentofacial Orthop. 1990;98(4):348-53.
329. Abu-Alhaija E, Jaradat M, Alwahadni A. An Ex-vivo Shear and tensile bond strengths of orthodontic molar tubes bonded using different techniques. J Clin Exp Dent. 2017;9(3):e448-e53.
330. Papadogiannis D, Iliadi A, Bradley TG, Silikas N, Eliades G, Eliades T. Viscoelastic properties of orthodontic adhesives used for lingual fixed retainer bonding. Dent Mater. 2017;33(1):e22-e7.
331. Stanford SK, Wozniak WT, Fan PL. The need for standardization of test protocols. Semin Orthod. 1997;3(3):206-9.
332. Mojtahedzadeh F, Akhoundi MS, Noroozi H. Comparison of wire loop and shear blade as the 2 most common methods for testing orthodontic shear bond strength. Am J Orthod Dentofacial Orthop. 2006;130(3):385-7.
333. Fox NA, McCabe JF, Buckley JG. A critique of bond strength testing in orthodontics. Br J Orthod. 1994;21(1):33-43.
334. Finnema KJ, Ozcan M, Post WJ, Ren Y, Dijkstra PU. In-vitro orthodontic bond strength testing: a systematic review and meta-analysis. Am J Orthod Dentofacial Orthop. 2010;137(5):615-22.e3.
335. Klocke A, Kahl-Nieke B. Influence of force location in orthodontic shear bond strength testing. Dent Mater. 2005;21(5):391-6.
336. Scherrer SS, Cesar PF, Swain MV. Direct comparison of the bond strength results of the different test methods: a critical literature review. Dent Mater. 2010;26(2):e78-93.
337. Littlewood SJ, Redhead A. Use of jigs to standardise orthodontic bond testing. J Dent. 1998;26(5-6):539-45.
338. Dos Santos PA, Garcia PP, Palma-Dibb RG. Shear bond strength of adhesive systems to enamel and dentin. Thermocycling influence. J Mater Sci Mater Med. 2005;16(8):727-32.
339. Bedran-De-Castro AK, Pereira PN, Pimenta LA. Long-term bond strength of restorations subjected to thermo-mechanical stresses over time. Am J Dent. 2004;17(5):337-41.
340. Iso TR. 11405 Dental materials—Guidance on testing of adhesion to tooth structure. International Organization for Standardization, Switzerland, Genf. 1994.
341. Ernst CP, Canbek K, Euler T, Willershausen B. In vivo validation of the historical in vitro thermocycling temperature range for dental materials testing. Clin Oral Investig. 2004;8(3):130-8.
342. Versluis A, Douglas WH, Sakaguchi RL. Thermal expansion coefficient of dental composites measured with strain gauges. Dent Mater. 1996;12(5):290-4.
343. Hengchang X, Wenyi L, Tong W. Measurement of thermal expansion coefficient of human teeth. Australian dental journal. 1989;34(6):530-5.
344. Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent. 1999;27(2):89-99.
345. Aguilar LT, Rezende NP, Reis A, Loguercio AD, Grande RH, Ballester RY, et al. Tensile bond strength of adhesive systems--effects of primer and thermocycling. Pesqui Odontol Bras. 2002;16(1):37-42.

346. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, et al. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials. 2005;26(33):6449-59.
347. Reis AF, Carrilho MR, Ghaname E, Pereira PN, Giannini M, Nikaido T, et al. Effects of water-storage on the physical and ultramorphological features of adhesives and primer/adhesive mixtures. Dent Mater J. 2010;29(6):697-705.
348. Shono Y, Terashita M, Shimada J, Kozono Y, Carvalho RM, Russell CM, et al. Durability of resin-dentin bonds. J Adhes Dent. 1999;1(3):211-8.
349. Fukushima T, Inoue Y, Miyazaki K, Itoh T. Effect of primers containing N-methylolacrylamide or N-methylolmethacrylamide on dentin bond durability of a resin composite after 5 years. J Dent. 2001;29(3):227-34.
350. Söderholm KJ. Water sorption in a bis(GMA)/TEGDMA resin. J Biomed Mater Res. 1984;18(3):271-9.
351. Vanlandingham MR, Eduljee RF, Gillespie JW. Moisture diffusion in epoxy systems. Journal of applied polymer science. 1999;71(5):787-98.
352. Soles CL, Chang FT, Gidley DW, Yee AF. Contributions of the nanovoid structure to the kinetics of moisture transport in epoxy resins. Journal of Polymer Science Part B: Polymer Physics. 2000;38(5):776-91.
353. Soles CL, Yee AF. A discussion of the molecular mechanisms of moisture transport in epoxy resins. Journal of Polymer Science Part B: Polymer Physics. 2000;38(5):792-802.
354. Bellenger V, Verdu J, Morel E. Structure-properties relationships for densely cross-linked epoxide-amine systems based on epoxide or amine mixtures. Journal of materials science. 1989;24(1):63-8.
355. Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med. 2001;12(2):136-51.
356. Momoi Y, McCabe JF. Hygroscopic expansion of resin based composites during 6 months of water storage. Br Dent J. 1994;176(3):91-6.
357. Vallittu PK. Effect of 180-week water storage on the flexural properties of E-glass and silica fiber acrylic resin composite. Int J Prosthodont. 2000;13(4):334-9.
358. Artun J, Bergland S. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. Am J Orthod. 1984;85(4):333-40.
359. Oliver RG. The effect of different methods of bracket removal on the amount of residual adhesive. Am J Orthod Dentofacial Orthop. 1988;93(3):196-200.
360. O'Brien KD, Watts DC, Read MJ. Residual debris and bond strength--is there a relationship? Am J Orthod Dentofacial Orthop. 1988;94(3):222-30.
361. Elnafar AA, Alam MK, Hasan R. The impact of surface preparation on shear bond strength of metallic orthodontic brackets bonded with a resin-modified glass ionomer cement. J Orthod. 2014;41(3):201-7.
362. Kim SS, Park WK, Son WS, Ahn HS, Ro JH, Kim YD. Enamel surface evaluation after removal of orthodontic composite remnants by intraoral sandblasting: a 3-dimensional surface profilometry study. Am J Orthod Dentofacial Orthop. 2007;132(1):71-6.
363. Ritter DE, Ritter AV, Bruggeman G, Locks A, Tulloch JF. Bond strengths and adhesive remnant index of self-etching adhesives used to bond brackets to instrumented and uninstrumented enamel. Am J Dent. 2006;19(1):47-50.
364. Montasser MA, Drummond JL. Reliability of the adhesive remnant index score system with different magnifications. Angle Orthod. 2009;79(4):773-6.
365. Robles-Ruíz JJ, Ciamponi AL, Medeiros IS, Kanashiro LK. Effect of lingual enamel sandblasting with aluminum oxide of different particle sizes in combination with phosphoric acid etching on indirect bonding of lingual brackets. Angle Orthod. 2014;84(6):1068-73.

366. Mews L, Kern M, Ciesielski R, Fischer-Brandies H, Koos B. Shear bond strength of orthodontic brackets to enamel after application of a caries infiltrant. Angle Orthod. 2015;85(4):645-50.
367. Bishara SE, Ostby AW, Laffoon JF, Warren J. Shear bond strength comparison of two adhesive systems following thermocycling. A new self-etch primer and a resin-modified glass ionomer. Angle Orthod. 2007;77(2):337-41.
368. Lee YK, Lim YK. Three-dimensional quantification of adhesive remnants on teeth after debonding. Am J Orthod Dentofacial Orthop. 2008;134(4):556-62.
369. Bishara SE, VonWald L, Laffoon JF, Warren JJ. Effect of a self-etch primer/adhesive on the shear bond strength of orthodontic brackets. Am J Orthod Dentofacial Orthop. 2001;119(6):621-4.
370. Grünheid T, Sudit GN, Larson BE. Debonding and adhesive remnant cleanup: an in vitro comparison of bond quality, adhesive remnant cleanup, and orthodontic acceptance of a flash-free product. Eur J Orthod. 2015;37(5):497-502.
371. Peixoto A, Mesquita MF, Costa HN, Carvalho PA, Manso A. Characterization of enamel surface after orthodontic brackets debonding: an in vitro study. Microscopy and Microanalysis. 2015;21:64-5.
372. Andrews LF. The six keys to normal occlusion. Am J Orthod. 1972;62(3):296-309.
373. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003;28(3):215-35.
374. Vinay S, Shivanna V. Comparative evaluation of microleakage of fifth, sixth, and seventh generation dentin bonding agents: An in vitro study. J Conserv Dent. 2010;13(3):136-40.
375. Sadek FT, Goracci C, Cardoso PE, Tay FR, Ferrari M. Microtensile bond strength of current dentin adhesives measured immediately and 24 hours after application. J Adhes Dent. 2005;7(4):297-302.
376. Kaaden C, Powers JM, Friedl KH, Schmalz G. Bond strength of self-etching adhesives to dental hard tissues. Clin Oral Investig. 2002;6(3):155-60.
377. Ajlouni R, Bishara SE, Oonsombat C, Denehy GE. Evaluation of modifying the bonding protocol of a new acid-etch primer on the shear bond strength of orthodontic brackets. Angle Orthod. 2004;74(3):410-3.
378. Eliades T, Brantley WA. The inappropriateness of conventional orthodontic bond strength assessment protocols. Eur J Orthod. 2000;22(1):13-23.
379. Bishara SE, Soliman M, Laffoon J, Warren JJ. Effect of changing a test parameter on the shear bond strength of orthodontic brackets. Angle Orthod. 2005;75(5):832-5.
380. Eliades T, Katsavrias E, Zinelis S, Eliades G. Effect of loading rate on bond strength. J Orofac Orthop. 2004;65(4):336-42.

Appendix 1. RM&G Consortium NHS Permission for Research

Birmingham and the Black Country
NIHR Comprehensive Local Research Network

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Tuesday, 16 August 2011

LETTER OF NHS PERMISSION

NHS Permission has been granted by the BBC CLRN RM&G Consortium Office on behalf of the BBC CLRN RM&G Consortium Trusts. The Chief Investigator named in this letter has permission to undertake the following research activity in the Consortium NHS Trust(s) identified below.

Chief Investigator Name: **Lorna Adams**

Original Date of Issue: **16.08.2011**

Project Title:	An investigation of the shear bond strength and bond failure mechanism of pre-mixed self-etching primers using metallic orthodontic brackets
Consortium R&D No.	**1467**
REC Ref.:	09.H0405.33
Project Start/End Dates:	**Start Date:** 01.11.10
	End date: 31.10.2012
Chief Investigator:	Laura Adams
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HTA Licence No.:	12313
NHS Trust Registered:	Birmingham Community Healthcare NHS Trust (BCHC)
Research Site:	Birmingham Dental Hospital
Trust Service/Directorate:	Special Services Division (Dental)

Thank you for informing the BBC CLRN RM&G Consortium of the above research.

If you require any further assistance, please call the CLRN RM&G Consortium office stating your **RM&G Reference Number 1449**.

We wish you success on completing your research.

Yours Sincerely,

Susie Fisher
CLRN RM&G Operational Manager (Consortium)
BBC CLRN RM&G Consortium

Appendix 2. Calculation for Mean Bracket Base Surface Area

Mean surface area (mm^2) for brackets was calculated using 20 representative sample brackets.

Bracket sample	Base surface Area (mm^2)
1	10.55
2	10.69
3	10.61
4	10.59
5	10.55
6	10.54
7	10.62
8	10.68
9	10.59
10	10.58
11	10.60
12	10.61
13	10.60
14	10.64
15	10.62
16	10.59
17	10.58
18	10.60
19	10.56
20	10.61
Mean (n=20)	10.60
SD	0.04

Appendix 3. Statistical Output Pre-cure SBS at Immediate Debonding

ANOVA

```
                Df Sum Sq Mean Sq F value  Pr(>F)
Adhesive.Type    2  50.41   25.21   5.082 0.00931 **
Residuals       57 282.72    4.96
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

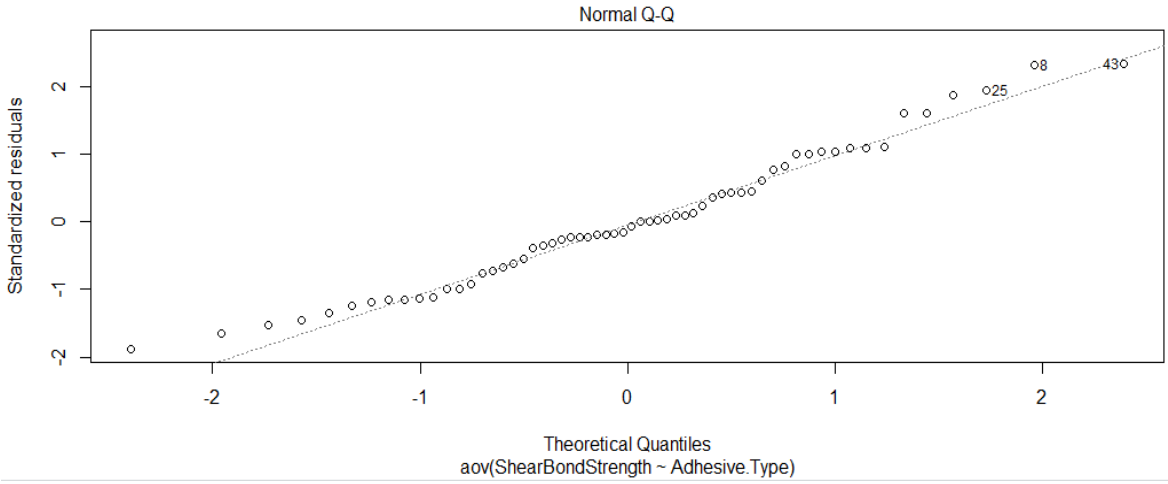
Tukey Multiple Comparison Test

```
  Tukey multiple comparisons of means
    99% family-wise confidence level

Fit: aov(formula = ShearBondStrength ~ Adhesive.Type, data = ID)

$Adhesive.Type
                       diff         lwr      upr     p adj
IBOND-CLEARFIL S3    0.5335 -1.60338893 2.670389 0.7303375
XENO V+-CLEARFIL S3 2.1555  0.01861107 4.292389 0.0092921
XENO V+-IBOND       1.6220 -0.51488893 3.758889 0.0634319
```

Quantile-Quantile plot (Q-Q)



A Q-Q plot (quantile-quantile) showing the data distribution of mean shear bond strengths for PC groups for Xeno V+, Clearfil S3 and iBond SE at immediate debonding. The points are close to the plotted line which indicates that the data is normally distributed.

Appendix 4. Statistical Output Co-cure SBS at Immediate Debonding

ANOVA

```
                Df Sum Sq Mean Sq F value   Pr(>F)
Adhesive.Type    3  142.1   47.35   6.847 0.000382 ***
Residuals       76  525.6    6.92
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

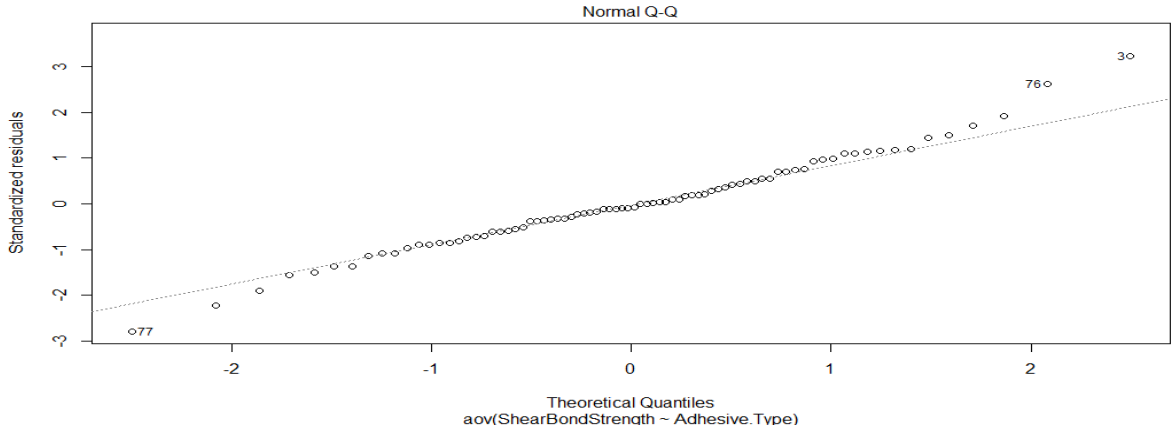
Tukey Multiple Comparison Test

```
  Tukey multiple comparisons of means
    99% family-wise confidence level

Fit: aov(formula = ShearBondStrength ~ Adhesive.Type, data = ID2)

$`Adhesive.Type`
                           diff         lwr      upr     p adj
IBOND-CLEARFIL S3     -1.0795 -3.75685306 1.597853 0.5669936
TRANSBOND-CLEARFIL S3  1.6940 -0.98335306 4.371353 0.1836762
XENO V+-CLEARFIL S3    2.2685 -0.40885306 4.945853 0.0387666
TRANSBOND-IBOND        2.7735  0.09614694 5.450853 0.0070840
XENO V+-IBOND          3.3480  0.67064694 6.025353 0.0007548
XENO V+-TRANSBOND      0.5745 -2.10285306 3.251853 0.9002983
```

Quantile-Quantile plot (Q-Q)



A Q-Q plot (quantile-quantile) showing the data distribution of mean shear bond strengths for CC groups for Xeno V+, Clearfil S3 and iBond SE at immediate debonding. The points are close to the plotted line which indicates that the data is normally distributed.

Appendix 5. Statistical Output Pre-cure and Co-cure SBS Immediate Debonding

Unpaired T-Test: Xeno V+

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20  7.73  3.41
2 Pre-Cure     20  7.86  2.27
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = -0.14839, df = 38, p-value = 0.8828
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.991315  1.719315
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                 7.7255                  7.8615
```

Unpaired T-Test: Clearfil S3

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20  5.46  1.93
2 Pre-Cure     20  5.71  2.43
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = -0.35888, df = 38, p-value = 0.7217
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.653572  1.155572
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                 5.457                   5.706
```

Unpaired T-Test: iBond SE

```
# A tibble: 2 x 4
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20  4.38  1.56
2 Pre-Cure     20  6.24  1.96
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = -3.3297, df = 38, p-value = 0.001942
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.9940465 -0.7299535
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                 4.3775                  6.2395
```

Appendix 6. Statistical Output Pre-cure SBS after Thermocycling

ANOVA

```
              Df Sum Sq Mean Sq F value   Pr(>F)
Adhesive.Type  2  138.5   69.25   14.49 8.15e-06 ***
Residuals     57  272.3    4.78
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

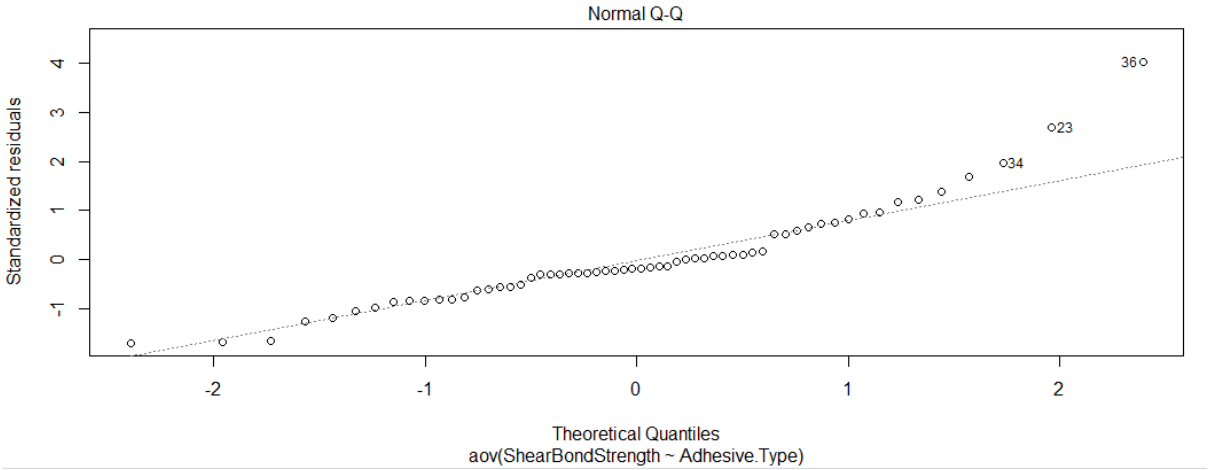
Tukey Multiple Comparison Test

```
  Tukey multiple comparisons of means
    99% family-wise confidence level

Fit: aov(formula = ShearBondStrength ~ Adhesive.Type, data = TC)

$Adhesive.Type
                          diff        lwr        upr     p adj
IBOND-CLEARFIL S3    -3.6925 -5.7898192 -1.5951808 0.0000050
XENO V+-CLEARFIL S3  -2.2475 -4.3448192 -0.1501808 0.0054012
XENO V+-IBOND         1.4450 -0.6523192  3.5423192 0.1008203
```

Quantile-Quantile plot (Q-Q)



A Q-Q plot (quantile-quantile) showing the data distribution of mean shear bond strengths for PC groups for Xeno V+, Clearfil S3 and iBond SE after thermocycling. The points are close to the plotted line which indicates that the data is normally distributed.

Appendix 7. Statistical Output Co-cure SBS after Thermocycling

ANOVA

```
              Df Sum Sq Mean Sq F value   Pr(>F)
Adhesive.Type  3   1018   339.5   14.65 1.27e-07 ***
Residuals     76   1760    23.2
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

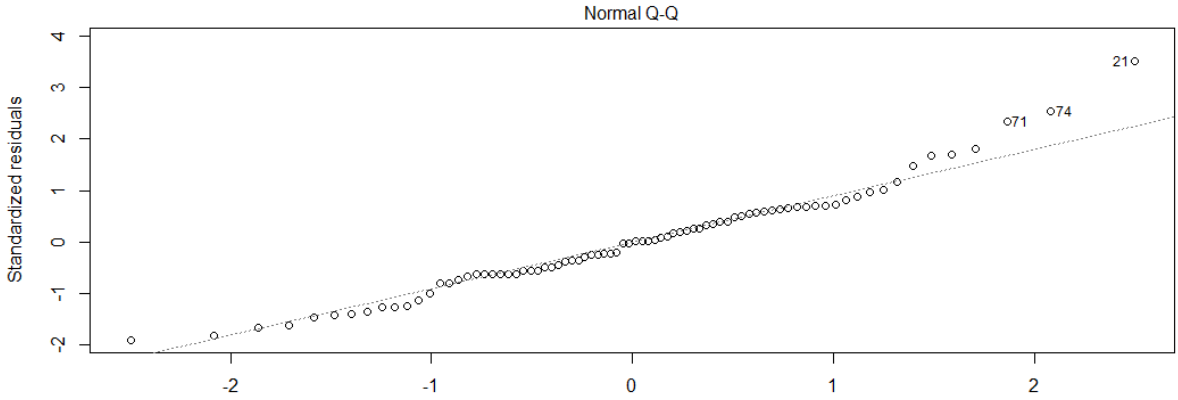
Tukey Multiple Comparison Test

```
  Tukey multiple comparisons of means
    99% family-wise confidence level

Fit: aov(formula = ShearBondStrength ~ Adhesive.Type, data = TC2)

$Adhesive.Type
                           diff       lwr         upr     p adj
IBOND-CLEARFIL S3     -3.7630 -8.662779  1.13677903 0.0724894
TRANSBOND-CLEARFIL S3  6.2075  1.307721 11.10727903 0.0006288
XENO V+-CLEARFIL S3    1.3525 -3.547279  6.25227903 0.8107312
TRANSBOND-IBOND        9.9705  5.070721 14.87027903 0.0000000
XENO V+-IBOND          5.1155  0.215721 10.01527903 0.0065447
XENO V+-TRANSBOND     -4.8550 -9.754779  0.04477903 0.0109005
```

Quantile-Quantile plot (Q-Q)



A Q-Q plot (quantile-quantile) showing the data distribution of mean shear bond strengths for CC groups for Xeno V+, Clearfil S3, iBond SE and Transbond Plus after thermocycling. The points are close to the plotted line which indicates that the data is normally distributed.

Appendix 8. Statistical Output Pre-cure with Co-cure SBS after Thermocycling

T-Test: Xeno V+

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20  8.05  3.72
2 Pre-Cure     20  2.11  1.73
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = 6.4776, df = 38, p-value = 1.263e-07
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 4.082232 7.793768
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                 8.0455                  2.1075
```

T-Test: Clearfil S3

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20  6.69  5.13
2 Pre-Cure     20  4.35  3.31
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = 1.7113, df = 38, p-value = 0.09518
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.4277593  5.1037593
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                  6.693                   4.355
```

T-Test: iBond SE

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20 2.93  3.09
2 Pre-Cure     20 0.662 0.616
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = 3.2135, df = 38, p-value = 0.002673
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.8390714 3.6959286
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                 2.9300                  0.6625
```

Appendix 9. Statistical Output Pre-cure SBS after Water Storage

ANOVA

```
                Df Sum Sq Mean Sq F value Pr(>F)
Adhesive.Type    2   44.4  22.202   2.703 0.0756 .
Residuals       57  468.2   8.214
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

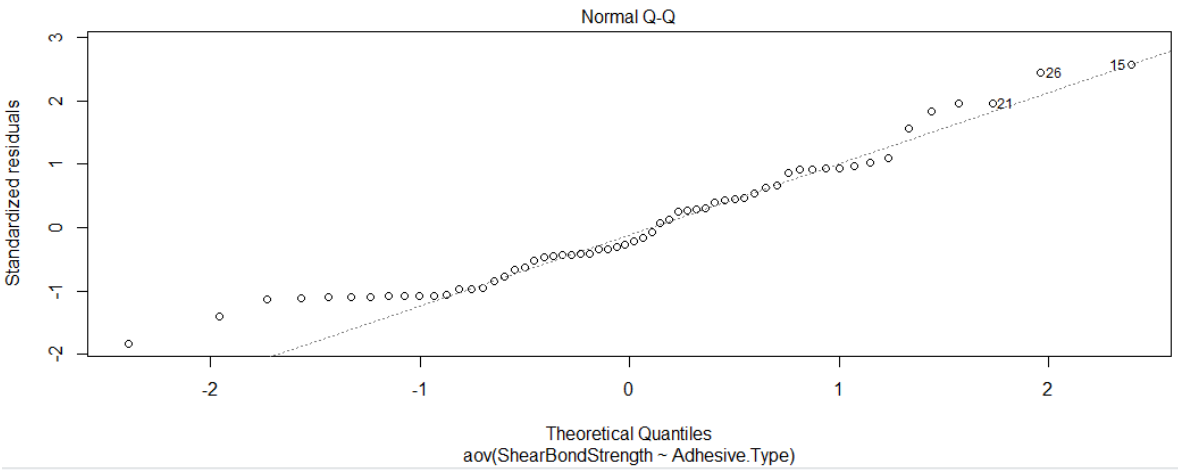
Tukey Multiple Comparison Test

```
  Tukey multiple comparisons of means
    99% family-wise confidence level

Fit: aov(formula = ShearBondStrength ~ Adhesive.Type, data = WS)

$Adhesive.Type
                         diff       lwr       upr     p adj
IBOND-CLEARFIL S3   -0.6810 -3.430948 2.0689479 0.7340229
XENO V+-CLEARFIL S3 -2.0675 -4.817448 0.6824479 0.0666418
XENO V+-IBOND       -1.3865 -4.136448 1.3634479 0.2847681
```

Quantile-Quantile plot (Q-Q)



A Q-Q plot (quantile-quantile) showing the data distribution of mean shear bond strengths for PC groups for 1-SEP Xeno V+, Clearfil S3 and iBond SE after water storage at 37°C for 7 days. The points are close to the plotted line which indicates that the data is normally distributed.

Appendix 10. Statistical Output Co-cure SBS after Water Storage

ANOVA

```
              Df Sum Sq Mean Sq F value   Pr(>F)
Adhesive.Type  3  716.8  238.93   15.81 4.42e-08 ***
Residuals     76 1148.8   15.12
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

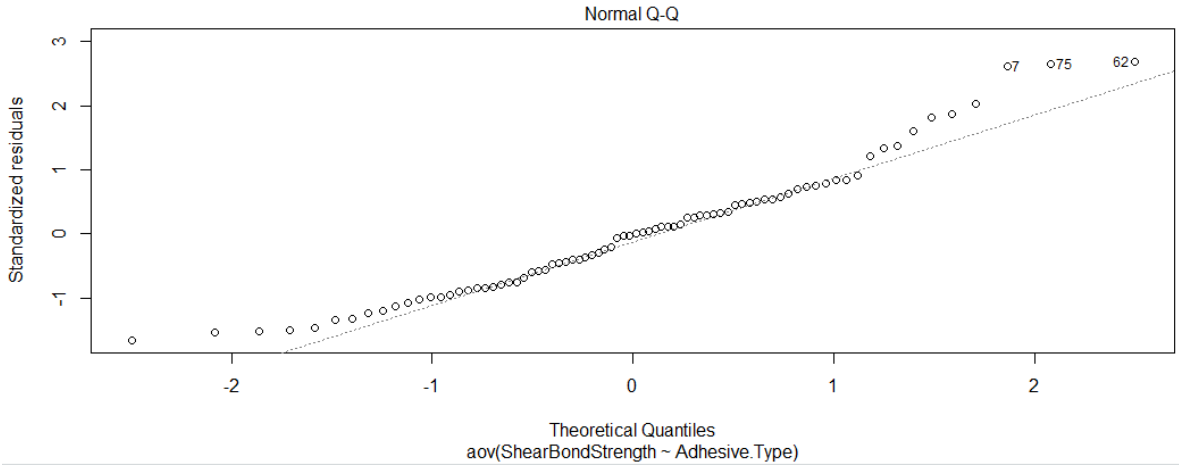
Tukey Multiple Comparison Test

```
  Tukey multiple comparisons of means
    99% family-wise confidence level

Fit: aov(formula = ShearBondStrength ~ Adhesive.Type, data = WS2)

$Adhesive.Type
                           diff        lwr       upr     p adj
IBOND-CLEARFIL S3     -7.0270 -10.985242 -3.068758 0.0000012
TRANSBOND-CLEARFIL S3 -0.3885  -4.346742  3.569742 0.9889908
XENO V+-CLEARFIL S3    0.0155  -3.942742  3.973742 0.9999993
TRANSBOND-IBOND        6.6385   2.680258 10.596742 0.0000043
XENO V+-IBOND          7.0425   3.084258 11.000742 0.0000011
XENO V+-TRANSBOND      0.4040  -3.554242  4.362242 0.9876586
```

Quantile-Quantile plot (Q-Q)



A Q-Q plot (quantile-quantile) showing the data distribution of mean shear bond strengths for CC groups for Xeno V+, Clearfil S3, iBond SE and Transbond Plus after water storage. The points are close to the plotted line which indicates that the data is normally distributed.

Appendix 11. Statistical Output Pre-cure with Co-cure SBS after Water Storage

Unpaired T-Test: Xeno V+

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20 13.1   4.01
2 Pre-Cure     20  3.10  3.08
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = 8.8286, df = 38, p-value = 9.671e-11
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  7.700449 12.282551
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                13.0870                  3.0955
```

Unpaired T-Test: Clearfil S3

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20 13.1   4.03
2 Pre-Cure     20  5.16  3.42
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = 6.6859, df = 38, p-value = 6.564e-08
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  5.513909 10.303091
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                13.0715                  5.1630
```

Unpaired T-Test: iBond SE

```
  CureStep  count  mean    sd
  <fct>     <int> <dbl> <dbl>
1 NoPreCure    20  6.04  2.37
2 Pre-Cure     20  4.48  1.85
```

```
        Two Sample t-test

data:  ShearBondStrength by CureStep
t = 2.3283, df = 38, p-value = 0.02532
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.2039313 2.9210687
sample estimates:
mean in group NoPreCure  mean in group Pre-Cure
                6.0445                  4.4820
```

Appendix 12. Statistical Output Pre-cure ARI at Immediate Debonding

Fisher's Exact Test

```
        Fisher's Exact Test for Count Data

data:  ImmediateDebondPC
p-value = 0.03203
alternative hypothesis: two.sided
```

Contingency Table

ARI	CLEARFIL S3	IBOND	XENO V+	Grand Total
0	7	14	14	35
1	13	6	5	24
2			1	1
3				0
Grand Total	**20**	**20**	**20**	**60**

Appendix 13. Statistical Output Co-cure ARI at Immediate Debonding

Fisher's Exact Test

```
        Fisher's Exact Test for Count Data

data:  ImmediateDebond
p-value = 7.658e-11
alternative hypothesis: two.sided
```

Contingency Table

ARI	CLEARFIL S3	IBOND	TRANSBOND	XENO V+	Grand Total
0	7	12		18	37
1	11	8	6	2	27
2	1		3		4
3	1		11		12
Grand Total	**20**	**20**	**20**	**20**	**80**

Appendix 14. Statistical Output Pre-cure ARI after Thermocycling

Fisher's Exact Test

```
        Fisher's Exact Test for Count Data

data:  ThermocyclingPC
p-value = 0.01734
alternative hypothesis: two.sided
```

Contingency Table

ARI	CLEARFIL S3	IBOND	XENO V+	Grand Total
0	7	15	15	37
1	13	5	5	23
2				0
3				0
Grand Total	**20**	**20**	**20**	**60**

Appendix 15. Statistical Output Pre-cure with Co-cure ARI after Thermocycling

Fisher's Exact Test

```
        Fisher's Exact Test for Count Data

data:  Thermocycling
p-value = 7.241e-06
alternative hypothesis: two.sided
```

Contingency Table

ARI	CLEARFIL S3	IBOND	TRANSBOND	XENO V+	Grand Total
0	8	17	3	8	36
1	7	3	17	12	39
2	4				4
3	1				1
Grand Total	**20**	**20**	**20**	**20**	**80**

Appendix 16. Statistical Output Pre-cure ARI after Water Storage

Fisher's Exact Test

```
        Fisher's Exact Test for Count Data

data:  WaterStoragePC
p-value = 0.001267
alternative hypothesis: two.sided
```

Contingency Table

ARI	CLEARFIL S3	IBOND	XENO V+	Grand Total
0	12	20	12	44
1	6		8	14
2	2			2
3				0
Grand Total	**20**	**20**	**20**	**60**

Appendix 17. Statistical Output Co-cure ARI after Water Storage

Fisher's Exact Test

```
        Fisher's Exact Test for Count Data

data:  WaterStorage
p-value = 4.667e-06
alternative hypothesis: two.sided
```

Contingency Table

ARI	CLEARFIL S3	IBOND	TRANSBOND	XENO V+	Grand Total
0	1	13	1	4	19
1	12	7	9	15	43
2	3		5	1	9
3	4		5		9
Grand Total	**20**	**20**	**20**	**20**	**80**

Appendix 18. Statistical Output Pre-cure with Co-cure ARI All Conditions

Xeno V+: Fisher's Exact Test and Contingency Table

```
        Fisher's Exact Test for Count Data

data:  XenoV
p-value = 0.07476
alternative hypothesis: two.sided
```

ARI	Co-cure	Pre-cure	Grand Total
0.00	30	41	71
1.00	29	18	47
2.00	1	1	2
3.00	0	0	0
Grand Total	**60**	**60**	**120**

Clearfil S3: Fisher's Exact Test and Contingency Table

```
        Fisher's Exact Test for Count Data

data:  Clearfils3
p-value = 0.005613
alternative hypothesis: two.sided
```

ARI	Co-cure	Pre-cure	Grand Total
0.00	16	26	42
1.00	30	32	62
2.00	8	2	10
3.00	6	0	6
Grand Total	**60**	**60**	**120**

iBond SE: Fisher's Exact Test and Contingency Table

```
        Fisher's Exact Test for Count Data

data:  IBond
p-value = 0.2003
alternative hypothesis: two.sided
```

ARI	Co-cure	Pre-cure	Grand Total
0.00	42	49	91
1.00	18	11	29
2.00	0	0	0
3.00	0	0	0
Grand Total	**60**	**60**	**120**

Appendix 19. Data for Pre-cure Groups at Immediate Debonding

XENO V+ PC 30 MINS DB			
	LOAD (N)	STRESS (MPa)	ARI
1	79.61	7.51	0
2	86.05	8.12	1
3	78.24	7.38	0
4	50	4.72	0
5	93.34	8.81	0
6	57.39	5.41	0
7	83.23	7.85	0
8	136.67	12.89	0
9	62.36	5.88	0
10	56.77	5.36	2
11	107.29	10.12	1
12	93.12	8.78	1
13	102.43	9.66	1
14	45.4	4.28	0
15	75.11	7.09	0
16	92.76	8.75	0
17	60.37	5.7	0
18	107.06	10.1	0
19	120.25	11.34	1
20	79.34	7.48	0
MEDIAN	81.42	7.68	
MEAN	83.34	7.86	
S.D	23.47	2.27	

CLEARFIL S3 PC 30 MINS DB			
	LOAD (N)	STRESS (MPa)	ARI
1	58.74	5.54	0
2	85.36	8.05	1
3	45.16	4.26	0
4	29.51	2.78	0
5	105.12	9.92	1
6	70.96	6.69	1
7	33.84	3.19	0
8	56.17	5.3	1
9	51.44	4.85	0
10	62.66	5.91	1
11	53.17	5.02	1
12	17.06	1.61	1
13	85.55	8.07	1
14	25.18	2.38	1
15	61.48	5.8	1
16	97.34	9.18	1
17	78.38	7.39	0
18	42.83	4.04	0
19	46.37	4.38	1
20	103.43	9.76	1
MEDIAN	57.46	5.42	
MEAN	60.49	5.71	
S.D	25.08	2.43	

IBOND PC 30 MINS DB			
	LOAD (N)	STRESS (MPa)	ARI
1	89.21	8.41	0
2	79.92	7.54	0
3	119.7	11.29	0
4	59.92	5.65	1
5	66.25	6.25	1
6	37.63	3.55	0
7	91.54	8.63	0
8	38.69	3.65	1
9	40.36	3.81	0
10	43.17	4.07	1
11	74.58	7.03	0
12	61.1	5.76	0
13	49.32	4.65	0
14	61.8	5.83	1
15	71.38	6.73	0
16	61.05	5.76	0
17	89.28	8.42	0
18	53.41	5.04	0
19	68.25	6.44	0
20	66.59	6.28	1
MEDIAN	64.03	6.04	
MEAN	66.16	6.24	
S.D	20.23	1.96	

Appendix 20. Data for Co-cure Groups at Immediate Debonding

XENO V+ CC 30 MINS DB			
	LOAD (N)	STRESS (MPa)	ARI
1	58.8	5.54	0
2	39.9	3.76	0
3	169.7	16.01	0
4	128.04	12.08	0
5	112.83	10.64	0
6	102.61	9.68	0
7	78.65	7.42	1
8	90.74	8.56	0
9	76.21	7.19	0
10	62.22	5.87	0
11	44.63	4.21	0
12	96.99	9.15	0
13	94.02	8.87	0
14	107.06	10.1	0
15	44.94	4.24	0
16	121.05	11.42	0
17	21.41	2.02	0
18	68.05	6.42	0
19	41.13	3.88	0
20	78.97	7.45	1
MEDIAN	78.81	7.44	
MEAN	81.9	7.73	
S.D.	35.25	3.41	

CLEARFIL S3 CC 30 MINS DB			
	LOAD (N)	STRESS (MPa)	ARI
1	89.56	8.45	1
2	55.13	5.21	1
3	76.89	7.25	0
4	35.97	3.39	1
5	28.58	2.67	0
6	63.48	5.99	1
7	65.6	6.19	2
8	38.74	3.65	1
9	31.41	2.96	1
10	63.2	5.96	0
11	109.78	10.36	1
12	59.2	5.58	1
13	37.97	3.58	0
14	77.2	7.28	0
15	71	6.7	0
16	48.5	4.58	1
17	41.14	3.88	0
18	57.98	5.47	1
19	52.77	4.98	3
20	53.16	5.01	1
MEDIAN	56.56	5.34	
MEAN	57.86	5.46	
S.D.	19.94	1.93	

IBOND CC 30 MINS DB			
	LOAD (N)	STRESS (MPa)	ARI
1	61.46	5.8	1
2	44.64	4.21	0
3	22.15	2.09	0
4	46.68	4.4	1
5	44.14	4.16	0
6	16.91	1.6	0
7	51.61	4.87	0
8	86.94	8.2	1
9	59.87	5.65	0
10	40.09	3.78	0
11	36.26	3.42	0
12	38.48	3.63	1
13	51.08	4.81	1
14	73.16	6.9	1
15	49.22	4.64	0
16	30.59	2.89	1
17	57.96	5.47	0
18	31.36	2.96	0
19	37.84	3.57	0
20	47.68	4.5	1
MEDIAN	45.66	4.31	
MEAN	46.41	4.38	
S.D.	16.09	1.56	

TRANSBOND CC 30 MINS DB			
	LOAD (N)	STRESS (MPa)	ARI
1	24.52	2.31	3
2	59.28	5.59	2
3	105.68	9.97	3
4	102.01	9.62	3
5	76.35	7.2	1
6	65.65	6.19	3
7	67.08	6.33	3
8	51.61	4.87	3
9	52.5	4.95	3
10	44.8	4.23	3
11	105.52	9.96	1
12	66.11	6.24	3
13	107.25	10.12	2
14	85.68	8.08	1
15	78.54	7.41	2
16	146.67	13.84	3
17	0.11	0.01	1
18	72.52	6.84	3
19	95.82	9.04	1
20	108.32	10.22	1
MEDIAN	74.44	7.02	
MEAN	75.8	7.15	
S.D.	32.44	3.14	

Appendix 21. Data for Pre-cure Groups after Thermocycling

XENO V+ PC THERMOCYCLING			
	LOAD (N)	STRESS (MPa)	ARI
1	2.95	0.28	0
2	49.01	4.62	0
3	16.3	1.54	0
4	53.64	5.06	0
5	43.79	4.13	0
6	15.39	1.45	1
7	49.61	4.68	1
8	3.17	0.3	1
9	0.42	0.04	0
10	8.72	0.82	0
11	18.98	1.79	0
12	40.74	3.84	0
13	35.81	3.38	1
14	0.37	0.34	0
15	38.91	3.67	0
16	22.69	2.14	1
17	10.86	1.02	0
18	3.83	0.36	0
19	24.53	2.31	0
20	3.98	0.38	0
MEDIAN	17.64	1.67	
MEAN	22.19	2.11	
S.D.	18.02	1.73	

CLEARFIL S3 PC THERMOCYCLING			
	LOAD (N)	STRESS (MPa)	ARI
1	43.28	4.08	1
2	33.32	3.14	0
3	106.93	10.09	1
4	41.89	3.95	0
5	8.21	0.77	1
6	37.82	3.57	0
7	17.64	1.66	1
8	31.99	3.02	1
9	22.6	2.13	1
10	62.89	5.93	1
11	7.66	0.72	1
12	84.3	7.95	1
13	67.1	6.33	1
14	90.41	8.53	0
15	8.89	0.84	1
16	136.96	12.92	0
17	28.58	2.7	0
18	39.75	3.75	0
19	19.51	1.84	1
20	33.74	3.18	1
MEDIAN	35.78	3.38	
MEAN	46.17	4.36	
S.D.	34.22	3.31	

IBOND PC THERMOCYCLING			
	LOAD (N)	STRESS (MPa)	ARI
1	10.02	0.95	1
2	7.41	0.7	0
3	1.57	0.15	0
4	2.03	0.19	0
5	21.7	2.05	0
6	2.11	0.2	0
7	2.74	0.26	1
8	0.43	0.04	0
9	8.46	0.8	0
10	18.9	1.78	0
11	10.49	0.99	0
12	0.67	0.06	0
13	0.51	0.05	0
14	9.28	0.88	0
15	8.81	0.83	0
16	18.79	1.77	1
17	0.34	0.03	1
18	5.93	0.56	0
19	6.95	0.66	0
20	3.16	0.3	1
MEDIAN	6.44	0.61	
MEAN	7.02	0.66	
S.D.	6.37	0.62	

Appendix 22. Data for Co-cure Groups after Thermocycling

XENO V+ CC THERMOCYCLING			
	LOAD (N)	STRESS (MPa)	ARI
1	67.61	6.38	1
2	143.51	13.54	1
3	86.76	8.18	0
4	57.9	5.46	0
5	115.95	10.94	0
6	89.63	8.46	1
7	86.13	8.13	1
8	46.07	4.35	0
9	46.12	4.35	1
10	18.69	1.76	0
11	125.25	11.82	1
12	67.33	6.35	0
13	118.95	11.22	1
14	75.21	7.1	1
15	114.77	10.83	1
16	0.52	0.49	0
17	104.75	9.88	1
18	158.47	14.95	1
19	102.72	9.69	1
20	74.48	7.03	0
MEDIAN	86.45	8.16	
MEAN	85.04	8.04	
S.D.	38.91	3.72	

CLEARFIL S3 CC THERMOCYCLING			
	LOAD (N)	STRESS (MPa)	ARI
1	245.25	23.14	2
2	120.98	11.41	0
3	84.35	7.96	1
4	0.42	0.04	0
5	56.65	5.34	1
6	114.23	10.78	0
7	69.42	6.55	2
8	102.49	9.67	1
9	42.79	4.04	3
10	21.82	2.06	0
11	106.23	10.02	0
12	58.32	5.5	0
13	69.32	6.54	2
14	34.8	3.28	1
15	8.13	0.77	1
16	79.75	7.52	2
17	37.37	3.53	1
18	76.6	7.23	1
19	81.61	7.7	0
20	8.22	0.78	0
MEDIAN	69.37	6.55	
MEAN	70.94	6.69	
S.D.	53.04	5.13	

IBOND CC THERMOCYCLING			
	LOAD (N)	STRESS (MPa)	ARI
1	44.23	4.17	0
2	0.34	0.03	0
3	0.52	0.05	0
4	0.6	0.06	0
5	50.88	4.8	1
6	0.47	0.04	0
7	9.34	0.88	0
8	64.51	6.09	0
9	0.41	0.04	0
10	7.02	0.66	0
11	40.6	3.83	1
12	0.43	0.04	0
13	58.32	5.5	0
14	66.74	6.3	0
15	114.27	10.78	1
16	63.4	5.98	0
17	3.19	0.3	0
18	33.17	3.13	0
19	56.14	5.3	0
20	6.55	0.62	0
MEDIAN	21.255	2.01	
MEAN	31.06	2.93	
S.D.	31.97	3.09	

TRANSBOND CC THERMOCYCLING			
	LOAD (N)	STRESS (MPa)	ARI
1	226.84	21.4	1
2	221.2	20.87	1
3	152.59	14.4	1
4	63.83	6.02	1
5	137.25	12.95	1
6	124.76	11.77	1
7	125.51	11.84	1
8	165.37	15.6	1
9	160.93	15.18	0
10	67.13	6.33	1
11	253.53	23.92	1
12	46.88	4.42	1
13	117.66	11.1	1
14	262.89	24.8	0
15	42.11	3.97	1
16	80.43	7.59	1
17	184.94	17.45	1
18	75.53	7.13	0
19	171.26	16.16	1
20	54.19	5.11	1
MEDIAN	131.38	12.4	
MEAN	136.74	12.9	
S.D.	67.65	6.38	

Appendix 23. Data for Pre-cure Groups after Water Storage

XENO V+ PC WATER STORAGE			
	LOAD (N)	STRESS (MPa)	ARI
1	58.33	5.5	0
2	30.4	2.87	0
3	0.63	0.06	0
4	22.72	2.14	0
5	9.98	0.94	1
6	14.3	1.35	1
7	0.75	0.07	1
8	34.95	3.3	1
9	51.56	4.86	0
10	46.88	4.42	1
11	1.15	0.11	0
12	60.69	5.73	0
13	0.47	0.04	0
14	0.42	0.04	0
15	109.02	10.28	0
16	0.58	0.06	1
17	79.43	7.49	0
18	87.34	8.24	1
19	20.54	1.94	0
20	26.22	2.47	1
MEDIAN	24.47	2.31	
MEAN	32.82	3.1	
S.D.	31.86	3.08	

CLEARFIL S3 PC WATER STORAGE			
	LOAD (N)	STRESS (MPa)	ARI
1	112.65	10.63	0
2	83.34	7.86	0
3	26	2.45	0
4	20.85	1.97	1
5	46.53	4.39	0
6	127.07	11.99	1
7	67.98	6.41	2
8	12.88	1.22	0
9	42.49	4.01	0
10	41.65	3.93	0
11	63.47	5.99	2
12	67.28	6.35	1
13	42.03	3.97	1
14	112.6	10.62	0
15	22.6	2.13	0
16	74.2	7	1
17	0.35	0.03	0
18	21.37	2.02	1
19	87.08	8.22	0
20	21.97	2.07	0
MEDIAN	44.51	4.2	
MEAN	54.72	5.16	
S.D.	35.38	3.43	

IBOND PC WATER STORAGE			
	LOAD (N)	STRESS (MPa)	ARI
1	19.18	1.81	0
2	63.47	5.99	0
3	33.6	3.17	0
4	22.39	2.11	0
5	59.13	5.58	0
6	55	5.19	0
7	56.74	5.35	0
8	42.76	4.03	0
9	31.97	3.02	0
10	77.8	7.34	0
11	18.51	1.75	0
12	74.93	7.07	0
13	51.06	4.82	0
14	75.43	7.12	0
15	37.63	3.55	0
16	34.35	3.24	0
17	38.3	3.61	0
18	27.76	2.62	0
19	74.73	7.05	0
20	55.42	5.22	0
MEDIAN	46.91	4.43	
MEAN	47.51	4.48	
S.D.	19.07	1.85	

Appendix 24. Data for Co-cure Groups after Water Storage

XENO V+ CC WATER STORAGE			
	LOAD (N)	STRESS (MPa)	ARI
1	95.69	9.03	2
2	203.05	19.16	0
3	89.34	8.43	1
4	120.85	11.4	1
5	85.4	8.06	1
6	125.69	11.86	1
7	243.73	22.99	1
8	170.11	16.05	1
9	128.99	12.17	1
10	156.71	14.78	1
11	141.93	13.39	1
12	169.25	15.97	0
13	172.31	16.26	0
14	175.02	16.51	1
15	149.04	14.06	1
16	122.7	11.58	1
17	139.94	13.2	1
18	78.8	7.43	0
19	90.77	8.56	1
20	115.04	10.85	1
MEDIAN	134.47	12.69	
MEAN	138.72	13.09	
S.D.	42.54	4.01	

CLEARFIL S3 CC WATER STORAGE			
	LOAD (N)	STRESS (MPa)	ARI
1	167.79	15.83	3
2	79.39	7.49	1
3	102.51	9.67	1
4	107.92	10.18	1
5	104.6	9.87	3
6	192.42	18.15	3
7	150.45	14.19	3
8	76.68	7.23	1
9	172.46	16.27	1
10	110.92	10.46	2
11	121.01	11.42	1
12	166.66	15.72	2
13	98.85	9.33	1
14	213.92	20.18	1
15	219.93	20.75	0
16	119.89	11.31	1
17	97.76	9.22	1
18	161.51	15.24	1
19	163.62	15.44	1
20	142.94	13.48	2
MEDIAN	131.98	12.45	
MEAN	138.56	13.07	
S.D.	41.65	4.03	

IBOND CC WATER STORAGE			
	LOAD (N)	STRESS (MPa)	ARI
1	76.8	7.25	0
2	112.55	10.62	0
3	64.15	6.05	0
4	25.98	2.45	0
5	77.76	7.34	0
6	56.02	5.28	1
7	52.42	4.95	0
8	83.07	7.84	1
9	119.41	11.27	0
10	29.86	2.82	1
11	49.07	4.63	0
12	62.72	5.92	0
13	76.54	7.22	0
14	83.81	7.91	1
15	47.69	4.5	0
16	33.49	3.16	1
17	68.39	6.45	0
18	62.94	5.94	0
19	66.06	6.23	1
20	32.45	3.06	1
MEDIAN	63.55	6	
MEAN	64.06	6.04	
S.D.	24.44	2.37	

TRANSBOND CC WATER STORAGE			
	LOAD (N)	STRESS (MPa)	ARI
1	111.86	10.55	3
2	242.24	22.85	1
3	80.3	7.58	3
4	207.69	19.59	2
5	145.01	13.68	3
6	89.22	8.42	3
7	132.18	12.47	1
8	140.48	13.25	1
9	138.95	13.11	1
10	67.78	6.39	3
11	73.42	6.93	1
12	146.12	13.78	2
13	155.95	14.71	1
14	98.78	9.32	2
15	240.77	22.71	1
16	95.17	8.98	2
17	101.39	9.56	2
18	156.15	14.73	0
19	154.68	14.59	1
20	110.84	10.46	1
MEDIAN	135.57	12.79	
MEAN	134.45	12.68	
S.D.	49.02	4.74	