The Effect of Application Mode and Aging on the Micro-tensile Bond Strength of Universal Adhesives to Enamel of Primary Teeth.

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1 INTRODUCTION

The prevalence of caries in children especially early childhood caries (ECC) and severe early childhood caries (S-ECC) is still higher than expected [16], and requires extra attention from healthcare professionals [88].

Introducing dental adhesion shifted the treatment of carious deciduous teeth away from extraction, which was the only available option earlier [73]. Additionally, they have played an important role in replacing the traditional cavity preparation paradigm named 'extension for prevention' with a more conservative approach to tooth structure [72].

The increased demand for practical and durable bonding systems led to the rapid development of multi-mode single bottled universal adhesives (UA) that can work in self-etching (SE), etch-and-rinse (ER) as well as selective-etching modes [9]. Those UAs are not as technique sensitive or time consuming as the old generations, thus, fulfilling a key point in management of children which is decreasing the appointment length to ensure cooperation [46].

UAs have been studied thoroughly on permanent teeth. Yet, the available scientific evidence of the effectiveness of adhesives in permanent teeth must not be taken as a guideline for primary teeth owing to the differences in their tooth morphologies and size [6]. Moreover, studying the effect of UAs on primary enamel is crucial due to the increased interest in direct tooth colored composite resin restorations and compomers [13], indirect restorations on extensively decayed primary anterior teeth such as composite shell crowns [77], and on posterior teeth such as onlays [118], endocrowns [66], and chairside indirect composite restorations [32].

Enamel has a homogeneous structure that is easier to bond to when compared to dentin, yet it can still be challenging when using SE adhesives or universal adhesives in SE mode. The effect of selective enamel etching on the bond strength of UAs has been studied extensively on permanent human teeth [95], as well as permanent bovine teeth [14], but scarce literature was available on its effect on primary teeth [9].

Furthermore, many variations of selective etching methods such as different etching times and acids [121], as well as variation in the addition of the bonding agent itself such as active rubbing [64], and the addition of an extra bonding layer [2] have been studied in

permanent teeth in an attempt to improve the bonding strength of universal adhesives to enamel. Yet their evaluation in primary teeth was found to be very rare [18]. To our knowledge, no single study has tested the effect of UAs on sound enamel of primary posterior human teeth neither regarding selective etching and their variations nor the etching time.

Also, the effect of aging on the long-term performance of the adhesives to resin restorations cannot be denied. Ignoring the effect of aging can lead to reporting higher bond strength values than the real ones due to overlooking the effect of factors such as thermal stresses and normal daily functions on the bond strength. [28]

Thus, our study has evaluated some application modes of UAs and their effect on the enamel bond strength, together with its stability after 6 months storage.

2 REVIEW OF LITERATURE

2.1 Prevalence and burden of caries in children

Epidemiological data from Germany, other European and non-European countries have proven, that dental caries and periodontal diseases are highly prevalent [51, 89]. Those studies have ranked oral diseases among the widely spread noncommunicable diseases in our society [47]. Despite the general decline in caries in children in Germany in the last 10 years, higher proportion of untreated caries were observed [102].

Germany has witnessed a decline in dmft in 6-7-year-old children from 2.89 in 1994 to 1.73 in 2016 [102], DMFT of 12-year-old children from 6.8 in 1980 to 0.7 in 2005 in the western states [100], and from 3.4 to 1.1 in the same years in the eastern states, yet dental caries is still presenting a great burden on the health care system. That's why, the WHO new global goals for oral health in children aging 12 years old is attaining a DMFT index below 1.0. [47]

The acute pain caused by dental caries has significant multidimensional impacts on children, their families and the surrounding society [113]. Presence of ECC and S-ECC entails a higher risk of development of new carious lesions in a child's primary and permanent dentition, increased treatment time and costs, and diminished oral health related quality of life. [57]

Premature loss of primary molars due to caries can result in loss of arch space and crowding of the permanent teeth thereby requiring orthodontic correction, which is even financially more consuming. Added to this, restoration and/or extraction of carious teeth can be a traumatic experience for very young children. [29]

Psychological problems have been associated with caries due to the affection of the child's appearance and speech. Children suffering from oral and dental diseases are more likely to feel shy, unhappy, or sad, and are less likely to socialize compared to those having good oral health. [26]

Among the significant consequences of caries are dental pain, presence of dental abscesses, and inability to chew food well, causing malnourishment and delayed growth and development of the child. Moreover, established associations were found between oral

infections in children together with diabetes, heart disease, stroke, and low birth weight. [45] Combined together, these dental, physical, and psychological effects are more likely to cause a decline in school performance of a child due to lack of attention and frequent absenteeism [42].

Therefore, continuous efforts must be marshaled together to focus on combating the serious emerging increase in incidence of dental caries. [12]

2.2 Enamel of primary teeth

Enamel is a non-vital, insensitive tissue, permeable for ionic exchange with the oral environment, thus allowing its mineralization. Despite its hardness and wear resistance, it is non-self-reparable. So, the development of adhesives is a crucial step in enamel repair and preservation. [15]

The outer surface of enamel is a prismless area called the aprismatic enamel, which is more frequently seen in primary teeth with a thickness of 16-45 μm, while in permanent teeth it is less than 5 μm. [96] This characteristic interferes with the acid etching pattern making adhesion more complicated than in permanent teeth, therefore, the idea of grinding the aprismatic layer before etching and bonding was introduced in the research field [50].

Histologically, enamel is more mineralized than dentin. Enamel consists of approximately 96% hydroxyapatite (HAp) by weight, 3% water, and 1% organic material, while dentin is only 70% hydroxyapatite by weight, 12% water, and 18% organic material (predominantly type I collagen). These percentages vary greatly according to the depth of those structures, the age of the tooth, and the pathological/traumatic history of the tooth, making bonding to both structures completely different. [3]

Furthermore, enamel differs from deciduous to permanent teeth. Enamel of deciduous teeth is thinner, yet more consistent in thickness than in permanent teeth, with an average thickness of 0.5-1.3 mm versus 2.5 mm in permanent teeth. Also, deciduous teeth differ in general, anatomically from the permanent teeth, since they are smaller in size and exhibit constant forms with less morphological variations that their permanent successors. Their crowns are generally more bulbous with narrow cervix tending to form cervical ridges at the cementoenamel junction of the tooth and their cusps are usually

more pointed. Accordingly, evidence of bonding to permanent teeth can't be generalized and adopted for primary teeth. [15]

Moreover, adhesion to enamel is further complicated because enamel structure and properties vary in different regions of the tooth. Surface enamel, for example is denser, less soluble, and harder than subsurface enamel and those properties decrease towards the interior of the tooth structure and also from the incisal tip to the cervical margin. [15] One study compared the bond strength of enamel in different regions and found out that the location of the enamel bond has significantly influenced the bond strength, where the highest bond strength values were on the occlusal third and the lowest on the cervical third [105]. That's why, introducing universal adhesives was important to face the enamel bonding challenges and create efficient bonding to both enamel and dentin.

2.3 Introduction of universal adhesives

Despite the improvement in adhesion, the weakest area of a restoration remains the bonding interface of dental substrates with restoratives and luting agents. [75]. This fact directed the focus of contemporary dental research to develop materials with long term performance and better bonding strength [111].

The concept of adhesion in dentistry was first discovered by Buonocore in 1955, he explained the ability of phosphoric acid etching to achieve a suitable retention of the acrylic-based resins to enamel [23]. This concept was further developed by Gwinnett, and Matsui, who introduced the concept of micromechanical retention via resin tags formation. [22]

Dental adhesives have passed through several developmental stages so called generations aiming to increase their performance. The first three generations focused mainly on dentin bonding and the idea of etching the enamel started first with the introduction of the fourth generation in market. [71] This generation consisted mainly of etchant, primer, and bond in 3 different bottles, which should be applied separately and in the mentioned sequence. Etching was done for 15-20 sec, rinsed then dried, then the primer infiltrated the dentin to form the hybrid layer. Yet, this method was technique sensitive and time consuming due to the multiple bottles used. [13]

In an attempt to overcome the disadvantages of the fourth generation, the fifth-generation primer and adhesive were mixed in one bottle. The lack of controlling the application of the hydrophilic primer led to more postoperative sensitivity, water sorption, and didn't improve the bond strength. [107]

The sixth-generation adhesives started in the 2000s with the introduction of the self-etching primers, so the etching step was eliminated. This created a lower bond strength on enamel although satisfactory on dentin and brought up the idea of selective etching. [9]

The seventh-generation adhesives were introduced directly before the introduction of UAs and has attempted to combine all the ingredients in one bottle, yet they had a lot of incorporated water in their formulas, which made them more prone to hydrolysis. This generation polymerized to create a more hydrophilic combination when compared to two step self-etch-adhesives. So, they were prone to water sorption and this caused limiting the resin infiltration depth in the substrates. [7]

In fact, the introduction of UAs (so called the 8th generation), enhanced the ability of dentists to bond various materials in a satisfactory manner to both enamel and dentin at the same time. This strategy was responsible for promoting the placement of direct and indirect restorations, veneers, and other aesthetic materials and blossoming the so called (Cosmetic revolution) in dentistry. [3]

2.3.1 Definition of universal adhesives

UAs are the latest innovation marketed for bonding of dental restorations to tooth structure [129]. They are known as ‘‘universal’’ or ‘‘multi-mode’’ or 1-step SE adhesives [64]. They can be identified as a single bottle, no mix adhesives that can be used in ER, SE or selective-etch modes depending on both the operator's preferences and the clinical situation. In addition, they can be used for both direct and indirect restorations, and are capable of bonding to dentin, enamel and other substrates such as zirconia, composites, and metals. [83]

Although the definition of universal adhesives implies that they should bond to all the above-mentioned substrates, many of the products marketed as universal adhesives need a separate primer to optimize bonding to porcelains and zirconia. [3]

2.3.2 Composition of universal adhesives

UAs consist of several components that help them maintain a good bond strength and durability as seen in **Figure 1**(page 10). The most important of them is the functional monomer. The functional monomer used primarily in most UAs is the phosphate ester (R-O-PO_3H_2), which copolymerizes with different restoratives and cements helping the adhesives to interact with different substrates. To facilitate the dissolution of those monomers and promote the usage of the bonding agent in the SE adhesive mode, it was essential to incorporate water in the adhesive composition. The disadvantage of this water incorporation is that it can affect the bonding strength later, therefore solvents as ethanol or acetone were added, in order to help in the evaporation of water once the monomers dissolute and enhance resin wetting. Other components as polymerization initiators, fillers, and silane also play an important role in the adhesive composition. [13]

For UAs to be efficient, all the essential ingredients must be present in a balanced condition. For example, monomers should exhibit a balance between hydrophilicity to properly wet the substrates and hydrophobicity to prevent water sorption and discourage hydrolysis on the long run. [3] Also, excessive amounts of water can be difficult to evaporate resulting in decreased polymerization, increased hydrolysis and compromised bond strength [74]. Moreover, a balanced acidic pH is required to be able to demineralize tooth substrates in the SE mode without breaking down the polymerization initiators needed for the self- and dual curing cements. Some other important characters of universal adhesives vary according to the manufacturer as pH value, initiators, type of solvent, and monomers type and concentration, and all of them affect the bonding performance of adhesives. [108]

Speaking of monomers, they can be categorized into either cross-linking or functional monomers [59]. Cross linking monomers enhance inter and intra-molecular crosslinks providing a stable collagen scaffold, and hence increases the mechanical properties and decrease biodegradation [130]. Functional monomers are acidic molecules that serve in etching tooth substrates, through demineralizing the HAp layer and partially dissolving the smear layer, they also facilitate monomer penetration, and enhance the potential of adhesives for interacting with tooth substrates. [25]

Functional monomers consist of a functional group to wet and demineralize tooth structures and a polymerizable group. Functional groups able to release one proton or

more such as carboxylate and/or phosphate monomers can also chemically bond to dental substrates. [87] Principally, functional groups work either by decalcifying or bonding to the tooth structure according to the 'adhesion-decalcification' theory [124]. When the functional group ionically interacts with the calcium in the hydroxyapatite layer, the bond formed may either be stable and bond, or it may decompose resulting in decalcification of the tooth structure. [59]

Although functional monomers increase the adhesive potential in interacting with the dental substrates, they can decrease the degree of conversion of camphoroquinone/amine-curing adhesives. But this effect depends mainly on the type and concentration of the incorporated monomer. [69]

Among the prerequisites of functional monomers, is to regulate the hydrophobic/hydrophilic behaviour of the adhesives. A hydrophilic monomer is required initially as it induces more dentin wettability through water sorption, yet a hydrophobic one after polymerization is inevitable to avoid hydrolytic degradation of the adhesive bond. Challenges in achieving the right balance between both states made the manufactures blend more than one monomer together. The 10-MDP (10- methacryloyloxydecyl-dihydrogen-phosphate) was used as the main monomer, while 2-hydroxyethyl methacrylate (HEMA) (hydrophilic), and Bisphenol A- glycidylmethacrylate (Bis-GMA) (hydrophobic) were added to balance the hydrophilicity of the adhesive. [127]

HEMA is a low molecular weight monomer that can perfectly wet dentin and is soluble in ethanol, acetone and water [78]. Its advantages lie not only in its ability to improve monomer penetration and enhance the formation of the hybrid layer, thus, increasing the immediate bond strength of the adhesives, but also in its solvent like nature that helps prevent phase separation in the presence of water [65]. Yet, higher amounts in both uncured or polymerized states can absorb water leading to swelling, discoloration, and hydrolysis of the adhesive interface. [3]

Unlike HEMA, Bis-GMA is hydrophobic in nature and absorbs 3% water by weight into its structure. Bis-GMA was used for a long time as the main base monomer of dental adhesives and composites, yet the combination of Bis-GMA and HEMA alone lead to frequent failures at the tooth restoration interface and decrease durability, because of the hydrolysis of the ester functional groups in the resin's constituents and their high-water sorption. Also, a lot of health concerns were raised due to the unleashing of the unreacted

HEMA and the Bisphenol A (BPA) as a degradation product of the Bis-GMA. This combined durability and cytotoxicity issues lead the manufacturers decrease or totally omit their levels in the current adhesives. [122]

Although universal adhesives are considered a breakthrough in the field of adhesive dentistry, they have some disadvantages such as their low film thickness, which supresses the polymerization of the adhesive layer using oxygen and affects its stability by encouraging water sorption from the underlying dentin. Also, this low film thickness affects the ability of the adhesive layer to absorb stresses. [107] The incorporation of HEMA monomer makes the adhesive interface more prone to hydrolytic degradation [125]. Moreover, silane in most UAs help them bond to glass resins without the need for an extra silane layer, yet for its stability, pH levels have to be kept > 2.5, this high pH level affects the ability of the adhesive to etch enamel and hence their bonding efficacy. [123] Although 10-MDP is considered an effective functional monomer, MDP esters linking the methacrylate and phosphate groups to the spacer are prone to hydrolytic degradation [71].

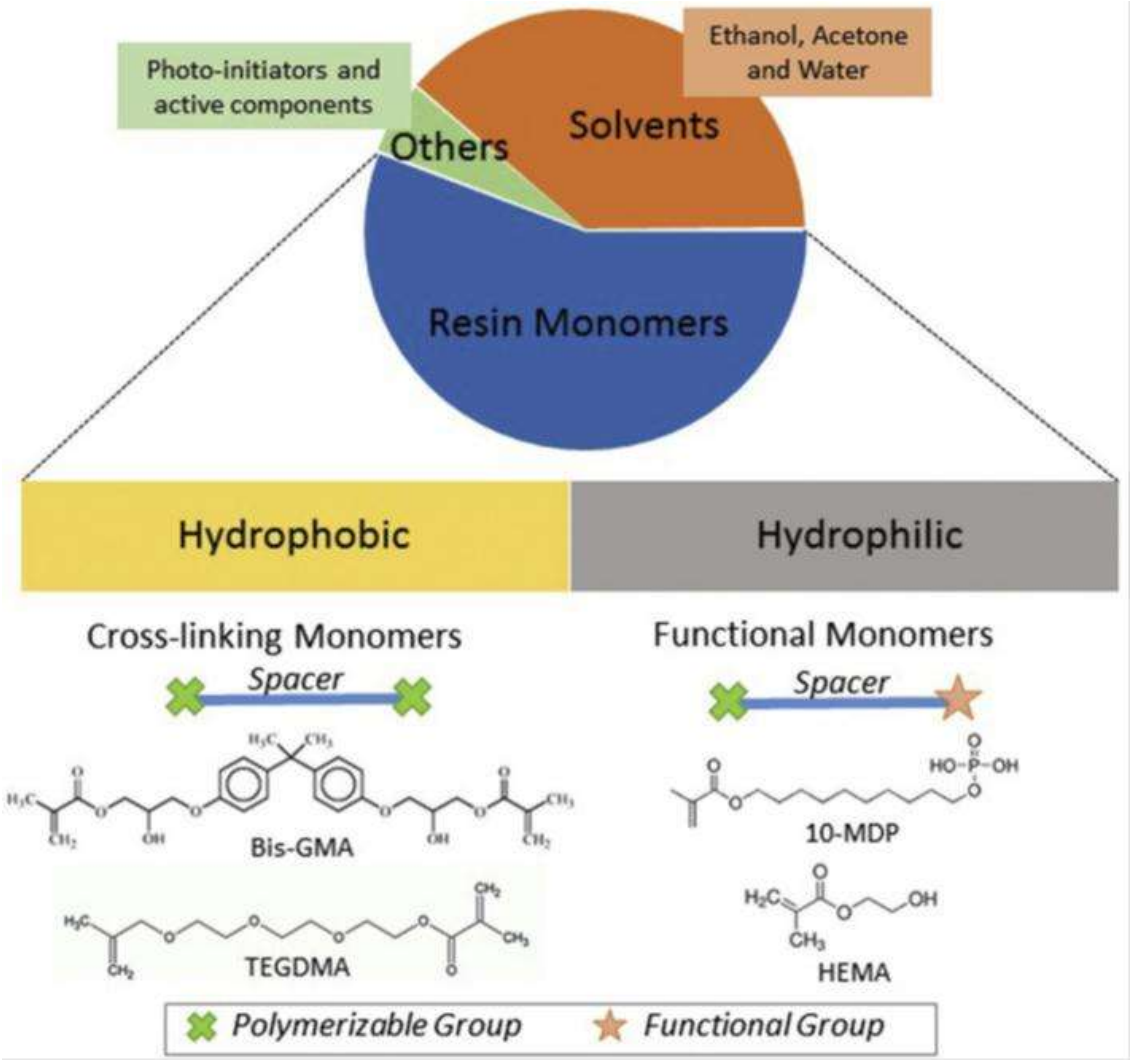

Figure 1: Composition of universal adhesives. Figure is taken from Bedran-Russo et al., 2017 [13].

2.3.3 Role of 10-MDP groups

10-MDP is a practical and proven phosphate ester functional monomer that was invented in the early 1980s by Kuraray, Japan to be used in their Panavia™ adhesive resin cement. Then, it subsequently spread in the market. [3]

It consists of a methacrylate group (hydrophobic) that can bond to restoratives and cements on one side and a phosphate group (hydrophilic) capable of bonding to tooth structure, zirconia, and metals on the other side. Additionally, the long carbon chain in-between both ends makes it the most hydrophobic of all functional monomers used in dental adhesives, preventing the hydrolytic breakdown of the adhesive interface over time. [71]

Ranking of functional monomers is mainly based on their chemical bonding potential, which is a reliable and stable function of 10-MDP [69]. The intense chemical interaction of MDP with HAp and the subsequent deposition of MDP-Ca salts, that are less soluble than those produced by other functional monomers [33], directed the attention of researchers to considering factors such as calcium salt stability, copolymerization, the ability to wet tooth substrates as essential selection criteria of the monomer. [25]

Although most functional monomers based on the adhesion-demineralization theory follow either a bonding route to tooth structure with minimal demineralization of the surface such as oxalic [109] and polyalkenoic acid [43], or a demineralization route as acetic or citric acids [17], 10-MDP implied the invention of a modified adhesion route because it ionically bonds to calcium in HAp crystals and at the same time produces adequate demineralization to release calcium, which in turn helps itself assemble into a 4-nm insoluble 10-MDP-Ca salt nanolayer. [33] Stable 10-MDP-Ca salt layer accounts for the durability of the nanolayer that can be incorporated in both the hybrid and the adhesive layers [71]. This self-assembled nano layered water stable structure was observed in adhesive systems containing 10-MDP on both enamel and dentin. Yet, there are still controversies regarding its role in the durability of MDP containing adhesives since it was not identified in all commercially available adhesive systems. [112]

In MDP containing adhesives, an acid-base resistant zone (ABRZ) was observed on the surface of dentin. This layer consists mainly of a combination of dentin and the adjacent hybrid layer created by penetration and polymerization of the acidic monomer after demineralization of dentin. Since this layer is believed to be acid resistant, it plays an important role in sealing restoration margins, preventing secondary caries, and promoting restoration's durability. [69] ABRZ was also reported in enamel, yet its position was different from dentin due to the structural differences of both substrates. In dentin it is formed below the hybrid layer, since the primers remove the smear layer, demineralize the dentin, and leave some HAp partially attached to the dentin collagen. On the other hand, enamel is highly mineralized with HAp crystals distributed all over the surface, therefore the ABRZ here is on the interface rather than below it, involving parts at the interface which were not dissolved after the acid-base challenge. [62]

2.3.4 Role of pH-value

Another key characteristic of universal adhesives is their pH values, which vary from extra mild (> 3) to mild (2- 3), indicating their low etching capabilities. This characteristic facilitates their bonding to dentin [120], yet controversies have been reported on bonding to enamel. [24, 31, 103] Few UAs possess intermediately strong (≈ 1.5) pH value with better etching capability. Thus, in our study we will be using two mild, and one intermediately strong universal adhesive.

Among the advantages named for mild SE adhesives were their ability to create a small hybrid layer of 1µm in thickness that can be easily penetrated with the adhesive in a short application time, they could also partially demineralize the dentin creating enough micromechanical retention while preserving the collagen from collapsing [76]. This hybrid layer is rich in HAp which can offer a better chance for interacting chemically, yet this step relies mainly on the functional monomer used [126]. On the other hand, they have several drawbacks such as insufficient micro-retention in enamel and the lack of sufficient hydrolytic stability of 10-MDP monomer. [74]

One study evaluated the effect of different application protocols, namely ER, SE for 20 s, and SE for 40 s, of three UAs with 2.3, 2.5, and 2.7 pH values on the microshear bond strength (µ-SBS) to enamel, in-situ degree of conversion and etching pattern. And it was found out that within this pH range, application of universal adhesives in SE mode for 20 s achieved lower bond strength values when compared to phosphoric acid etching. On the other hand, prolonged application in SE mode for 40 s has increased the bond strength values and improved the etch pattern with results comparable to the ER mode. [24]

PH effect is not limited to bonding to tooth substrates, but it also affects the ability of the adhesive to bond to self and dual-cured resin cements and composites [101]. In general, the more acidic the adhesive is, the more readily it deactivates the aromatic tertiary amines responsible for the chemical curing of the material, the less its compatibility with the self-cure modes of dual cured resin materials [56]. Therefore, different combinations of different pH values with variable application modes are continuously tested to achieve the best bond strength values of UAs.

2.3.5 Variations in using universal adhesives

I. Selective etching

Selective or hybrid etching technique is the application of phosphoric acid to enamel, avoiding dentin before using the adhesive. [30]

The principal mechanism of ER systems is the diffusion based micromechanical interlocking. Etching of the enamel with phosphoric acid creates intraprismatic pores causing the resin tags during infiltration. When the enamel prism cores of the HAp crystals demineralize, they result in macro pits that appear on the surface in which the bonding agent is drawn with capillary action. The process of polymerization interlocks the resin tags in place helping for the durability of adhesive joint. [71]

On the contrary phosphoric acid is considered an aggressive variant for dentin etching that demineralizes dentin to a depth of 4-6 µm. Therefore, it should be limited to no more than 15 s in time, because the more demineralized the dentin surface, the less readily the penetration of the monomer to this demineralization depth and the more exposed the collagen is. [71]

Therefore, the most reliable technique to improve bonding of 1-step SE adhesives and UAs to enamel is the separate enamel etching, which led manufacturers to invent the term (selective etching of enamel), yet its main drawback is the accidental contamination of dentin. [24]

The depth of enamel layer removed by etching depends on the type of acid, acid concentration, duration of etching, and the substrate to be etched [7]. Since bonding to enamel is mainly dependant on the micromechanical interlocking of resin into the microporosities, the extent and depth of etching pattern influences the bonding performance of the adhesive massively. [39]

Also, the etching capacity of adhesives is affected by several factors namely the substrate where it is applied, the incorporated monomer (MDP) and other functional monomers such as (4-META, and Phenyl-P). [25]

Although selective acid etching of enamel can achieve higher immediate bond strength values when compare to the SE mode, earlier signs of degradation of the adhesive joint were reported [68], which led researchers to investigate other solutions to increase the

bond strength such as active rubbing, increasing the application time of the adhesives, or applying more than one adhesive layer.

II. Etching time and concentration

The extent and depth of demineralization caused by acid etching is influenced by the etching time and concentration [98]. There is a great controversy concerning the optimal etching time of primary enamel [92]. Earlier, it was suggested that a prolonged etching time of 180 s is necessary to establish a proper etch pattern and increase the resin-tag formation [20]. Yet this paradigm has changed in the late 80s, when a study found out that a 120 s etching time produced a bond strength that wasn't significantly different from 15 s, 30 s, and 60 s. The authors of the study have observed a massive increase in enamel loss under SEM at 120 s and suggested that there is no need for the additional enamel loss since there is no increase in the bond strength. [92] Also, increasing the etching time was found to decrease the hardness of enamel from 4.21 GPa to 3.41 GPa for 15 s etching and 1.15 GPa after 120 s etching. This decrease can affect the enamel interface and cause early failure of bonding. [128]

On the other hand, studies have reported that the etching concentration did not affect the bond strength massively and concluded that etching acids of 27% or more are sufficient to demineralize the enamel surface. [37, 60]

III. Active application of the adhesive

Complete evaporation of the water and the solvent from the adhesive is a complex process that cannot be achieved by air blowing alone, due to their low vapor pressure when mixed with hydrophilic monomers [34]. The idea of active application of the bonding agent through scrubbing it to the surface aims mainly at promoting the evaporation of the water/solvent, so that more monomers are impregnated in the smear layer, and thus an improvement in the adhesive-interface occurs. [110]

A study has compared the effect of scrubbing of two one step SE adhesives on the μ-TBS of dentin. Both adhesives used had the same HEMA, MDP functional groups, as well as water/ethanol as solvents. Here, it was concluded that employing a scrubbing method can enhance monomer infiltration in dentin only in ultra-mild SE adhesives, while the technique was not found to be beneficial as the pH value falls. [110]

Similarly, active application of seven different universal adhesives to the enamel surface was studied and the μ-SBS, in-situ degree of conversion as well as enamel etching patterns were compared. It was found out that active application increased the bond strength of 5 out of 7 tested adhesives. The authors suggested that the scrubbing technique had enhanced the solvent evaporation especially in adhesives containing solvents with low vapor pressure such as water and ethanol, which in turn increased polymer crosslinking and the degree of conversion. [64] Yet they refused the assumptions of other studies claiming that the increase in bond strength was attributed to deeper penetration of the adhesive into enamel layers causing deeper demineralization, since the etching pattern was not significantly different between the active and passive application techniques. [7, 114]

2.3.6 Durability of universal adhesives

The fundamental requirement of dental restoration placement in dentistry is successful adhesion and it is directly related to the hybrid layer durability. Thus, improving the stability of the bonding interface is crucial to prolong the adhesive lifetime. [34]

Immediate bond performance of UAs as regards their bond strength and their marginal integrity was proven to be successful, yet their long-term performance needs more research. [71]

A systematic review evaluating the strength of evidence of adhesives to primary enamel was published in 2015 and found out that most of the research for bond strength degradation/aging was done only on the dentin substrate. [61]

2.3.7 Examples of modern universal adhesives

I. Scotchbond Universal Adhesive (SU)

Scotchbond™ Universal adhesive (3M ESPE) was used as an example of universal adhesives with extra mild etching capabilities. It is packaged in either a bottle form for multiple usage or an L-Pop™ delivery device for single use. Its main difference from the previous generations of 3M is that the phosphorylated methacrylate monomers (MDP) partially replaced the methacrylate monomers (UDMA and GDMA) to enhance its self-etching properties. Also, it contains a Vitrebond™ Copolymer, which is believed to yield a resistant system against the detrimental effects of humid conditions during storage. Its

solvent system is an ethanol/water-based solvent system, which is less volatile and helps maintain the viscosity of the product while in use. [1]

Although the manufacturers' claim that SU is a UA that can bond to different substrates without the use of additional materials, yet it requires a separate "activator" when used in combination with other manufacturer's self- or dual-cure resin cement during placement of indirect restorations.[3] Also, its primary use is with light-cured materials, however, it requires separate activation with Scotchbond Universal Dual Cure Activator (DCA) to bond to self- or dual-cure composite and cement materials. Although the manufacturer recommended the use of self-etch technique on uncut enamel, the results of our study showed different results. [1]

II. Clearfil Universal Bond Quick (CU)

Clearfil™ Universal Bond Quick (Kuraray) is an example of mild universal adhesives, which is marketed by the manufacturer as a (No Waiting, No extensive rubbing, No multiple layers) bond. This advantage made it very interesting in Paediatric dentistry and hence, it was chosen in our study. [55]

The no waiting property of the bond is claimed to be due to mixing the MDP monomer with a hydrophilic amide monomer that offers optimum hydrophilicity for the penetration of the bond in the dentin structure and at the same time high stability due to its high cross linking after polymerization. [55]

Like SU, CU is also supplied in a unit dose as well as bottles. Also, they require an additional activator (CLEARFIL™ DC ACTIVATOR), when used for dual curing resin cements of another manufacturer during the placement of indirect restorations. [3]

III. iBond Universal Adhesive (iBU)

iBOND Universal is a light-curing, all-in-one strong universal adhesive for use in adhesive restorative dentistry. The adhesive can be applied in a film thickness of approx. 5 – 10 μm. [54]

iBU differs from the other two adhesives used, in the fact that 4-META is used as a functional monomer together with 10-MDP. Also, a UDMA/TEGDMA combination is used as cross liking monomer instead of Bis-GMA/HEMA and acetone is used as a

solvent instead of ethanol, which is said to enhance water evaporation through its high vapor pressure. [54]

Unlike the other two adhesives used, iBU is compatible with self-cured and dual cured composites and resin cements without the use of any additional activator thanks to its photoinitiator system, yet the use of iBOND Ceramic Primer is needed when used with ceramic restorations. [83]

2.4 Bond strength testing methods

Bond strength testing can be classified as macro and micro testing. Macro bond strength tests whether shear or tensile were excluded from the studies although their specimen preparation was very easy to obtain, due to the lack of standardization of the testing parameters that lead to invalid comparisons between the studies. Additionally, it was found out that micro tests can measure higher interfacial bond strengths, the variances and means can be calculated for the whole tooth, it permits testing of small surfaces as well as irregular surfaces, and facilitates examinations under SEM/TEM. [21] With the wide spread of μ-SBS and μ-TBS tests, it was necessary to standardize the testing procedure and to understand the relationship between the measured bond strength and the stress distribution during the test and the associated clinical performance [11].

μ-SBS creates less damage during specimen preparation and therefore it should be recommended for brittle substrates as enamel and glass ionomers, yet it was found that the tensile stresses generated within were responsible for crack initiation, non-uniform stress distribution and underestimated measured bond strengths. [94] Therefore, μ-TBS was preferred. Although μ-TBS is very technique sensitive and is affected by the different test parameters including the gripping device, specimen geometry, specimen preparation, and test speed, yet it was created to overcome the limitations of the μ-SBS through ensuring uniform stress distribution on the small bonding areas and avoiding the occurrence of cohesive failures within the substrate. [97]

To overcome the limitations of the μ-TBS, this study has followed the standardized test recommendations by Armstrong et al., to decrease the errors created by the test parameters and ensure the reproducibility of our results. [10]

3 AIM OF THE STUDY-NULL HYPOTHESES (H0)

The aim of the present study was the suggestion of an adhesive protocol for universal dental adhesives on the enamel of primary teeth in-vitro through:

a) Testing the effect of selective acid etching of primary enamel prior to the application of universal adhesives on its μ-TBS and determining its stability after 6 months aging in an aqueous solution.

b) Measuring the effect of acid etching time of primary enamel prior to the application of universal adhesives on its μ-TBS.

c) Evaluating the effect of active application of universal adhesives to primary enamel on its μ-TBS.

Null hypotheses

a) Selective acid etching has no effect on the μ-TBS of universal adhesives to enamel of primary teeth.

b) Acid etching time has no effect on the μ-TBS of universal adhesives to enamel of primary teeth.

c) Active application of the universal adhesive has no effect on the μ-TBS to enamel of primary teeth.

d) Artificial aging of primary enamel treated with acid etching in different etching times for 6 months in an aqueous solution has no effect on its μ-TBS.

4 MATERIALS AND METHODS

This study was conducted at the Department of Paediatric Dentistry, School of Dentistry, University Medical Centre Giessen and Marburg, Campus Giessen, Justus-Liebig-University Giessen, Germany. The protocol was written according to the guidelines of the Ethical Committee of the Faculty of Medicine (Department 11), Justus-Liebig-University Giessen, and an approval was granted on the 3rd of May 2021 (AZ: 143/09). The study protocol was written taking the Declaration of Helsinki for the use of human body material in medical research into consideration [36].

4.1 Materials

The following table (**Table 1**, page 19) shows a summary of the materials used, their application protocols, and their composition according to the manufacturers' instructions.

Table 1: Material's data and application guidelines according to the manufacturers.

Material	Composition	Manufacturer	Instructions of use	LOT number
1. DeTrey® Conditioner 36 (pH< 2)	Phosphoric acid 36%, highly dispersed silicon dioxide, detergent, pigment, water	Dentsply DeTrey GmbH, Konstanz, Germany	Clean enamel with a pumice slurry, apply for at least 15 s, rinse for 15 s, dry.	2107000965 2010000659
2. 3M™ Scotchbond Universal Adhesive (pH= 2.7)	MDP, dimethacrylate resin, HEMA, Bis-GMA, Vitrebond copolymer, filler, ethanol, water, initiators, silane	3M™ Oral Care, Seefeld, Germany	Apply with gentle agitation for 20 s, airdry for 5 s, polymerize for 10 s.	8830691 8090379 8113907 7646399
3. Clearfil™ Universal Bond Quick (pH= 2.3)	MDP, HEMA, Bis-GMA, hydrophilic amide monomers, colloidal silica, silane, sodium fluoride, dl-camphorquinone, ethanol, water	Kuraray Noritake Dental Inc., Okayama, Japan	Apply with gentle agitation (no waiting time), airdry for 5 s, polymerize for 10 s.	000032 000011
4. iBond® Universal Adhesive (pH= 1.6-1.8)	4-META, MDP, UDMA, acetone, water	Heraeus Kulzer GmbH, Hanau, Germany	Apply with gentle agitation for 20 s, airdry to a thin layer, polymerize for 10 s	K010047 K010045
5. Filtek™ Z250 Universal Restorative System, Shade: A3	Bis-GMA, UDMA, TEGDMA, Bis-EMA, and PEGDMA	3M™ Oral Care, Neuss, Germany	Place in 2 mm increments, polymerize for 20 s.	NE15669 NE67430 NE50366 NE83781

MDP: 10-Methacryloyloxydecyl Dihydrogen Phosphate, **HEMA:** 2-Hydroxyethyl Methacrylate, **Bis-GMA:** Bisphenol A- glycidylmethacrylate, **4-META:** 4-Methacryloyloxyethyl Trimellitate Anhydride, **UDMA:** Urethane Dimethacrylate, **TEGDMA:** Triethylene Glycol Methacrylate, **Bis-EMA:** Ethoxylated Bisphenol-A Dimethacrylate, **PEGDMA:** Polyethylene Glycol Dimethacrylate.

4.2 Methods

4.2.1 Teeth collection and storage

One hundred thirty-two teeth halves were collected out of 119 freshly extracted primary molars and were distributed as follows:

a) Four tooth halves for investigating the enamel surface preparations (4.2.4, page 25).

b) One tooth half for measuring remaining enamel thickness (4.2.4, page 26).

c) Sixty-nine tooth halves for phase I (storage for 24 h in distilled water) (4.2.5, page 27).

d) Forty-four tooth halves for phase II (storage for 6 months in distilled water) (4.2.5, page 27).

e) Fourteen tooth halves for SEM examination (4.2.7, page 32).

Teeth collected were extracted by a verbal informed consent of the parents and were either extracted upon a clear clinical indication such as caries, pathological root resorption, over-retention, and serial extraction, or were normally exfoliated.

Inclusion criteria:

a) Primary molars having buccal and/or oral surfaces free from any form of carious lesions, hypocalcification, cracks or restorations.

b) No history of previous root canal treatment was determined.

c) Proximal lesions such as spot caries or hypomineralization lines were only accepted if they did not extend to the examined surface, buccal or oral, in a mesiodistal direction.

Exclusion criteria:

a) Severely decayed or mutilated primary molars.

b) Evidence of previous restoration on the surface to be examined or evidence of root canal treatment.

c) Evidence of caries or hypomineralization on the surface to be examined.

Collected teeth were stored in 0.5% Chloramine-T-solution (Chloramin T trihydrate, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) at +4-7 ˚C directly after their extraction for a maximum period of 30 days. Teeth were then transferred to the laboratory, cleaned with a sickle shaped scaler (H6/H7 Hygienist scaler, Hu-Friedy Mfg. Co., LLC., Tuttlingen, Germany) under running water to remove any hanging soft tissues, and they were inspected at 4X magnification under a LED magnifier glass lamp (Magnifier Glass Lamp 1.75/4X, Model No: 8093. Bulb: 12W, MBFZ Toolcraft, Spalt, Germany) to ensure their conformation to the inclusion criteria. Finally, eligible teeth were numbered and frozen in sequentially numbered containers in distilled water at -22 ˚C till the conduction of the experiment.

Samples were assigned to the subgroups in a block randomization technique with a block size of 10 teeth to compensate for the dropouts on tooth level. In case both tooth halves of one tooth met the inclusion criteria, the tooth halves were assigned to two consecutive blocks to make sure that only one tooth half per tooth was assigned to the same experimental subgroup.

4.2.2 Study design

The study was conducted in two phases according to the incubation time of the samples, where phase I was incubated for only 24 h and phase II for 6 months at +37 ˚C in distilled water. The 24 h group was tested first as a reference for the results and only the subgroups that yielded the best results in this phase were tested in phase II. In the 24 h group, SU was tested in 4 application modes (self-etch, 30 s selective-etching, 15 s selective-etching, and active application of the tested adhesives), while CU and iBU were tested only in 3 application modes, excluding the active application of the tested adhesives. On the other hand, only the15 s selective-etching and SE modes were tested for all adhesives in phase II due to limited number of teeth.

Figure 2 (page 22) shows a schematic representation of the study groups elaborating the number of tooth halves used in each subgroup. Differences between the subgroups in the number of tooth halves tested, aimed at equalizing the different number of sticks yielded from each tooth half, produced by tooth-size variations.

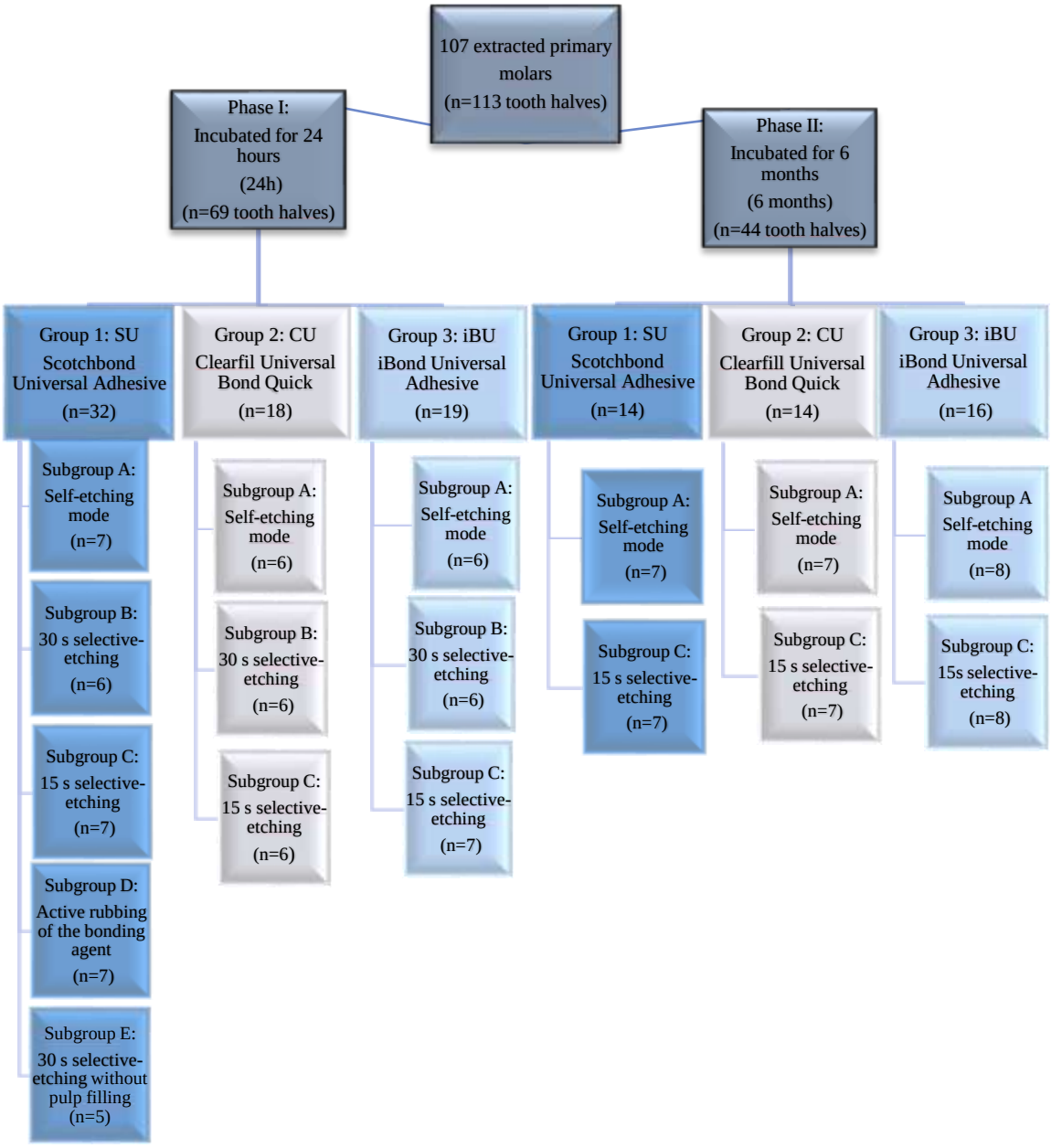

Figure 2: Schematic representation of the study groups showing the number of tooth halves tested in each group. SU: Scotchbond Universal adhesive, CU: Clearfil Universal Bond Quick, iBU: iBond Universal adhesive, SE: Self-etch mode, 30s: selective phosphoric acid etching for 30 s, 15s: selective phosphoric acid etching for 15 s, act: active rubbing of the bonding agent, 24h: storage for 24 h in distilled water, 6 months: storage for 6 months in distilled water.

4.2.3 Tooth preparation

Before starting with the tooth preparation, teeth were allowed to defreeze in a container containing distilled water at room temperature for approximately 30 min. To remove the roots, teeth were fixed using dental wax (Supradent-Wachs, Chemisches Dental-Labor Oppermann-Scwedler, Pluradent, Offenbach am Main, Germany) to the

holder of a cutting machine (Isomet 1000 Precision Saw, Buehler GmbH, Uzwil, Switzerland) so that the occlusal surface is facing the holder (**Figure 3**a, page 24). The whole structure was then fixed to the saw's table with screws, and the device was operated at a speed of 975 rpm with a weight of 75 g using a cutting blade (Isomet Diamond Wafering Blades 15LC Diamond [127 x 0.4 mm], Buehler, Uzwil, Switzerland) until the root was completely separated from the crown. Afterwards, soft pulpal tissues were removed using a hand scaler (H6/H7 Hygienist scaler, Hu-Friedy) and the pulp chamber was bonded with SU (3M™ Scotchbond Universal adhesive, 3M™ Oral Care) according to the manufacturer's instructions in a SE mode and filled with resin composite (Filtek™ Z250 Universal Restorative System, Shade: A3, 3M™ Oral Care) (**Figure 3**b& c, page 24).

To measure the effect of pulp filling on the μ-TBS, extra 5 tooth halves were prepared without filling their pulp chambers and they followed the exact same preparation steps as the other teeth. Those teeth were prepared as the SU-30s-24h group, and their results were compared.

The teeth were then fixed again with their buccal surfaces facing the holder of the saw, and the same cutting process was repeated to split the tooth mesiodistally into buccal and oral halves. To ensure that both halves were equal, a line joining the mesial and distal surfaces of the tooth and passing through the central occlusal groove was drawn with a permanent marker, and this line was then positioned on top of the cutting blade (**Figure 3**d& e, page 24).

Moreover, the total bonding area of the surface to be tested was measured with a periodontal probe and tooth halves with a total area < 4 × 4 mm were excluded. Finally, eligible teeth were distributed to the assigned groups in a block randomization. (**Figure 2**, page 22).

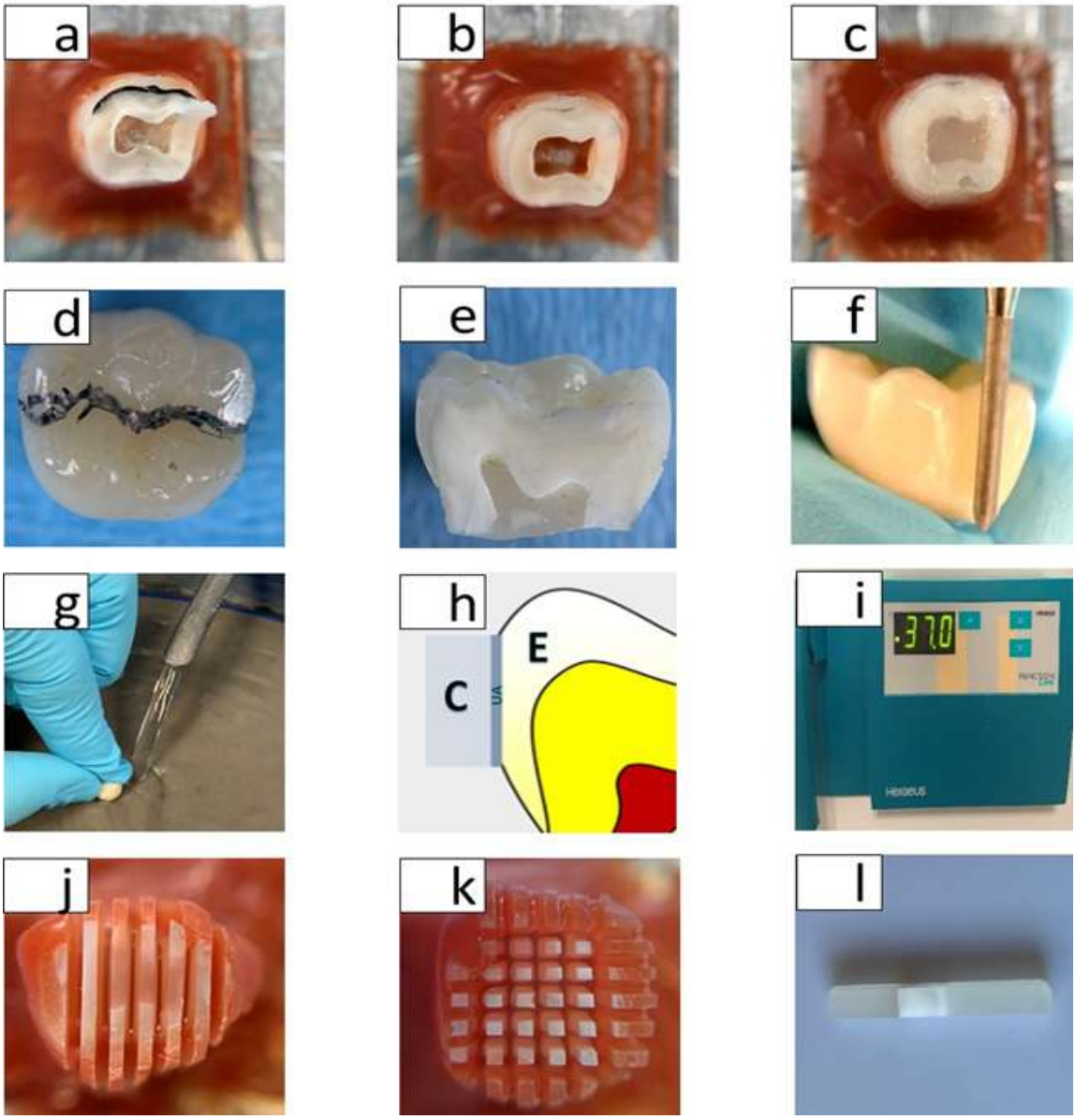

Figure 3: Step-by-step representation of tooth preparation and sectioning. a. removal of the root portion of the tooth, b. cleaning of the pulp chamber, c. filling of the pulp chamber with composite resin, d. marking the central occlusal groove, e. splitting the tooth mesiodistally into two equal halves, f. removal of the aprismatic enamel using yellow ring flame finishing stone, g. flattening the surface with a sandpaper under water coolant, h. schematic representation for the addition of the tested materials and the build-up of composite resin (C: composite, UA: universal adhesive, E: enamel), i. incubation of the samples at + 37°C, j. sawing the sample in a mesiodistal direction, k. sawing the sample in a bucco-oral direction, l. sticks production.

4.2.4 Investigating different enamel surface preparations

Several methods of enamel surface preparation were tested to investigate the best method to smoothen the enamel and remove the aprismatic layer without exposing the underlying dentin. Tooth halves in this study were flattened using a yellow ring finishing stone (Diamant FG878EF, 012G, Busch & Co. GmbH, Engelskirchen, Germany) then by two consecutive sandpapers in sizes P1200 (600-grit) and P4000 (Silicon carbide-Sandpaper, Buehler, Uzwil, Switzerland) respectively to smoothen the preparation lines produced by the stone [50]. (**Figure 3**f& g, page 24)

I. Etching pattern analysis

To analyse the enamel etching pattern, the enamel of 4 extra tooth halves was prepared with different methods as follows:

a) Yellow ring flame finishing stone (Diamant FG878EF, 012G, Busch) mounted in a high-speed handpiece (EXPERTmatic LUX E25 L (Mat. Nr. 1.007.5550), Kavo Dental GmbH, Biberach, Germany) with a speed of 40,000 rpm. This was done by moving the stone for 2-3 times on the enamel surface in one direction in a swiping motion to minimize building up any irregularities on the surface.

b) Red ring flame finishing stone (Diamant FG878, 012G, Busch) used as mentioned in a).

c) Enamel surface was flattened with silicon carbide sandpapers in a roughness of P1200 (600-grit) and P4000 respectively, which were fixed to a grinding machine (Beta Grinder-Polisher, Buehler, Uzwil, Switzerland) operated at a speed of 100 rpm under water irrigation, flattening was completed for 1 min manually in a figure of 8 shape, then the surface was rinsed for another minute using air water spray.

d) Yellow ring flame finishing stone as in a), followed by flattening with two silicon carbide sandpapers in a roughness of P1200 (600-grit) and P4000 respectively as in c).

Teeth were then etched with 36 % phosphoric acid (Detrey Conditioner 36, Dentsply DeTrey GmbH, Konstanz, Germany) for 30 s, rinsed for 60 s, then dried with an air-water syringe for 10 s. Samples proceeded to SEM evaluation as described in section (4.2.7, page 32).

II. Measuring remaining enamel thickness

An additional exemplary tooth half was assigned to group d) in the last heading (4.2.4, page 25) to determine the quantity of enamel removed, thus the thickness of the remaining enamel as follows: This tooth half was divided into two parts in an occluso-cervical direction without removing the root. The tooth was embedded in acrylic resin (Technovit 4071, Heraeus Kulzer GmbH, Wertheim, Germany) using the support of a silicon mould so that only half of the root is covered. Three marks were made with a permanent marker on the dentin surface near the dentino-enamel junction in the middle of the cervical, middle, and occlusal one-thirds to create a guide for repeated measurements. Using a light microscope (AZ 100M, Nikon, Tokyo, Japan) with an objective 1X and zoom 1X, the enamel thickness was measured with the help of NIS-Elements AR 5.11.00 (64 bit) software with pixel size 3.45 × 3.45 µm from the center of the 3 marks to the outer surface of the tooth creating a straight line, then the inner end of the straight line was pulled to the dentino-enamel junction for the final measurement. This process was repeated once at baseline (before enamel surface preparation) and once after enamel was prepared.[82] This step assured that the amount of enamel removed throughout the study was approximately 50 µm, which corresponds to the amount of aprismatic enamel in primary teeth.[96] (**Figure 4**, page 26)

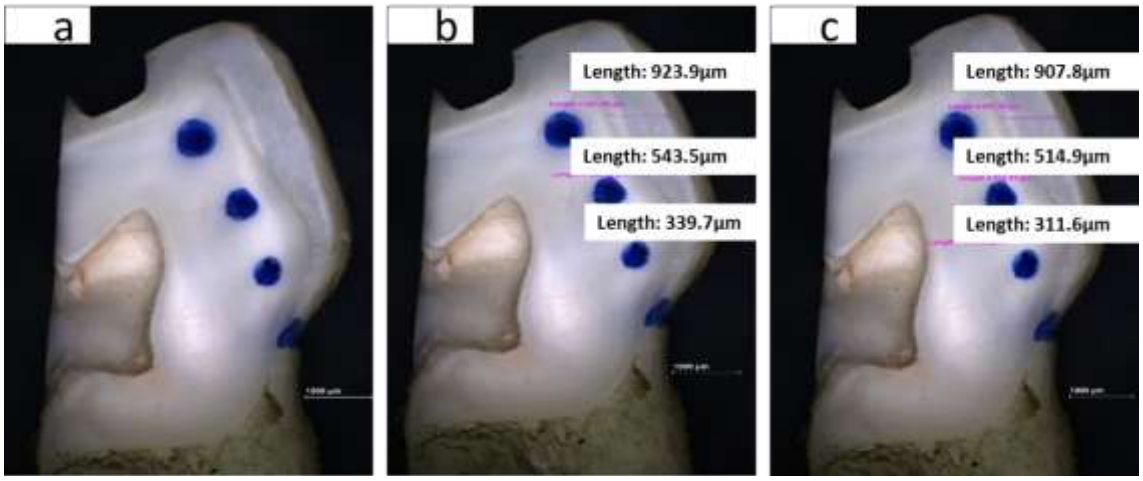

Figure 4: Exemplary measurements for the remaining enamel thickness under light microscope at 6X magnification. a. shows the placement of guiding marks on the dentin surface, b. baseline measurement, c. measurement after surface preparation.

4.2.5 Addition of the tested materials

Teeth halves were fixed on a flat metal holder 2.5 × 2.5 cm in dimension using flowable compomer (Dyract Flow, Dentsply DeTrey GmbH, Konstanz, Germany) exposing a free enamel surface to facilitate the addition of tested materials without contamination.

Depending on the assigned group, Detrey conditioner 36 etchant (DeTrey® Conditioner 36, Dentsply DeTrey GmbH) was applied directly from the tip of the syringe to enamel for 15 s or 30 s with continuous agitation using a microbrush, then it was rinsed for 30 s and 60 s, respectively, and dried for 10 s. Afterwards 3 universal adhesives were used in the form of single-doses and their entire content was applied by dropping without touching the surface in subgroups A, B, C& E, while in subgroup D, the bond was rubbed with a microbrush for the period specified by the manufacturers (**Table 2**, page27). The surface was then gently dried with an airway syringe placed at a 45° angle to the surface from the center of the tooth to the periphery until no movement of the bond was observed. Polymerization of the bonding agent was done by LED curing unit (Bluephase® Style LED Curing Light, Ivoclar Vivadent GmbH, Ellwangen, Germany), which was calibrated and checked for light intensity using a visible curing light meter (CURE RITE®, Visible Curing Light Meter, Dentsply Caulk, Bensheim, Germany) before and after each subgroup, and the reported average light irradiance was 1325.9 ± 16.2 mW/cm^2.

Table 2: Etchgel and adhesives' application directions

	3M™ Scotchbond Universal Adhesive	Clearfil™ Universal Bond Quick	iBond® Universal Adhesive
Subgroup A	3	5	3
Subgroup B	1, 3	1, 5	1, 3
Subgroup C	2, 3	2, 5	2, 3
Subgroup D	4	6	4
Etch: 1-apply 30 s with agitation, rinse 60 s, dry 10 s, 2-apply 15 s with agitation, rinse 30 s, dry 10 s. **Bond:** 3-apply passively, leave 20 s, dry 10 s, polymerize 20 s, 4-rub for 20 s, dry 10 s, polymerize 20 s, 5-apply passively, no waiting time, dry 10 s, polymerize 20 s, 6-rub actively, no waiting time, dry 10 s, polymerize 20 s.			

Composite resin (Filtek™ Z250 Universal Restorative System, Shade: A3, 3M™ Oral Care) was built up using a double flat hand instrument (Pluline Heidemannspatel narrow (2401), Pluradent, Tübingen, Germany) in a first layer of 0.5 mm thickness then adapted well to the surface by a comporoller (Comporoller 5300, Kerr Dental, Rastatt, Germany) before it was cured for 40 s. Consecutive layers of composite of 1 mm thickness were then condensed after one another and polymerized to reach a height of 6-7 mm (**Figure 3**h, page 24).

Finally, all samples were stored soaked in distilled water inside sealed containers in an incubator (Incubator IP20 Function Line, Heraeus, Hanau, Germany) at +37 °C for 24 h in phase I, or 6 months in phase II to stabilize the composite's water sorption. In phase II, distilled water was checked every two months, but was not changed or added to throughout the whole period (**Figure 3**i, page 24).

4.2.6 Microtensile bond strength testing

I. Calibration for µ-TBS testing

Internal calibration was done with two reference groups, once on dentin of permanent molars and once on enamel of anterior permanent teeth based on the methodology of Krämer et al.[52]

- For dentin calibration, 5 human third molars were collected. Their roots as well as their occlusal one thirds were removed to expose mid-coronal dentin. Dentin surfaces were flattened with silicon carbide paper P600 (360-grit), then P1200 (600-grit) (Silicon carbide- Sandpaper, Buehler) for 1 min to create a standard smear layer. Etching of the surface was performed for 15 s, rinsed for 15 s, and dried without desiccation. SU single dose adhesive (3M™ Scotchbond Universal adhesive, 3M™ Oral Care) was applied with its own microbrush with active rubbing for 20 s, followed by drying till no movement of the bond was seen, then polymerized for 20 s.
- For enamel calibration, 5 permanent anterior teeth were collected. Their roots were removed, then the labial surface was cut with a yellow ring flame finishing stone (Diamant FG878EF, 012G, Busch). Etchgel was applied for 30 s, rinsed for 30 s, then dried. SU (3M™ Scotchbond Universal adhesive, 3M™ Oral Care) was used exactly as mentioned in the dentin calibration.

For both calibrations, composite build up (4.2.5, page 27), sectioning of the sample (4.2.6, page 29), as well as µ-TBS testing (4.2.6, page 30) were performed exactly as in the main experiment. Then, the specimen preparation, sawing, and µ-TBS test parameters were modified until a standard deviation between sticks of the same tooth of 35-50 % was reached and the standardized parameters were applied to our study.

II. Fabrication of the sticks

Tooth halves were removed from the incubator directly before sawing and special care was taken to keep them moist throughout the whole operation by soaking them in distilled water in between the manipulation steps. Their dimensions were measured with a clinical caliper (Seitz und Haag, Zuercher Model, Germany) to be able to adjust the cutting depth and the number of slabs during sectioning. These measurements were further checked and adjusted automatically by the saw before cutting.

Samples were fixed with the composite resin build up facing towards the holder of the saw (Isomet High Speed Pro Precision Cutter, Buehler) using dental wax (Supradent-Wachs, Chemisches Dental-Labor Oppermann-Scwedler, Pluradent), which was melted with an electric wax knife (Pro Waxer duo 414-0000, YETI Dentalprodukte GmbH, Engen, Germany), so that all surfaces of the tooth were covered except the upper edge. The tooth holder was then screwed to a precision saw (Isomet High Speed Pro Precision Cutter, Buehler) and the cutting parameters were adjusted to a rotating speed: 4250 rpm / cutting speed: 5 mm/min /specimen size: 0.75 mm, while the depth of cut and the number of cuts were adjusted individually according to the measurements of each specimen. Sectioning proceeded at first in a mesiodistal direction then the holder was rotated 90 ˚ and they were further sectioned into bucco-oral direction perpendicular to the first cut to produce sticks of 0.7 × 0.7 mm in thickness and approximately 10-12 mm in height (**Figure 3**j& k, page 24). Sticks were then carefully removed from the surrounding wax with a fine scalpel (Surgical Disposable Scalpel, B Braun, Melsungen, Germany) and the first line of peripheral sticks from all sides was discarded (**Figure 3**l, page 24). Each tooth half yielded an average of 7-16 sticks.

The dimensions of all sticks were rechecked using a dental caliper (Schnelltaster Kroeplin, Schlüchtern, Germany). Afterwards, they were inspected under a LED magnifying glass lamp (Magnifier Glass Lamp 1.75/4X, Model No: 8093. Bulb: 12W, MBFZ Toolcraft) prior to testing and discarded if the following drop out criteria were met:

Drop-outs at the stick-level prior to testing:

1. Insufficient remaining tooth structure (< 2mm).
2. Insufficient length of the tooth half including the pulp filling (< 4mm).
3. Fractures in composite resin or tooth structure.
4. Sticks showing no enamel structure.
5. Non-uniform shaped sticks (i.e., not perfectly rectangular in cross-section) due to bevelling of the perimeter areas by the saw or partial coverage of the stick with the surrounding wax.
6. Air voids present in the adhesive layer or in composite resin.

III. µ-TBS measurement

µ-TBS measurements were performed on day 1 (24 h) and after (6 months) storage in distilled water under +37 ˚C using the operating software (TC-550 Zug-/Druck-Messsoftware V3_1, Munich, Germany) attached to a bond strength testing machine (Syndicad TC-550) and 1 mm was kept constant between plates which hold the sticks.

Sticks were blotted dry to remove excess moisture, then were fixed one by one to the metallic plates by applying the composite resin side always to the left plate and the enamel side to the right, keeping the adhesive joint centralized between both plates. Both ends of the stick were attached to the plates with flowable compomer (Dyract Flow, Dentsply DeTrey GmbH) cured for 20 s on each side, followed by a small drop of SU (3M™ Scotchbond Universal adhesive, 3M™ Oral Care) cured for 10 s, then again, the same flowable compomer cured for 20 s. All materials were cured with the same curing lamp (Bluephase® Style LED Curing Light, Ivoclar Vivadent) taking special care not to touch the adhesive interface. (**Figure 5**, page 31)

µ-TBS test was performed under the following parameters: Specimen: rectangular (a. 0.700 mm / b. 0.700 mm), Force Direction: Tensile Force, Maximum load: 50 N, Crosshead speed: 1 mm / min. The maximum force of fracture of the sticks was saved by the software in Newton (N) and converted to Mega Pascal (MPa). Sticks which broke during cutting the sample or at any step before testing were regarded as pre-test failures (ptf) and were evaluated as "zero" MPa. Moreover, sticks with the following criteria were again considered as dropouts and were excluded from the results.

Drop-outs at the stick-level during and after testing:

1. Failure due to improper handling of the sticks (manipulation failure) was recorded but excluded from the results.
2. Failure between tooth structures and the composite resin filling the pulp cavity during μ-TBS testing.
3. Debonding of one or both sides of the stick during μ-TBS testing.

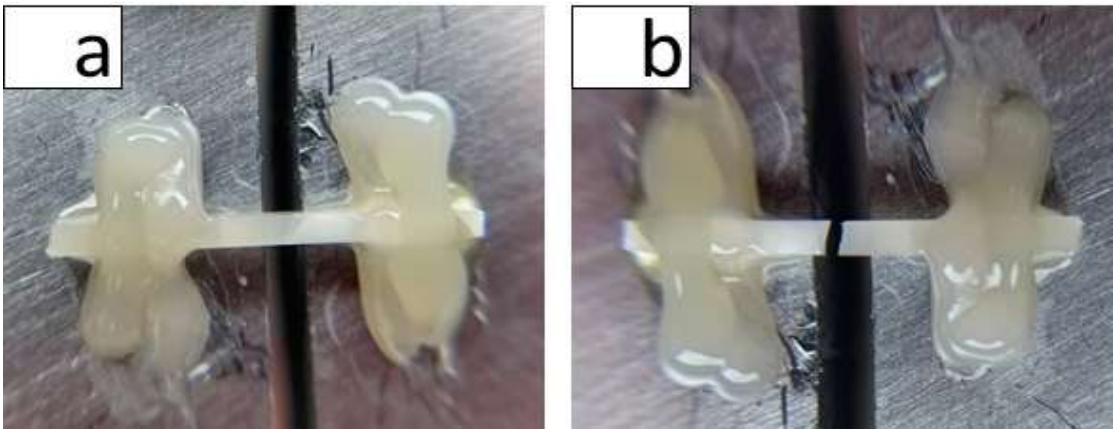

Figure 5: Fixation of the sticks for μ-TBS testing. a. before testing, b. after fracturing.

Failure analysis was done under a LED magnifier glass lamp with 4X magnification (Magnifier Glass Lamp 1.75/4X, Model No: 8093. Bulb: 12W, MBFZ Toolcraft) by a single examiner, and was categorized into:

1. Adhesive
2. Cohesive in composite resin
3. Cohesive in enamel
4. Mixed in enamel-adhesive interface
5. Mixed in composite resin-adhesive interface
6. Mixed in composite resin and enamel.

Then, fractured sticks were stored in sealed tubes (SafeSeal Reagiergefäß 0.5 ml, Sarsted, Germany) in distilled water.

After testing all sticks of the same tooth, mean bond strength value and standard deviation were calculated. And the whole tooth was excluded if it met the following criteria:

Drop-outs at the tooth-level:

1. The drop-out rate at the stick-level ≥ 50 % in relation to the total number of sticks.
2. Teeth yielding less than seven measurable sticks.

4.2.7 Microscopic evaluation

Microscopic evaluation was done first for debonded sticks under light (LM) and florescence microscope (FM) to identify the type of failure, then for intact sticks under scanning electron microscope (SEM) to identify the characteristics of the adhesive joint.

I. Light and florescence microscopic evaluation

Overview images of the debonded halves of the sticks were captured first under LM (AZ 100M, Nikon), then under FM (AZ 100M, Nikon) using a FITC filter (excitation filter 465 – 495 nm, blocking filter 512 – 558 nm) with an exposure time of 100-400 ms.

Fractured sticks were imaged directly after μ-TBS testing while still fixed to the holders of the testing machine. Each stick was photographed once with the debonded halves directed upwards towards the lens with an objective 4X and zoom 3X (magnification= 72X), then with both sides facing each other with an objective 4X, zoom 3X (magnification= 72X), and the scale was recorded under each image.

Magnification of the images was calculated using the following equation: (Objective × Zoom × Tube factor × Ocular), given that the tube factor of our microscope is 0.6X and the ocular is 10X [19].

II. Scanning electron microscopic evaluation

Etching pattern evaluation

To evaluate the enamel etching pattern, teeth were immersed in alternating baths of acetone (Acetone extra pure (CH_3COCH_3) K30376813 211, Merck, Darmstadt, Germany), distilled water, ethanol 96% (Ethanol absolute ≥ 99.8 32205-1L- M, Sigmaaldrich, UK), and distilled water again for 5 min each. Then, they were dehydrated by immersing them in a series of rising ethanol (60-70-80-90 % for 20 min each, 100 % for 1 h). Finally, they were dried in 1,1,1,3,3,3 hexamethyldisilazane (Merk Schuchardt, Hohenbrunn, Germany) for 10 min and allowed to air dry further overnight.

To observe the sticks under the SEM, they were fixed on aluminum stubs (Nr. G301, Agar Scientific Ltd., Wetzlar, Germany) with carbon conductive cement (Leit-C nach Göcke, Plano GmbH, Wetzlar, Germany) and were gold-sputtered with argon gas vacuum (POLARON SC502 SPUTTER COATER, Peter Liebscher, Wetzlar, Germany) under 1000VA E 100%. Aluminum stubs holding the specimen were placed in SEM device (SEM Amray Model 1810, Amray, Bedford, MA, USA) and images were taken at 150X, 1250X, and 4000X magnifications.

Enamel-resin interface evaluation

For evaluating enamel-resin interface, 3 extra exemplary intact sticks per subgroup, not loaded for µ-TBS testing, were prepared and proceeded to qualitative SEM evaluation. The surface opposite to the one being evaluated was marked with a waterproof marker to facilitate positioning and handling of the sample, while the surface to be evaluated was manually polished with sandpaper of decreasing roughness (P1200- P4000 Silicon Carbide Grinding Paper, Buehler) under water irrigation, in order to attain a smooth surface, followed by polishing with a diamond paste with synthetic diamond powder of particle size ranging between 2 and 4 µm (DirectDia Paste, Diamond Polishing Paste, SHOFU INC., Kyoto, Japan). Finally, all sticks were cleaned in an ultrasonic device with 50 W, 220-240 V, 50-60 Hz operating parameters for 5 min (Halbwellenbetrieb Ultraschallreinigungsgeraet, Easy Home Laundry, Aldi, Jonschwil, Switzerland).

Sticks were demineralized in 36 % phosphoric acid (Detrey Conditioner 36, Dentsply DeTrey GmbH) for 30 s for partial removal of the inorganic content, rinsed with distilled water, then dehydrated [41]. Afterwards, fixation was done using carbon conductive tabs (Leit Tabs nach Göcke, Nr. G3347, 12mm, Plano GmbH), and sputtering was done exactly as mentioned in etching pattern evaluation section (4.2.7, page 32), then images were captured at 700X, 1250X, 4000X, 6000X, and 10000X magnification.

Resin-tags evaluation

For evaluating resin tags, another 3 extra exemplary intact sticks per subgroup, not loaded for μ-TBS testing, were prepared and proceeded to qualitative SEM evaluation. Specimens were immersed directly in 37 % HCL (Sigma-Aldrich, Steinheim, Germany) for at least 3 h till the whole enamel substrate was dissolved. Afterwards, sticks were washed with distilled water and left to dry at room temperature overnight. Fixation, sputtering and examination under SEM was done exactly as mentioned above (4.2.7, page 32) and images were taken at 700X, 1250X, 4000X, 6000X, and 10000X magnifications.

Then, the etching patterns were classified as described by Silverstone et al. into 5 different types as follows [106]:

Type 1: preferential dissolution of the prism cores, resulting in a honeycomb like appearance. Type II: preferential dissolution of the prism peripheries, giving a cobblestone like appearance. Type III: a mixture of types I and II patterns. Type IV: pitted enamel surfaces as well as structures that look like unfinished puzzles, maps, or networks. Type V: flat, smooth surfaces.

4.2.8 Statistical analysis

I. Sample size estimation

Sticks were used as the experimental unit of the study. The guidance published by the Academy of Dental Materials was followed recommending that a minimum of 3 teeth per subgroup should be included [(10)]. Due to difficulties in collecting sound primary molars with caries-free buccal and oral surfaces, we used 6 tooth halves from different teeth per subgroup instead of 3 complete teeth and tooth dependency of each stick was accounted for in the statistical analysis.

II. Analysis of μ-TBS data

Data were gathered and analysed statistically using software SPSS 26.0 (IBM SPSS Statistics for Windows, Version 26.0, IBM Corp, Armonk, NY, USA). Statistical analysis of μ-TBS data for both phase I and phase II was first evaluated separately using linear mixed models (LMM) with the restricted maximum likelihood (REML) method, where the tooth was modelled as the random variable and the group assignment (Material) was chosen as the fixed factor. This method was used to account for dependencies in the data based on the fact that multiple sticks from one tooth half were analysed. Residuals were checked for normal distribution (Kolmogorov-Smirnov test and Shapiro-Wilk test) and variance homogeneity (Levene test). In the case of heterogeneity of variances, heteroscedasticity was modelled and permitted in the statistical analysis (REPEATED = Material| SUBJECT (Tooth*Row) COVTYPE (DIAG))[1].

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[1] SPSS code:

```
MIXED MPA BY Material
/FIXED=Material | SSTYPE (3)
/METHOD=REML
/RANDOM=INTERCEPT | SUBJECT (Tooth) COVTYPE(VC)
/REPEATED=Material | SUBJECT (Tooth*Row) COVTYPE (DIAG)
/SAVE=RESID
/EMMEANS= TABLES (Material) COMPARE ADJ (SIDAK)
```

The RANDOM statement defines the random intercept model (variance component model) with "Tooth" as random factor and "Material" as the fixed factor; the REPEATED statement with covariance type "Diagonal" (DIAG) calculates the variances separately for all combinations of the fixed factor "Material". In order to achieve this, the "SUBJECT" in this statement has to be defined as "Row" which refers to the number of sticks tested per tooth half. Since "Tooth" is defined as subject in the RANDOM statement already, it has to be included in the REPEATED subject as well, otherwise, the model would give a warning message only.

Sidak adjustment for multiple testing was used to for multiple pairwise comparisons of data and their corrections.

Then, a comparison of the μ-TBS value change in the different subgroups between phase I (24 h aging) and phase II (6 months aging) was performed, where the two fixed factors (Aging; 24 h and 6 months) (Material) and their interaction was tested. Additionally, the differences between the two phases were tested for all materials. Again, variance heterogeneity was modelled and permitted for statistical analysis (REPEATED=Material*Aging| SUBJECT (Tooth*Row) COVTYPE(DIAG))[2].

The exact same tests were used to investigate the effect of the examined surface (buccal vs. oral), the total bonding area of each tooth, as well as the effect of pulp chamber filling on the μ-TBS with only changing the fixed variable. Spearman's rho test was used to find the correlation between the total bonding area/tooth and the number of sticks yielded. Then, the total dropouts were calculated and compared between the groups using one-way analysis of variance (ANOVA).

Moreover, a post hoc sensitivity analysis was conducted to assess the robustness of findings retrieved by μ-TBS testing. The results of the sensitivity analysis showed that a population-based effect size of at least Cohen's d = 0.48 (medium effect) would be necessary to detect statistically reliable differences between two of the test groups using this study design with 7 tooth halves/material and 10 observations (sticks)/tooth half. In

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[2] This was achieved by adding the REPEATED statement to the model:
SPSS code:
MIXED MPA BY Material Aging
/FIXED=Material Aging Material*Aging | SSTYPE (3)
/METHOD=REML
/RANDOM=INTERCEPT | SUBJECT (Tooth) COVTYPE(VC)
/EMMEANS=TABLES (Material*Aging) COMPARE (Aging)
/REPEATED=Material*Aging | SUBJECT (Tooth*Row) COVTYPE(DIAG).

The RANDOM statement defines the random intercept model (variance components model) with "Tooth" as random factor; the REPEATED statement with covariance type "Diagonal" (DIAG) calculates the variances separately for all combinations of the two fixed factors "Material and Aging". In order to achieve this, the "SUBJECT" in this statement has to be defined as "Row" which refers to the number of sticks tested per tooth half. Since "Tooth" is defined as subject in the RANDOM statement already, it has to be included in the REPEATED subject as well, otherwise, the model would give a warning message only.

the most conservative model, a population-based effect size of at least Cohen's d = 0.65 (medium effect) would be necessary to detect statistically reliable differences between two of the test groups using this study design with 6 tooth halves/material and 7 observations (sticks)/tooth half. An ICC of 0.018 was used for this analysis, which is indicative of a very small tooth dependency.

The level of significance was set at $\alpha < 0.05$.

Fracture modes were calculated in percent and were descriptively presented.

5 RESULTS

In this study, 107 extracted primary molars were gathered for the main experiment. Those yielded 113 tooth halves and were distributed as 5 tooth halves to test the effect of pulp chamber filling, 64 tooth half (611 stick) for phase I, and 44 tooth half (448 stick) for phase II. (**Figure 2**, page 22) shows an overview of the tooth halves distribution, while (**Table 3**, page 41) and (**Table 4**, page 44) show an overview of the number of sticks per subgroup.

In addition, extra 4 tooth halves were used for investigating different enamel surface preparations (4.2.4, page 25), 1 tooth half for measuring the remaining enamel thickness (4.2.4, page 26) , 4 tooth halves for etching pattern evaluation for the four application methods, and 10 tooth halves for the enamel resin interface evaluation (30 stick) and resin tag evaluation (30 stick) together (4.2.7, page 32).

5.1 Calibration of µ-TBS

5.1.1 µ-TBS of permanent molars' dentin

Estimated mean µ-TBS data of 5 human 3rd molars with 95% confidence interval (CI) of the main operator in this study (50.1 MPa; [44.1, 56.1]) were compared with a previous experienced and calibrated operator (54.2 MPa; [48.3, 60.1]). The residuals showed normal distribution (Kolmogorov-Smirnov, $p = 0.2$; Shapiro-Wilk test, $p = 0.812$) with heterogenicity of variance (Levene's test, $p = 0.047$). The compared bond strength values of the two operators were not significantly different from each other (LMM, REML, $p = 0.299$). Fracture modes were also descriptively compared, and the most common fracture of the main operator versus the calibrated one was the adhesive fracture (90.9 %, 81.7 %) followed by the cohesive fracture in composite (3.4 %, 12.7%) respectively.

5.1.2 µ-TBS of permanent incisors' enamel

Estimated mean of µ-TBS data of 5 permanent incisors of the main operator in this study (33.5 MPa; [29.3, 37.6]) was compared with an already calibrated operator (34.5 MPa; [30.6, 38.5]). The residuals showed heterogenicity of variance (Levene's test, $p <$

0.001), that was allowed and modulated. The compared bond strength values of the two operators (LMM, REML, $p = 0.662$), as well as their fracture modes (Fisher's exact test, $p = 0.324$) showed no significant difference.

5.2 μ-TBS

5.2.1 Investigating different enamel surface preparations

Four methods of enamel surface preparations were examined under SEM to investigate the best method to remove the aprismatic enamel and flatten the surface without exposing the underlying dentin. (**Figure 6**, page 40)

a) Yellow ring flame finishing stone showed regular and deep satisfactory etching pattern.

b) Red ring flame finishing stone showed almost the same intensity and distribution of enamel demineralization produced by the yellow ring stone.

c) Silicon carbide sandpapers with thickness P1200 and P4000 respectively showed irregular and mild etching pattern, that was not enough to create acceptable bond strength values.

d) Yellow ring flame finishing stone followed by silicon carbide sandpapers with thickness P1200 and P4000 respectively showed etching pattern comparable to the yellow stone alone, yet with flatter and smoother enamel surface, hence easier layering of the composite on the surface.

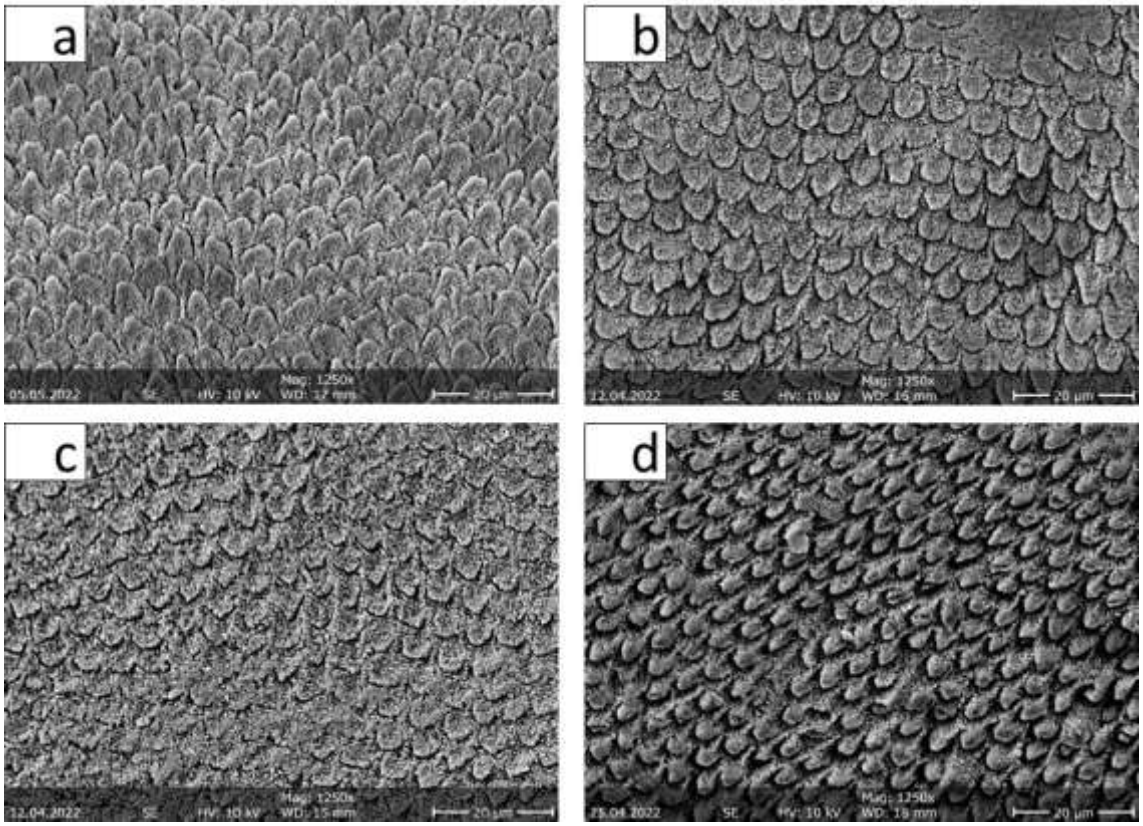

Figure 6: SEM representation of the etching pattern of different enamel surface preparations at 1250X magnification. a. yellow ring finishing stone, b. red ring finishing stone, c. silicon carbide sandpapers, d. yellow ring finishing stone followed by silicon carbide papers.

5.2.2 Pulp chamber filling

A comparison of the μ-TBS values of teeth with filled pulp chambers (SU-30s-24h) versus 5 exemplary teeth with unfilled pulp chambers (SU-30s-24h-without pulp filling) was done only for the SU adhesive with 30 s etching. The data showed homogeneity of variance (Levene's test, $p = 0.919$) and pulp chamber filling was proven to have no significant effect on the bond strength values (LMM, REML, $p = 0.53$).

5.2.3 Phase I (after 24 h storage)

The actual size of the sticks produced was 0.64 ± 0.4 mm for both phases. Our results showed that the application mode used had a significant difference on the μ-TBS of all tested adhesives (LMM, REML, $p < 0.001$). Mean values of the μ-TBS of the tested groups were calculated with a CI of 95 % and are presented in (**Table 3**, page 41). The residuals were not normally distributed (Kolmogorov-Smirnov, $p < 0.001$; Shapiro-Wilk test, $p < 0.001$) and showed heterogenicity of variance (Levene's test, $p < 0.001$), that

was allowed and modulated. Thus, significant differences were calculated and multiple comparisons between the groups were done using Sidak corrections method.

Table 3: Summary of μ-TBS values after 24 h storage in distilled water

Groups	Number of sticks	Estimated marginal mean (MPa)	Standard error	Confidence interval (95 %)	
				Lower limit	Upper limit
SU-SE	57	12 [a]	1.4	9.2	14.8
SU-30s	56	29.2 [b]	1.6	25.9	32.4
SU-15s	62	34.9 [b]	1.8	31.4	38.5
SU-act	64	14.80 [a]	1.9	11	18.5
CU-SE	58	9.3 [a]	1.1	7	11.7
CU-30s	62	25.5 [b]	1.4	22.7	28.3
CU-15s	61	30.4 [b]	1.6	27.2	33.7
iBU-SE	67	16.9 [a]	1.7	13.5	20.3
iBU-30s	62	28.3 [b]	1.4	25.3	31.2
iBU-15s	62	28.9 [b]	1.5	25.9	31.9

∗ Different superscript letters indicate significant differences within the same adhesive, while different cell borders indicate differences between different adhesives (LMM, REML, $p < 0.05$). SU-SE: Scotchbond Universal applied passively in self-etch mode, SU-30s: Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching, SU-15s: Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching, SU-act: Scotchbond Universal applied actively, CU-SE: Clearfil Universal Bond Quick applied passively in self-etch mode, CU-30s: Clearfil Universal Bond Quick applied passively preceded by 30 s phosphoric acid etching, CU-15s: Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching, iBU-SE: iBond Universal applied passively in self-etch mode, iBU-30s: iBond Universal applied passively preceded by 30 s phosphoric acid etching, iBU-15s: iBond Universal applied passively preceded by 15 s phosphoric acid etching.

As seen in (**Table 3**, page 41), SU showed high significant differences in μ-TBS values between subgroup SU-SE when compared to SU-30s (Sidak, $p < 0.001$) and SU-15s (Sidak, $p < 0.001$). Also, significant differences were found between SU-act in comparison to SU-30s (Sidak, $p < 0.001$) and SU-15s (Sidak, $p < 0.001$), while no significant differences between SU-SE and SU-act (Sidak, $p < 1.000$) as well as SU-30s and SU-15s (Sidak, $p < 0.576$) were observed.

In CU, significant differences were detected when comparing CU-SE to subgroups CU-30s (Sidak, $p < 0.001$) and CU-15s (Sidak, $p < 0.001$), while no differences were found between subgroups CU-30s and CU-15s (Sidak, $p = 0.692$). Similarly, iBU showed differences between subgroup iBU-SE with iBU-30s (Sidak, $p < 0.001$) and iBU-15s

(Sidak, $p < 0.001$) but not between subgroups iBU-30s and iBU-15s (Sidak, $p < 1.000$). On the other hand, significant differences between adhesives were only detected between CU-SE and iBU-SE (Sidak, $p = 0.029$). An overview of the µ-TBS values of the 3 adhesives can be seen in (**Figure 7**, page 42).

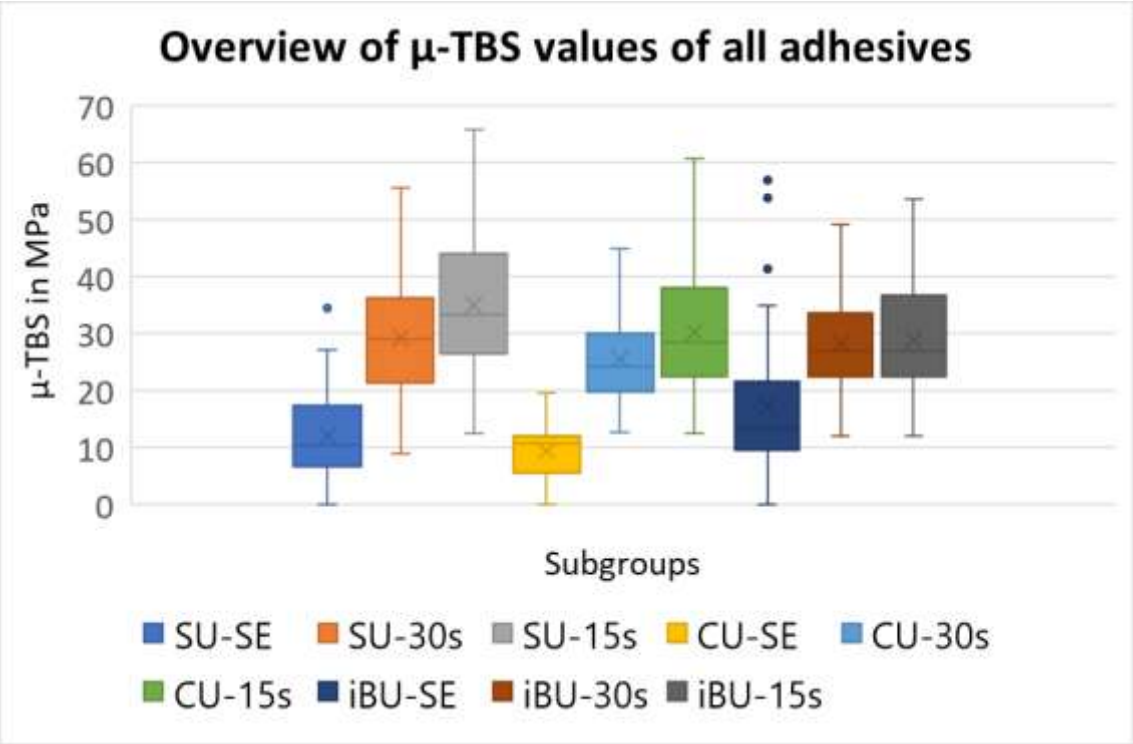

Figure 7: Comparison of the µ-TBS values of the 3 adhesives in different application modes. SU-SE: Scotchbond Universal applied passively in self-etch mode, SU-30s: Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching, SU-15s: Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching, CU-SE: Clearfil Universal Bond Quick applied passively in self-etch mode, CU-30s: Clearfil Universal Bond Quick applied passively preceded by 30 s phosphoric acid, CU-15s: Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching, iBU-SE: iBond Universal applied passively in self-etch mode, iBU-30s: iBond Universal applied passively preceded by 30 s phosphoric acid etching, iBU-15s: iBond Universal applied passively preceded by 15 s phosphoric acid etching.

Moreover, the effect of the examined surface (buccal vs. oral) on the μ-TBS values was analysed. Mean μ-TBS values for the buccal and oral surfaces were calculated and were found to be 23.73 MPa [22.31, 25.14] and 22.32 MPa [20.91, 23.72] respectively, indicating that there were no significant differences between both surfaces in all subgroups for the three adhesives (LMM, REML, $p = 0.17$).

Furthermore, the total bonding area per examined tooth half showed no significant effect on the μ-TBS value of the tooth in all tested adhesives in all application modes (LMM, REML, $p = 0.971$). Meanwhile, a middle strong positive correlation was found between the total bonding area of examined tooth halves and the number of yielded sticks (Spearman's rho test, correlation coefficient = 0.365) with a level of significance ($p = 0.003$).

When comparing the total dropouts per tooth in the different subgroups, the data were found to be homogenous (Levene's test, $p = 0.688$) and they showed no significant differences in all adhesives (ANOVA, $p = 0.167$).

5.2.4 Phase II (after 6 months storage)

Mean values of the μ-TBS of the tested groups are presented in (**Table 4**, page 44). The residuals were not normally distributed (Kolmogorov-Smirnov, $p < 0.001$; Shapiro-Wilk, $p < 0.001$), and heterogenicity of variance was observed and modulated (Levene's test, $p < 0.001$). Thus, significant differences were calculated and multiple comparisons between the groups were done using Sidak corrections method.

As expected, pairwise comparisons showed highly significant differences between subgroup A (SE) versus subgroup C (15s) in all tested adhesives (Sidak, $p < 0.001$), while there were not any differences detected between the same subgroups of different adhesives, except between CU-SE and iBU-SE (Sidak, $p = 0.014$).

Table 4: ***Summary of μ-TBS values after 6 months storage in distilled water***

Groups	Number of sticks	Estimated marginal mean (MPa)	Standard error	Confidence interval (95 %)	
				Lower limit	Upper limit
SU-SE	79	10.4 [a]	1	8.5	12.2
SU-15s	75	28.9 [b]	1.6	25.8	32.1
CU-SE	74	9.1 [a]	1	7.2	11.1
CU-15s	73	27.4 [b]	1.4	24.7	30
iBU-SE	76	15.1 [a]	1.5	12.2	18
iBU-15s	71	26.1 [b]	1.4	23.3	29

∗Different superscript letters indicate significant differences within the same adhesive, while different cell borders indicate differences between different adhesives. Statistical analysis was done using LMM and REML ($p < 0.05$). SU-SE: Scotchbond Universal applied passively in self-etch mode, SU-15s: Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching, CU-SE: Clearfil Universal Bond Quick applied passively in self-etch mode, CU-15s: Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching, iBU-SE: iBond Universal applied passively in self-etch mode, iBU-15s: iBond Universal applied passively preceded by 15 s phosphoric acid etching.

The interactive effect of the examined surface (buccal vs. oral) together with the subgroup was found to have a significant effect on the μ-TBS ($p = 0.023$), yet the examined surface alone had a lower influence on the μ-TBS ($p = 0.431$) when compared to the effect of subgroup alone ($p < 0.001$). Since, the effect of the examined surface was very small, the data weren't modelled.

Furthermore, the total bonding area per tooth half showed no significant effect on the μ-TBS value of the tooth in all tested adhesives in all application modes (LMM, REML, $p < 0.717$). Meanwhile, a middle strong positive correlation was found between the total bonding area of examined tooth halves and the number of yielded sticks (Spearman's rho test, correlation coefficient = 0.483) with a level of significance ($p < 0.001$).

The differences in total dropouts per tooth between different subgroups were significantly different (ANOVA, $p = 0.010$), yet the data showed heterogenicity of variances and high standard deviation levels, therefore the analysis was not reliable.

5.2.5 Comparison between phase I and phase II

Results showed that there were not any significant interactions between the factor material and aging (LMM, REML, $p < 0.446$). Mean values of the μ-TBS between aged groups for 24 h and aged groups for 6 months in distilled water were calculated and the residuals showed lack of normal distribution (Kolmogorov-Smirnov, $p < 0.001$; Shapiro-Wilk test, $p < 0.001$). The heterogenicity of variance (Levene's test, $p < 0.001$) was significant, thus, the differences were calculated and multiple comparisons between the groups were done using Sidak corrections method (**Figure 8**, page 46).

μ-TBS values of all subgroups were decreased after aging. A decline was shown in SU-15s from 34.99 MPa [31.60, 38.38] to 28.98 MPa [25.56, 32.39], CU-15s from 30.36 MPa [27.40, 33.32] to 27.21 MPa [24.25, 30.16], iBU-15s from 28.87 MPa [26.18, 31.56] to 26.10 MPa [23.02, 29.16], SU-SE from 11.98 MPa [9.53, 14.44] to 10.39 MPa [8.08, 12.70], CU-SE from 9.33 MPa [7.46, 11.21] to 9.13 MPa [6.80, 11.47], and iBU-SE from 16.91 MPa [13.78,20.05] to 15.06 MPa [11.87, 18.25].

From the results it can be concluded that, all groups have shown a change in values in the same direction, yet significant differences were only found in SU-15s (Sidak, $p = 0.014$). The interaction tested between the factors "Material*Aging" was meant to analyse if the differences among the adhesives were significantly different between the two phases. This development yielded no significant difference owing to the wide confidence interval range of the subgroups.

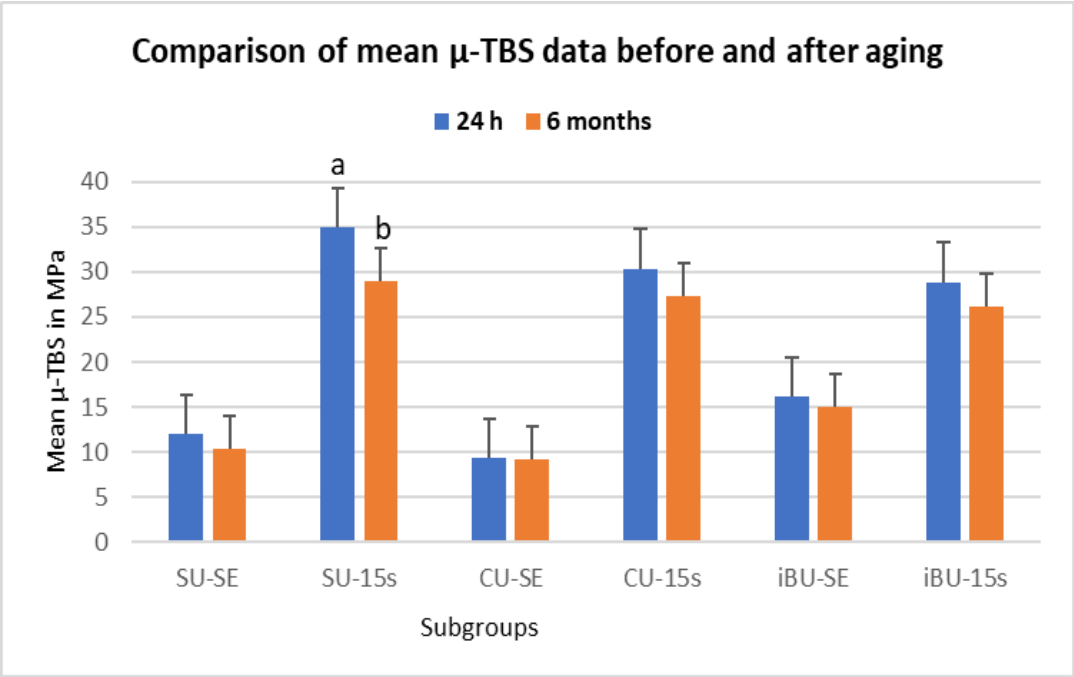

Figure 8: Comparison of the mean µ-TBS in MPa between 24 h and 6 months aging. SU-SE: Scotchbond Universal applied passively in self-etch mode, SU-15s: Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching, CU-SE: Clearfil Universal Bond Quick applied passively in self-etch mode, CU-15s: Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching, iBU-SE: iBond Universal applied passively in self-etch mode, iBU-15s: iBond Universal applied passively preceded by 15 s phosphoric acid etching. Different superscript letters indicate significant differences within the same subgroup. Error bars represent the standard error.

5.2.6 Failure patterns evaluation

Phase I

Failure patterns were analysed descriptively. In phase I, the adhesive fracture (78.7 %) represented the most dominant fracture mode of all groups (**Figure 9**, page 47) (**Figure 10**, page 48), followed directly by the mixed fracture in enamel and adhesive (10.6 %), mixed fractures in both adhesive and composite (3.8 %), cohesive fractures in enamel (3.1 %), then cohesive fractures in composite (2 %), and finally pre-test failures (1.8 %).

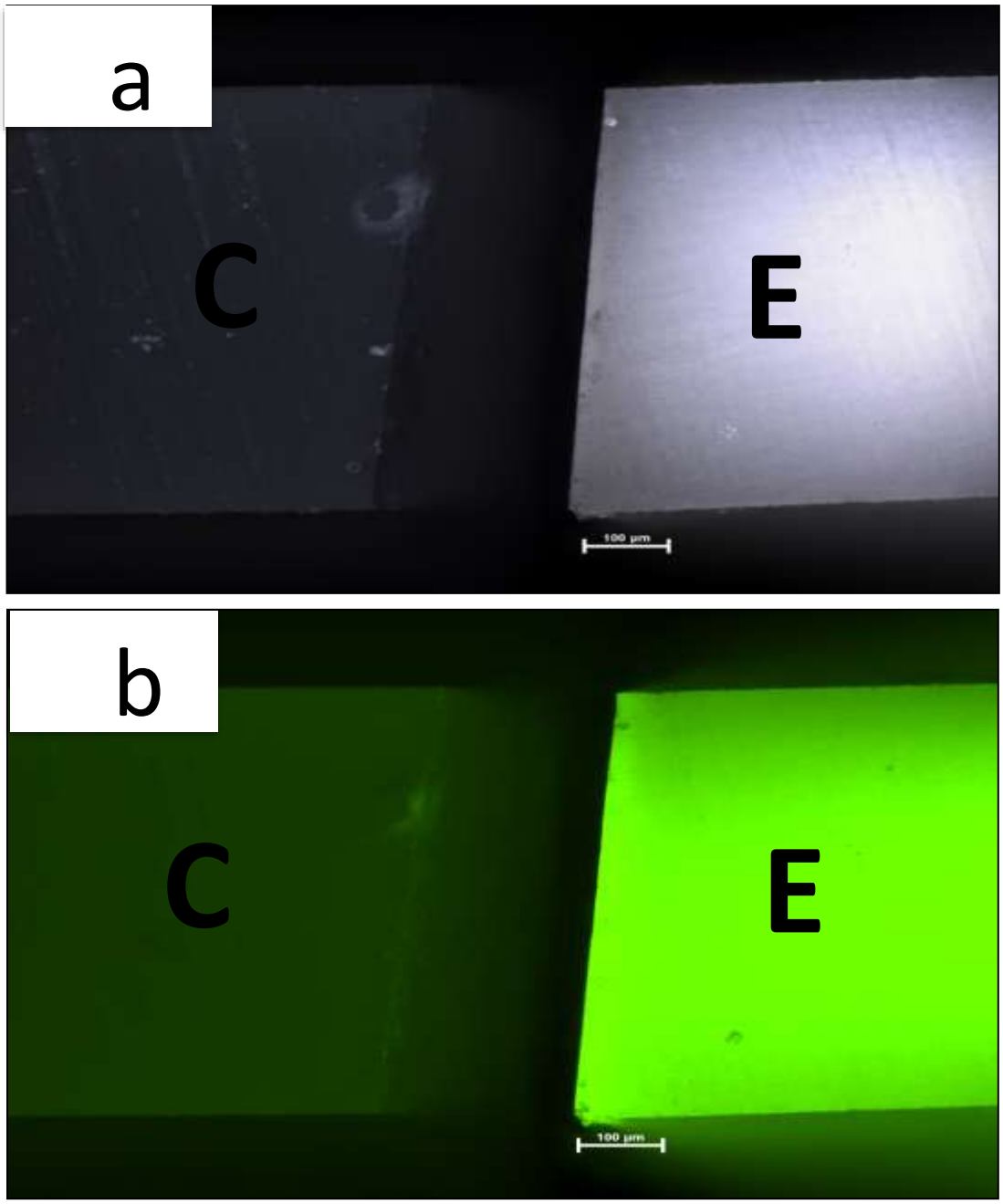

Figure 9: A side view of a stick fixed to the holders of the testing machine, after fracture, showing an adhesive failure. Image was taken for the subgroup iBU-15s-24h at 72X magnification. a: under light microscope, b: under florescent microscope. E: Enamel, C: composite.

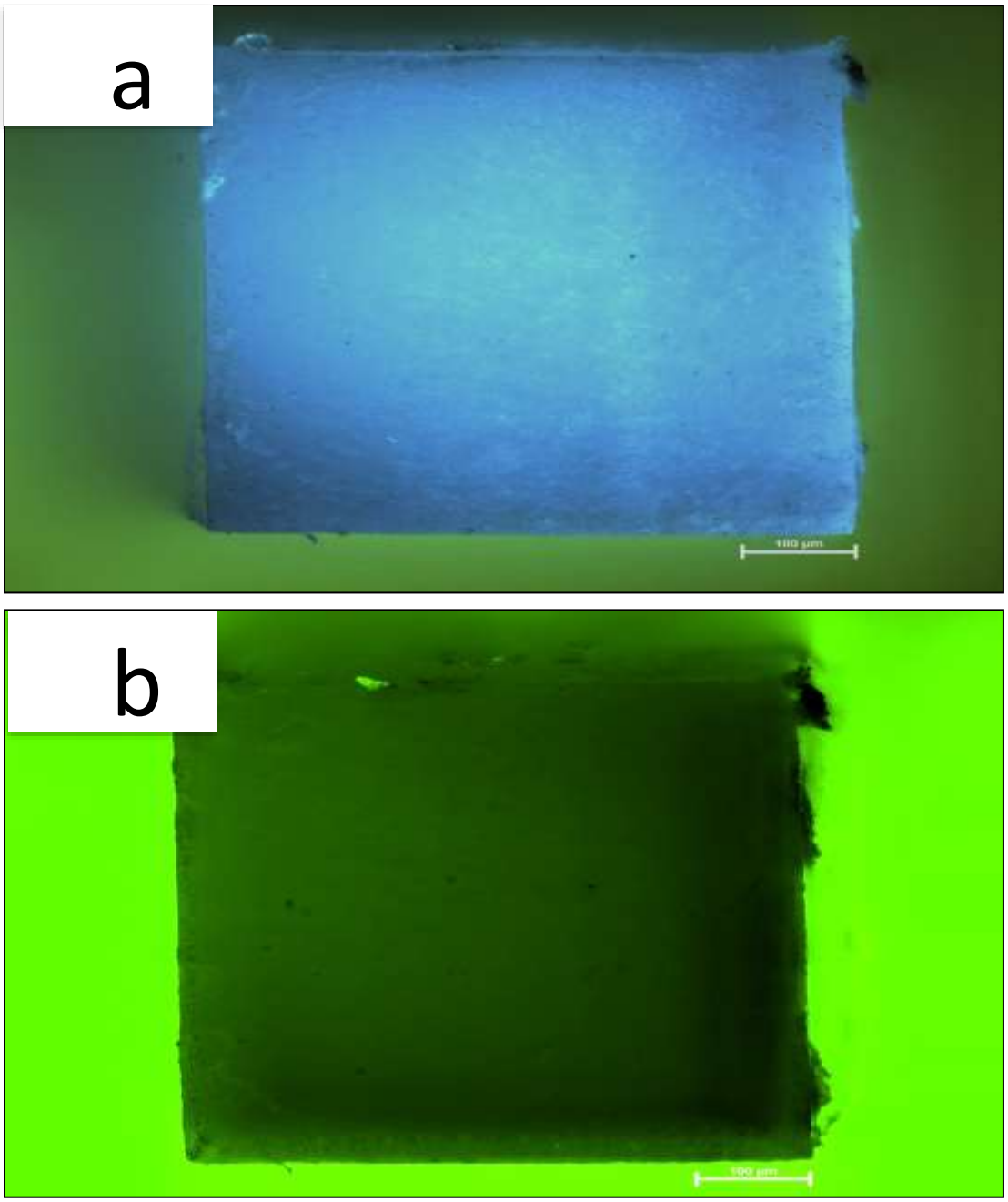

Figure 10: A top view of the enamel half of the stick, after fracture, showing an adhesive failure. Image was taken for the subgroup iBU-15s-24h at 72X magnification. a: under light microscope, b: under florescent microscope.

The distribution of different fracture modes between the subgroups in phase I is represented in (**Figure 11**, page 49). Within the same adhesive, adhesive fractures were predominant in CU-15s (83.6 %), and in SU-30s adhesive (87.5 %), as well as iBU-30s (87.1 %), while mixed fractures at the adhesive enamel interface were represented in SU-SE (12.3 %), CU-SE (10.3 %) and iBU-SE (14.9 %).

The rest three fracture modes were scarcely represented, and ptf were only found in SU-SE (7 %) and SU-act (7.8 %).

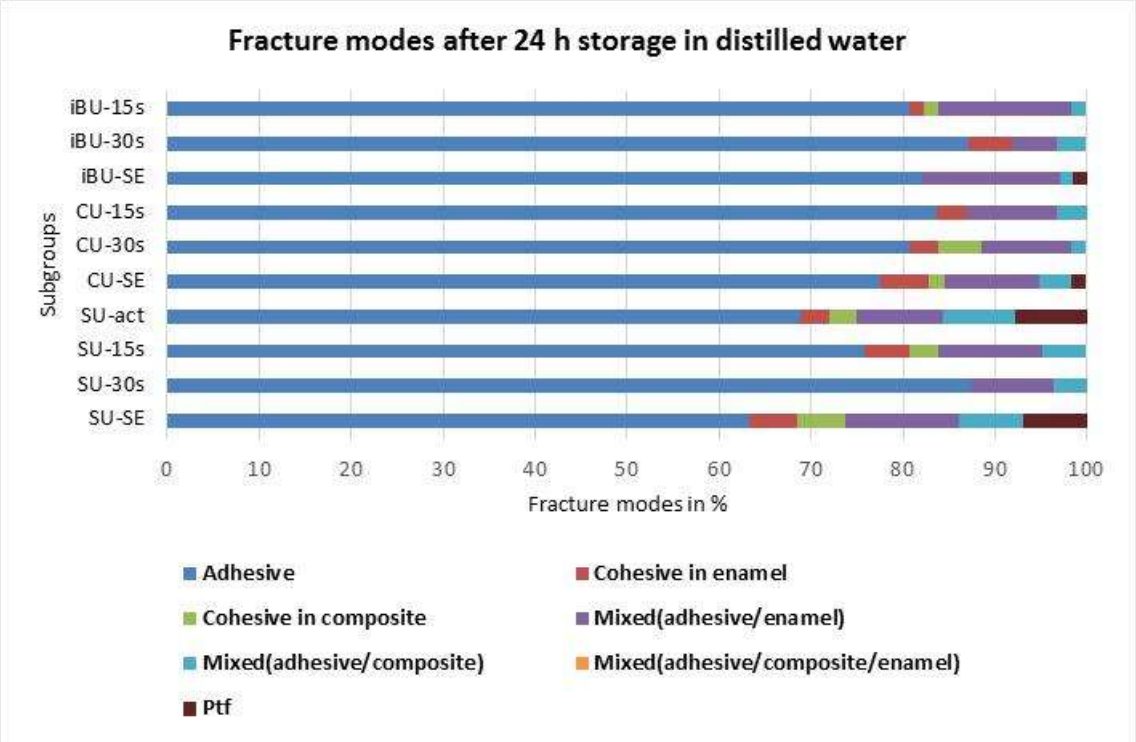

Figure 11: Mode of bond failure after 24 h storage in distilled water. Different types of fractures are presented in % and are differentiated through colour coding. SU-SE: Scotchbond Universal applied passively in self-etch mode, SU-30s: Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching, SU-15s: Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching, SU-act: Scotchbond Universal applied actively, CU-SE: Clearfil Universal Bond Quick applied passively in self-etch mode, CU-30s: Clearfil Universal Bond Quick applied passively preceded by 30 s phosphoric acid etching, CU-15s: Clearfil Bond Quick Universal applied passively preceded by 15 s phosphoric acid etching, iBU-SE: iBond Universal applied passively in self-etch mode, iBU-30s: iBond Universal applied passively preceded by 30 s phosphoric acid etching, iBU-15s: iBond Universal applied passively preceded by 15 s phosphoric acid etching.

Phase II

Unlike phase I, the most common total fracture mode of all groups was the mixed fracture in both adhesive and enamel (50.7 %) (**Figure *12***, page 50) (**Figure 13**, page 51), followed by adhesive fracture (29.7 %), then pre-test failures (12.9 %), cohesive fractures in enamel (2.9 %), mixed fractures in both composite and enamel (2.2 %), cohesive fractures in composite (1.3 %), and finally mixed fractures in composite and enamel (0.2 %).

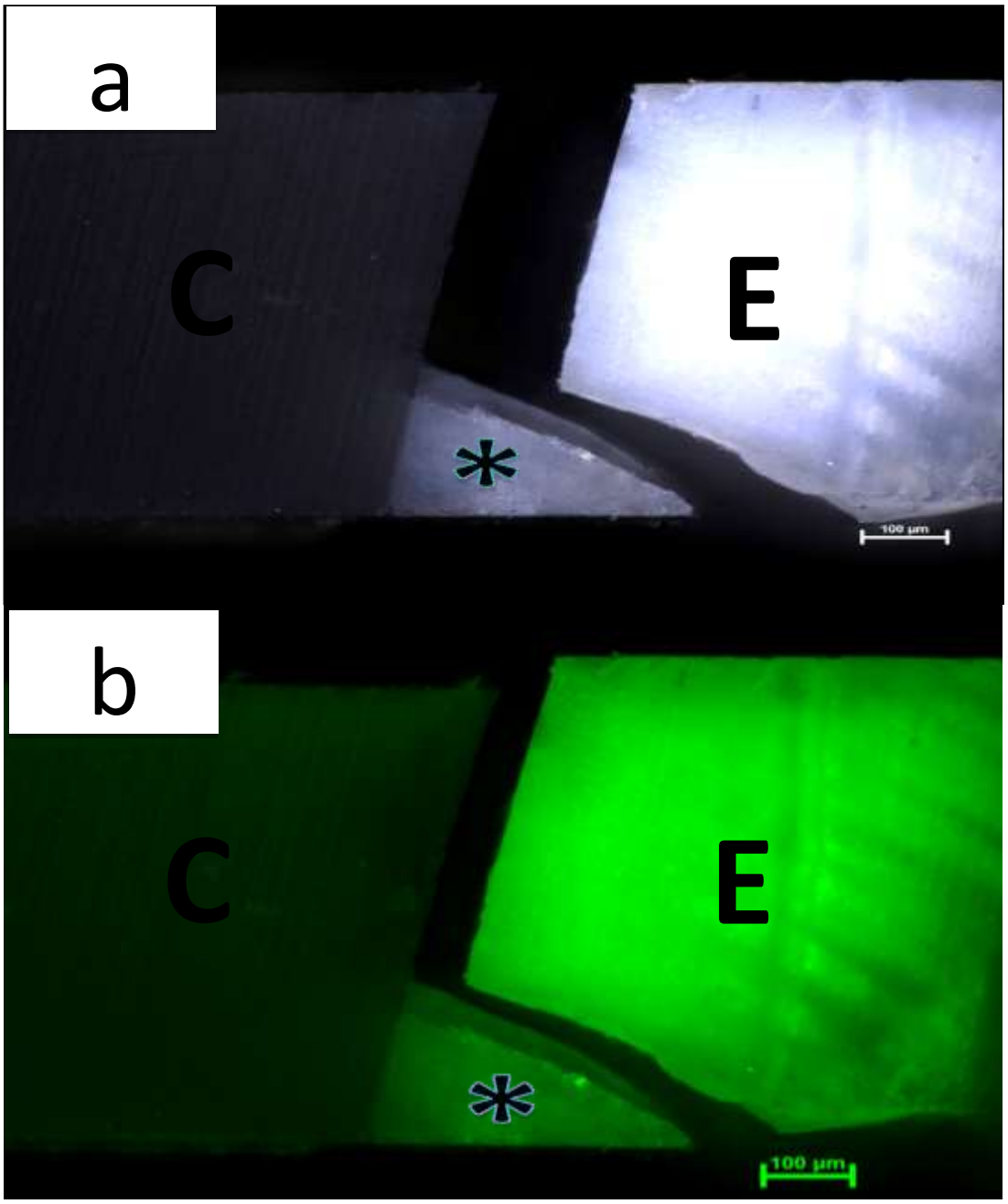

Figure 12: A side view of a stick fixed to the holders of the testing machine, after fracture, showing a mixed adhesive enamel failure. Image was taken for the subgroup CU-SE-24h at 72X magnification. a: under light microscope, b: under florescent microscope. E Enamel, C: composite, *: fractured enamel part hanging on the composite side.

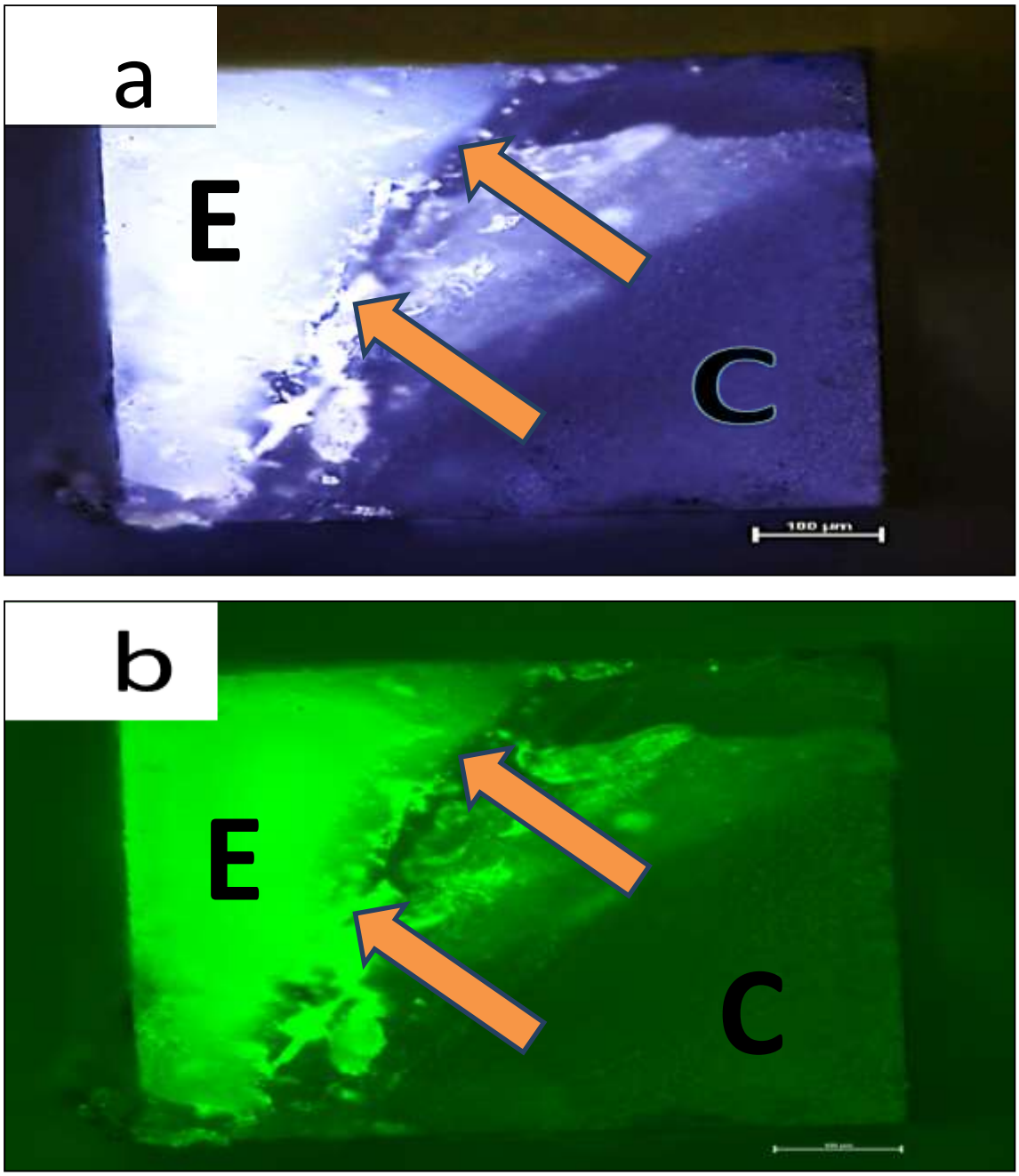

Figure 13: A top view of the composite half of the stick, after fracture, showing a mixed adhesive enamel failure. Image was taken for the subgroup iBU-15s-24h at 72X magnification. a: under light microscope, b: under florescent microscope. E: Enamel, C: composite, Arrows: demarcation line between enamel and composite.

The distribution of different fracture modes between the subgroups is represented in (**Figure 14**, page 52). Within the same adhesive, mixed fractures at the adhesive enamel interface as well as adhesive fractures are predominantly represented in SU-15s (57.3 %, 33.3 %), CU-15s (64.4 %, 26 %), and iBU-15s (52.1%, 36.6 %) respectively.

Although ptf were more distributed in phase II when compared to phase I, they were only observed in subgroups A (SE) of all three adhesives with percentages of 20.3 % in SU, 23 % in CU, and 32.9 % in iBU. Additionally, mixed fracture in composite and enamel were exclusively represented in phase II, but only in CU-15s (1.4 %)

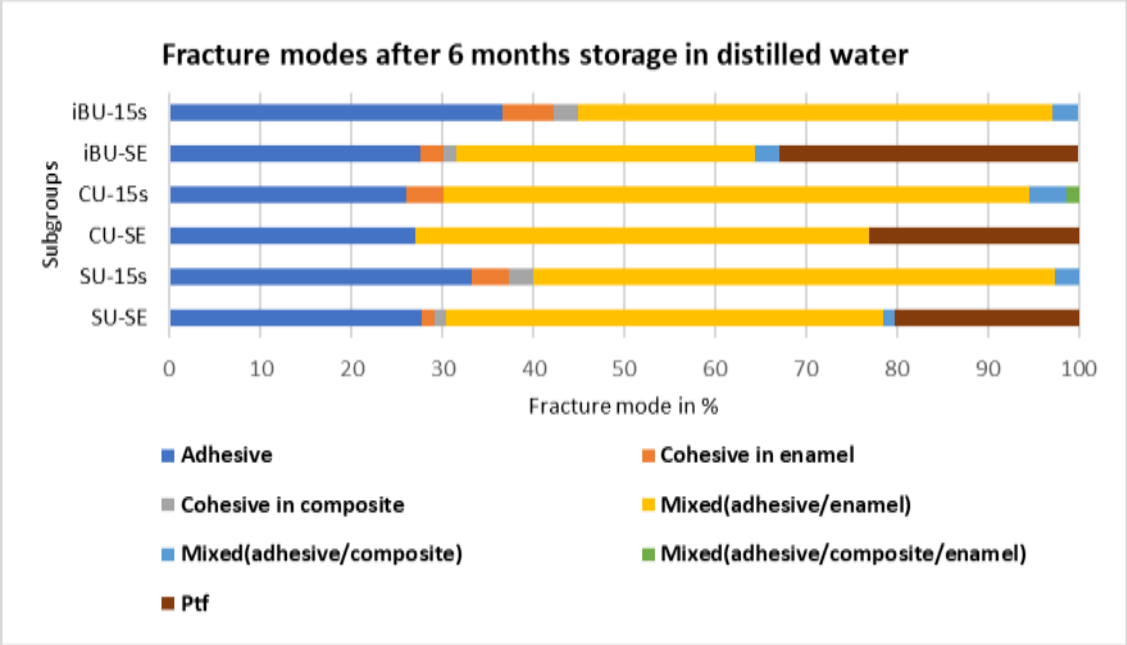

Figure 14: Mode of bond failure after 6 months storage in distilled water. Different types of fractures are presented in % and are differentiated through color coding. SU-SE: Scotchbond Universal applied passively in self-etch mode, SU-15s: Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching, CU-SE: Clearfil Universal Bond Quick applied passively in self-etch mode, CU-15s: Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching, iBU-SE: iBond Universal applied passively in self-etch mode, iBU-15s: iBond Universal applied passively preceded by 15 s phosphoric acid etching.

Generally, it can be observed that phase II showed a decrease in the adhesive fracture percentage of all adhesives in all subgroups, together with an increase in the percentage of mixed fractures in the adhesive enamel interface. Also, a noticeable increase in the ptf was observed in the SE mode of all adhesives in phase II when compared to phase I.

5.2.7 Qualitative SEM evaluation

Qualitative evaluation of the etching pattern, enamel-resin interface, and resin-tags was performed under scanning electron microscope using separate exemplary samples from phase I (after 24 h storage in distilled water) of the study.

I. Etching pattern evaluation

Etching pattern was evaluated using separate tooth halves treated with Scotchbond Universal adhesive in the 4 different application modes. (**Figure 15**, page 53) shows that subgroups B (30s) and C (15s) had similar and comparable etching patterns to one another with regular and pronounced demineralization of the enamel prisms all over the examined surface. While in subgroups A (SE) and D (act), a mild demineralization pattern combined with no demineralization areas can be observed on the surface.

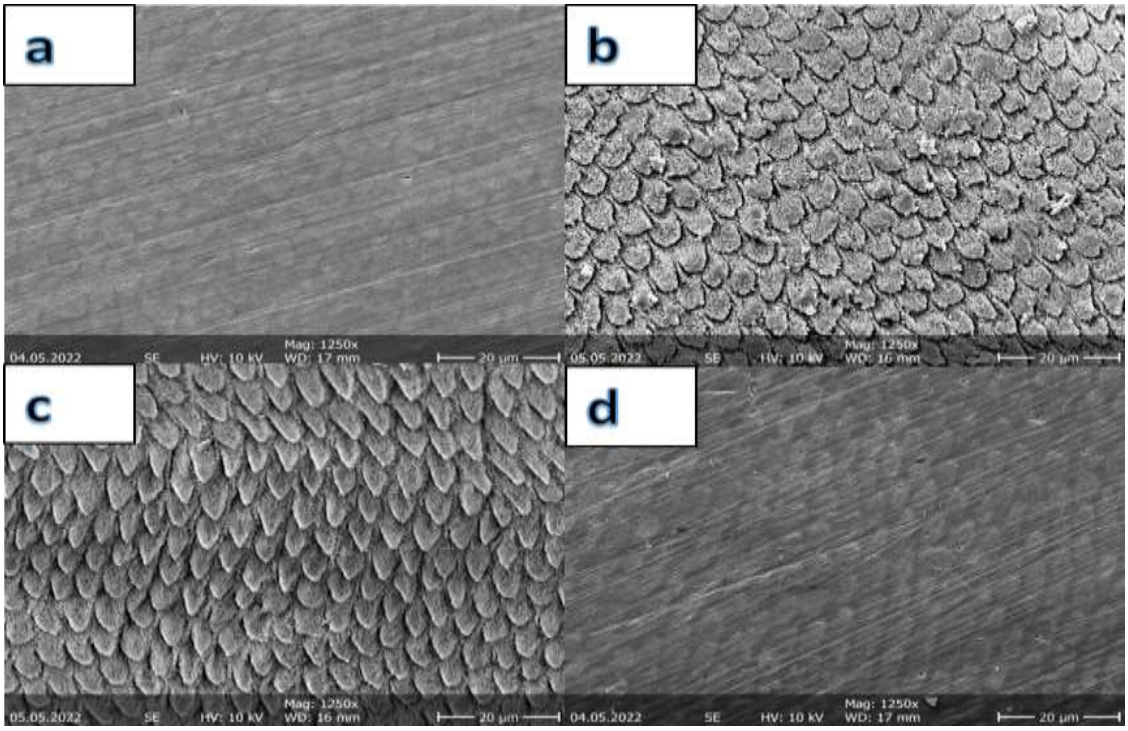

Figure 15: Morphological comparison of exemplary SEM images of the etching pattern of Scotchbond Universal adhesive at 1250X magnification. a. Scotchbond Universal applied passively in self-etch mode, b. Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching, c. Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching, d. Scotchbond Universal applied actively.

II. Enamel-resin interface evaluation

Intact exemplary sticks were used for evaluation of the enamel-resin interface at 700X and 4000X magnification, in which the abundance of resin-tags, hybrid layer width, and adhesive interface quality were compared between the different application modes in the tested adhesives.

Subgroup A (SE)

Self-etch mode showed a flat adhesive resin interface with absence of resin-tags and abundant voids especially in CU. (**Figure 16**, page 55)

The hybrid layer for all tested adhesives was generally thin. iBU showed the widest hybrid layer of all followed by SU, then CU, which matches the μ-TBS results of subgroup A. (**Table 3**, page 41)

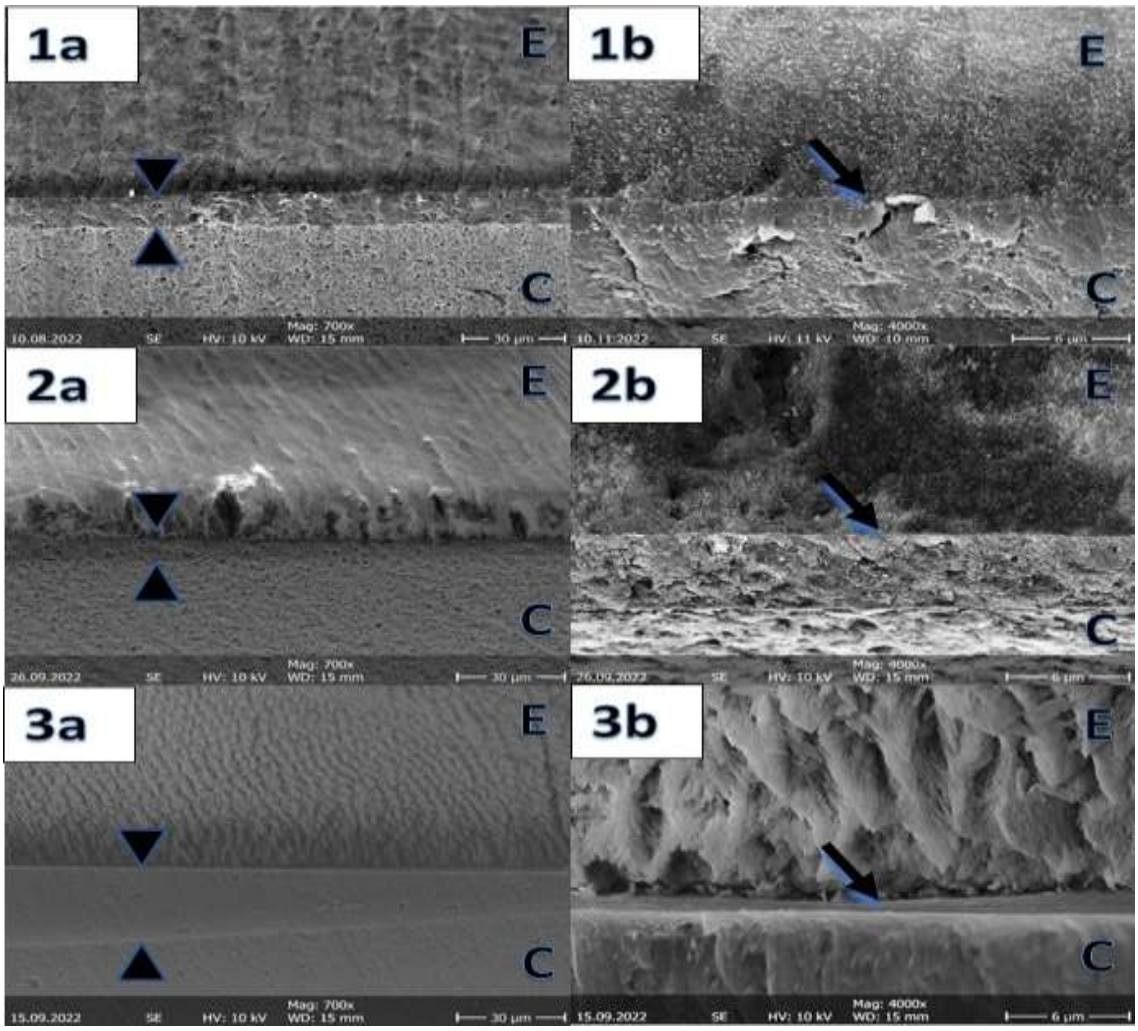

Figure 16: Morphological comparison of exemplary SEM images of the adhesive zone of samples not treated with phosphoric acid (subgroup A) showing flat adhesive joint without any obvious resin-tags. 1a. Scotchbond Universal applied passively in self-etch mode at 700X magnification, 1b. Scotchbond Universal applied passively in self-etch mode at 4000X magnification, 2a. Clearfil Universal Bond Quick applied passively in self-etch mode at 700X magnification, 2b. Clearfil Universal Bond Quick applied passively in self-etch mode at 4000X magnification, 3a. iBond Universal applied passively in self-etch mode at 700X magnification, 3b. iBond Universal applied passively in self-etch mode at 4000X magnification. E: enamel, C: composite, 1-arrow: lack of resin-tags, 2-arrowheads: adhesive joint.

Subgroup B (30s)

Though the μ-TBS values between the different adhesives in this subgroup did not significantly differ from each other, slight differences were seen in the adhesive joint under the SEM. A thick well-formed hybrid layer was seen in SU adhesive with numerous long resin-tags extending into the enamel prisms. This, was less pronounced with shorter and less resin-tags in CU. On the other hand, iBU showed the thickest hybrid layer, with

multiple resin-tags distributed all over the adhesive joint that seemed to be shorter than in SU. (**Figure 17**, page 56)

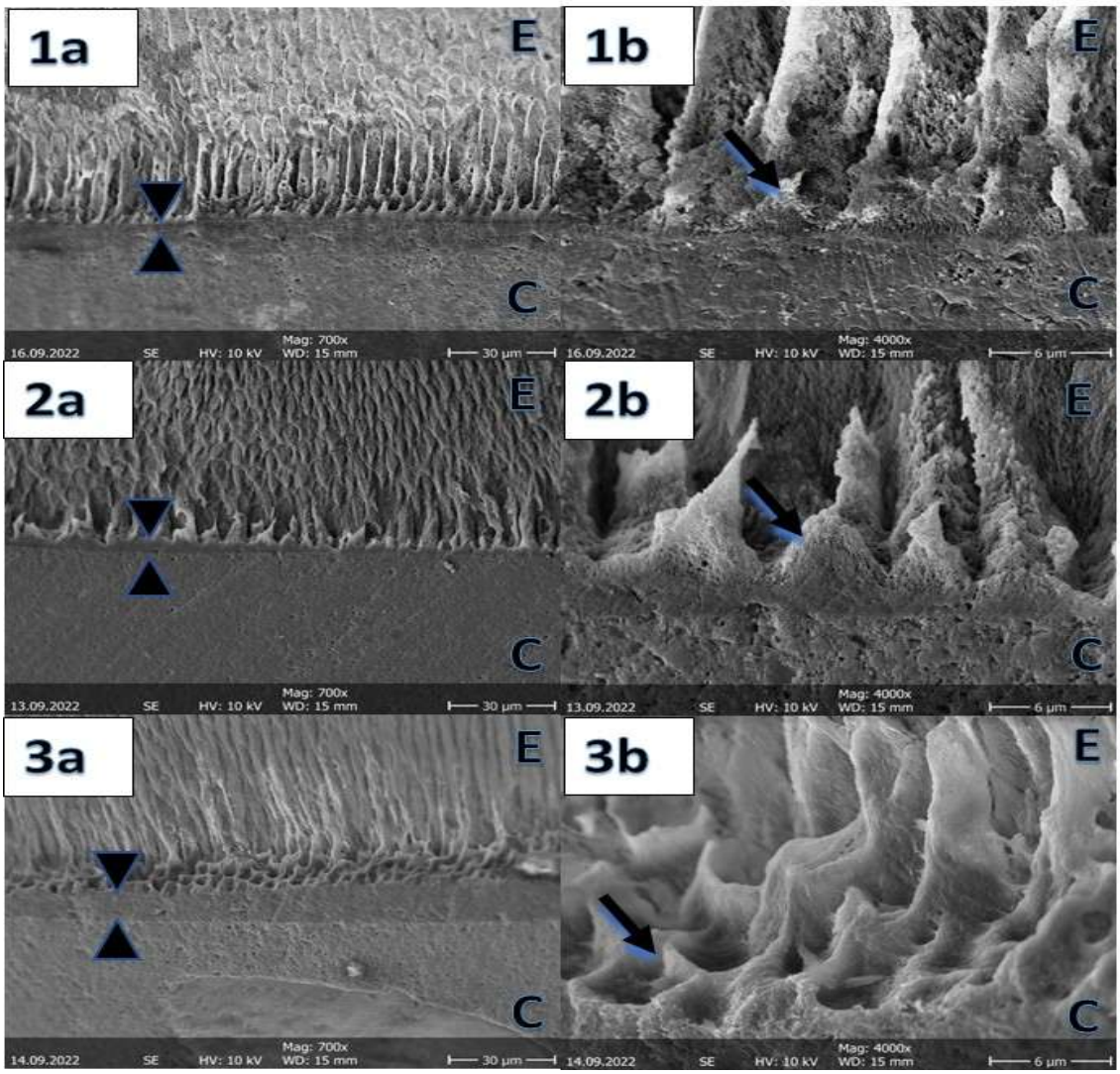

Figure 17: Morphological comparison of exemplary SEM images of the adhesive zone of samples etched with phosphoric acid for 30 s (subgroup B) showing well-formed adhesive joint with numerous resin-tags. 1a. Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching at 700X magnification, 1b. Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching at 4000X magnification, 2a. Clearfil Universal Bond Quick applied passively preceded by 30 s phosphoric acid etching at 700X magnification (Adhesive joint not obvious), 2b. Clearfil Universal Bond Quick applied passively preceded by 30 s phosphoric acid etching at 4000X magnification, 3a. iBond Universal applied passively preceded by 30 s phosphoric acid etching at 700X magnification, 3b. iBond Universal applied passively preceded by 30 s phosphoric acid etching at 4000X magnification. E: enamel, C: composite, 1-arrow: resin-tags, 2-arrowheads: adhesive joint.

Subgroup C (15s)

Like subgroup B (30s), subgroup C (15s) has shown well-formed hybrid layer with long numerous resin-tags in SU and iBU, while CU showed indefinite less pronounced hybrid layer with shorter resin-tags. However, no significant differences were found in the µ-TBS values. (**Figure *18***, page 57)

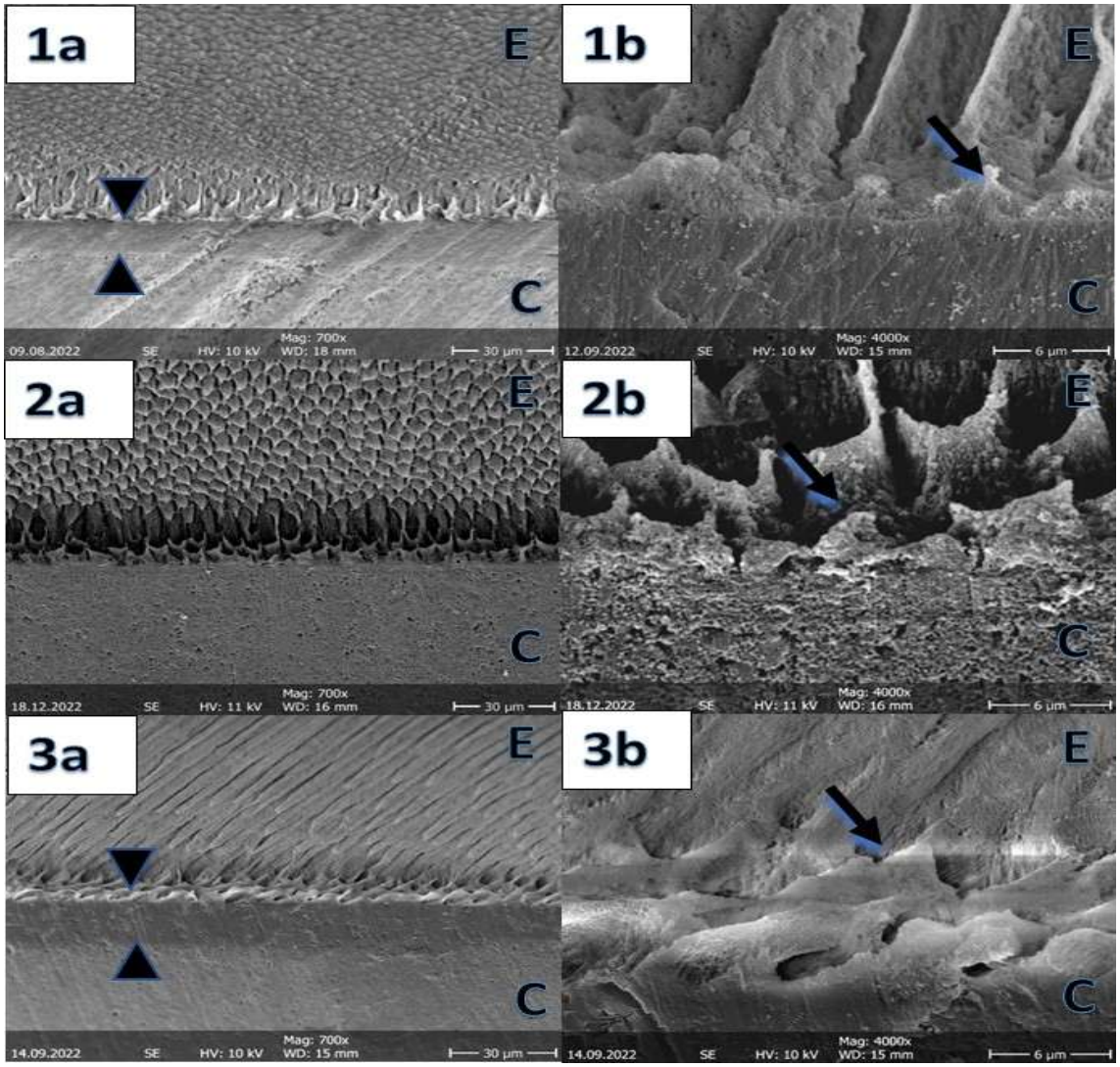

Figure 18: Morphological comparison of exemplary SEM images of the adhesive zone of samples etched with phosphoric acid for 15 s (subgroup C) showing well-formed adhesive joint with numerous resin-tags. 1a. Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching at 700X magnification, 1b. Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching at 4000X magnification, 2a. Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching at 700X magnification (Adhesive joint not obvious), 2b. Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching at 4000X magnification, 3a. iBond Universal applied passively preceded by 15 s phosphoric acid etching at 700X magnification, 3b. iBond Universal applied passively preceded by 15 s phosphoric acid etching at 4000X magnification. E: enamel, C: composite, 1-arrow: resin tags, 2-arrowheads: adhesive joint.

Subgroup D (act)

(**Figure 19**, page 58) showed that SEM images of subgroup D (act) were almost like subgroup A (SE), in the fact that hybrid layer was ill defined with lots of voids and cracks, and no resin-tags were observed.

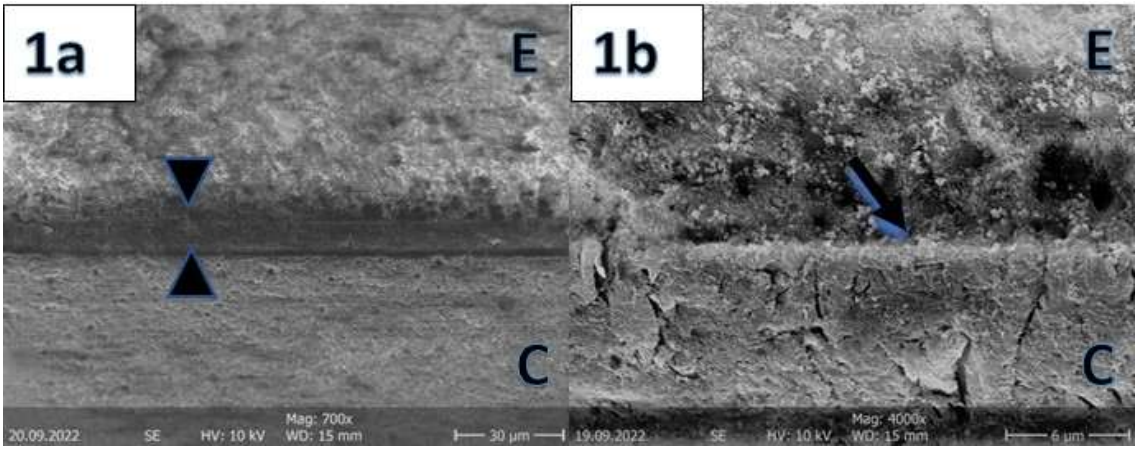

Figure 19: Morphological comparison of exemplary SEM images of the adhesive zone of samples treated with active rubbing of the bonding agent (subgroup D). 1a. Scotchbond Universal applied actively at 700X magnification, 1b. Scotchbond Universal applied actively at 4000X magnification. E: enamel, C: composite, 1-arrow: resin tags, 2-arrowheads: adhesive joint.

III. Resin-tags evaluation

Exemplary intact sticks were used to evaluate the resin-tags production after complete dissolution of the enamel layer.

Subgroup A (SE)

SEM images showed a combination of Type IV or V etching patterns, without any enamel dissolution regardless the adhesive used. Also, resin-tags were absent and the adhesive interface was shown to be flat (**Figure *20***, page 59).

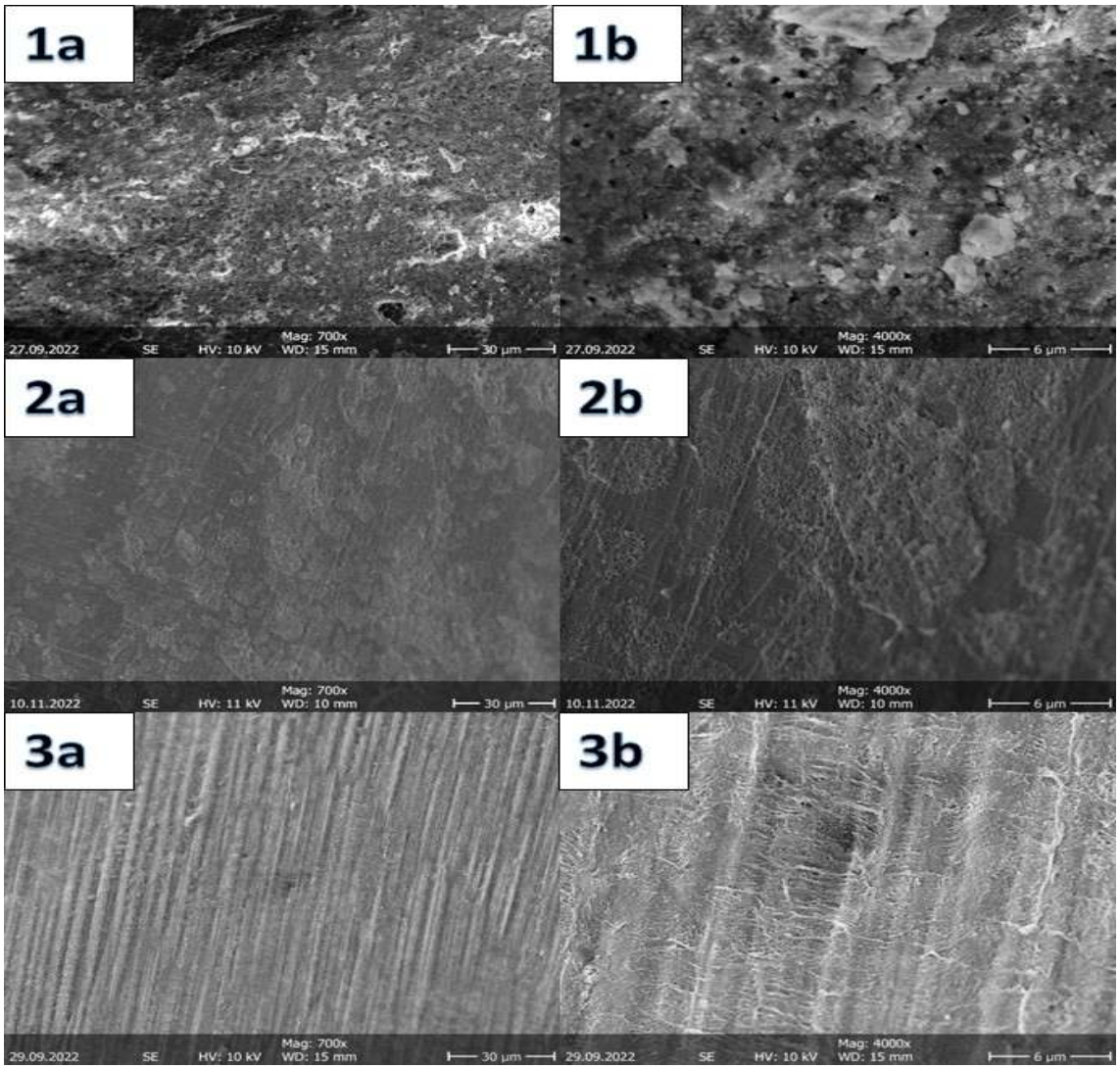

Figure 20: Morphological comparison of exemplary SEM images of the resin-tags after complete enamel dissolution of samples not treated with phosphoric acid (subgroup A). 1a. Scotchbond Universal applied passively in self-etch mode at 700X magnification, 1b. Scotchbond Universal applied passively in self-etch mode at 4000X magnification, 2a. Clearfil Universal Bond Quick applied passively in self-etch mode at 700X magnification, 2b. Clearfil Universal Bond Quick applied passively in self-etch mode at 4000X magnification, 3a. iBond Universal applied passively in self-etch mode at 700X magnification, 3b. iBond Universal applied passively in self-etch mode at 4000X magnification.

Subgroup B (30s)

(**Figure *21***, page 60) shows exclusively a Type I etching pattern in iBU bond, while SU showed a Type II etching pattern and CU showed a Type III etching pattern with a higher percentage of Type II.

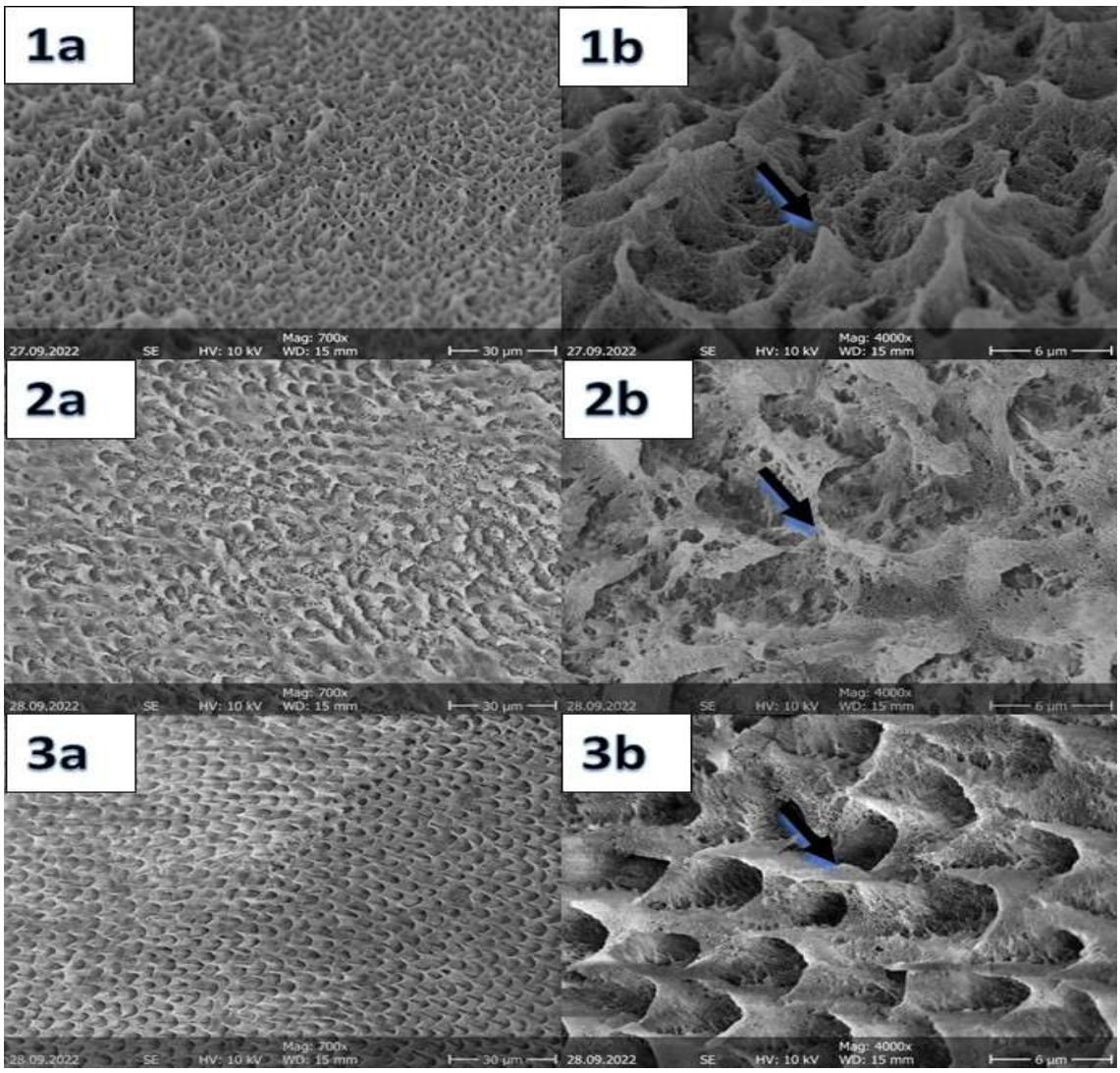

Figure 21: Morphological comparison of exemplary SEM images of the resin-tags after complete enamel dissolution of samples etched with phosphoric acid for 30 s (subgroup B). 1a. Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching at 700X magnification, 1b. Scotchbond Universal applied passively preceded by 30 s phosphoric acid etching at 4000X magnification, 2a. Clearfil Universal Bond Quick applied passively preceded by 30 s phosphoric acid etching at 700X magnification, 2b. Clearfil Universal Bond Quick applied passively preceded by 30 s phosphoric acid etching at 4000X magnification, 3a. iBond Universal applied passively preceded by 30 s phosphoric acid etching at 700X magnification, 3b. iBond Universal applied passively preceded by 30 s phosphoric acid etching at 4000X magnification. Arrow: resin tags.

Subgroup C (15s)

The resin-tag production of all adhesives including CU was well pronounced, and very clearly shown in all samples etched for 15 s prior to the application of the assigned adhesives. SU and iBU showed Type I etching pattern while CU showed Type III etching pattern. (**Figure *22***, page 61)

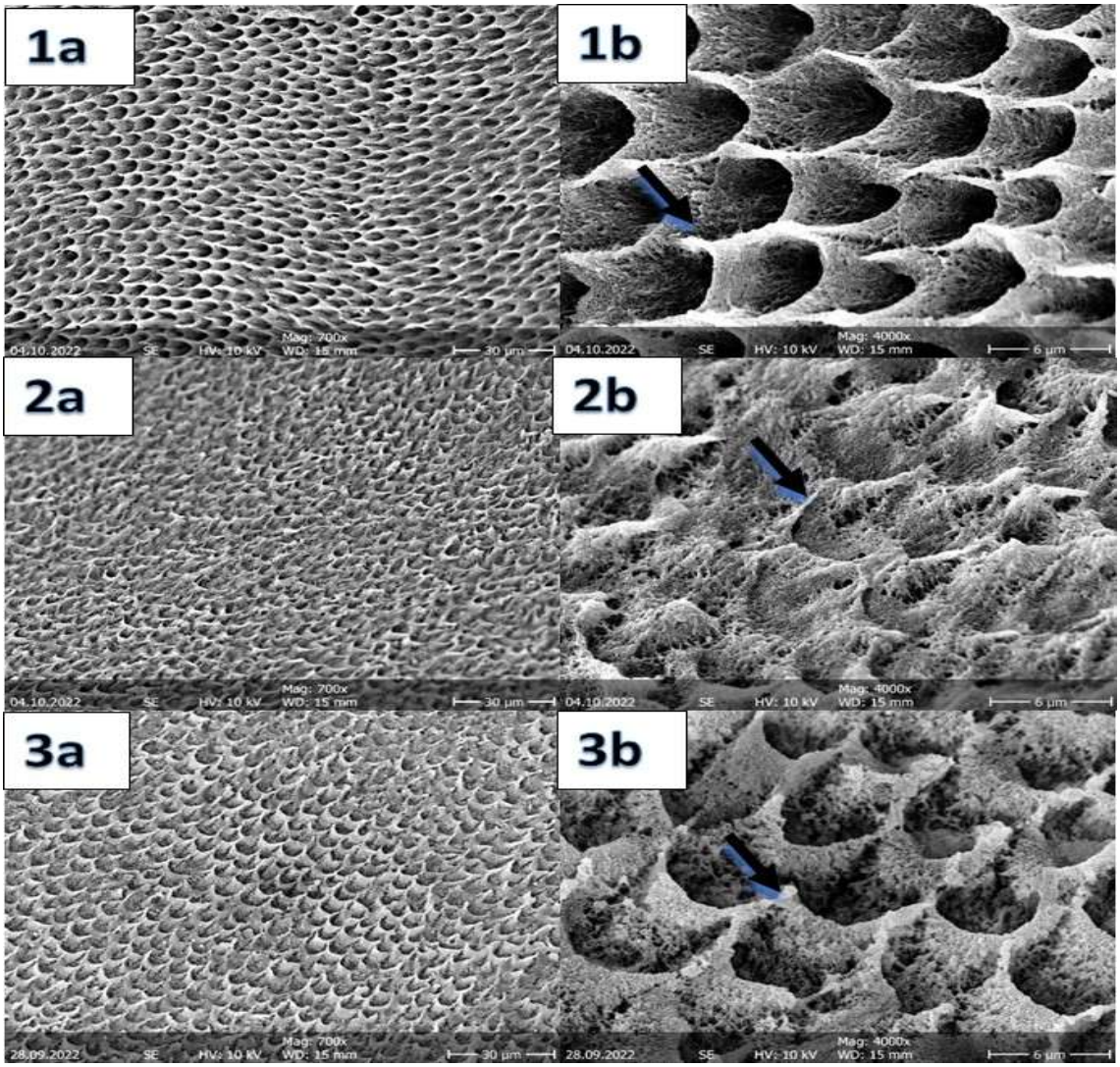

Figure 22: Morphological comparison of exemplary SEM images of the resin-tags after complete enamel dissolution of samples etched with phosphoric acid for 15 s (subgroup C). 1a. Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching at 700X magnification, 1b. Scotchbond Universal applied passively preceded by 15 s phosphoric acid etching at 4000X magnification, 2a. Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching at 700X magnification, 2b. Clearfil Universal Bond Quick applied passively preceded by 15 s phosphoric acid etching at 4000X magnification, 3a. iBond Universal applied passively preceded by 15 s phosphoric acid etching at 700X magnification, 3b. iBond Universal applied passively preceded by 15 s phosphoric acid etching at 4000X magnification. Arrow: resin tags.

Subgroup D (act)

Images of subgroup D (**Figure *23***, page 62) are similar to subgroup A (SE) in the absence of any resin-tags and the flat adhesive joint.

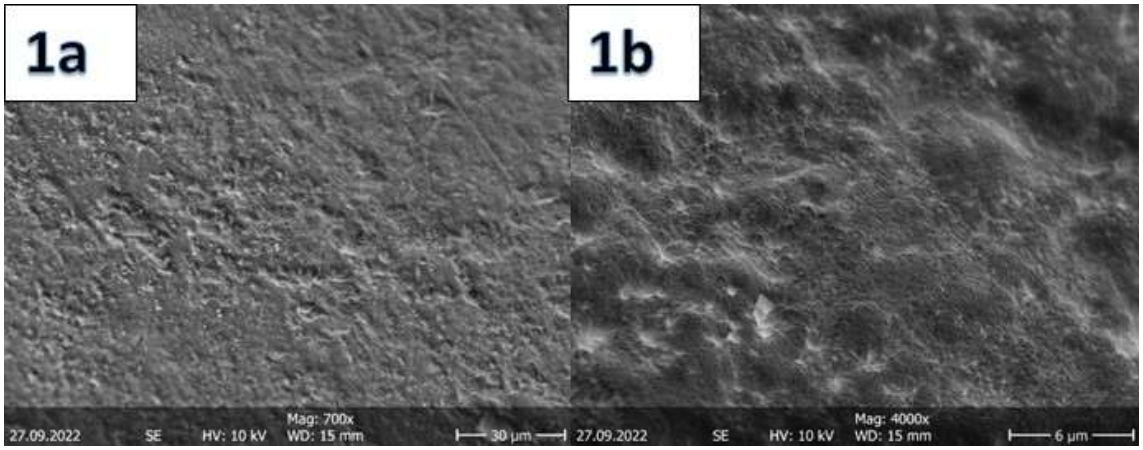

Figure 23: Morphological comparison of exemplary SEM images of the resin-tags after complete enamel dissolution of samples treated with active rubbing of the bonding agent (subgroup D). 1a. Scotchbond Universal applied actively at 700X magnification, 1b. Scotchbond Universal applied actively at 4000X magnification.

6 DISCUSSION

6.1 Discussion of materials and methods

A thorough understanding of the utilized materials, the substrate bonded to, and a precise clinical protocol are the key elements in the success of dental restorations. Proper management of the adhesive interface is a must learn trait for dentists. [3] Therefore, this study aimed at suggesting a protocol for bonding universal adhesives on primary molar's enamel in-vitro.

6.1.1 Selection of materials

A standard 36% phosphoric acid etchant (DeTrey® Conditioner 36, Dentsply) available in the dental clinic was used for the calibration process to resemble and standardize the process with the previous operator. Then its use was adopted for the main experiment, since phosphoric acid concentrations between 34- 37 % were proven to obtain extensive resin penetration and smoother transition from resin to enamel over several microns, that decreases crack propagation. [93]

UAs were particularly chosen for this study because they provide the operator with a free choice of selecting the adhesive strategy used, in addition to providing a promise for a chemical interaction capable of achieving long lasting stable bond without extra tooth preparation for macro-retention. [25]

Specific concern was made to choose adhesives containing 10-MDP functional monomer, since it was identified as capable of creating a stable chemical interaction with HAp, thus achieving better bond strength values. [33]

Adhesives present in the market have either extra mild, mild, or strong etching capabilities. These pH ranges present usually effective bonding to dentin, but equivocal research is available on its bonding to enamel.[3] That's why we have chosen in our study one extra mild (3M^{TM} Scotchbond Universal adhesive, 3M oral care; pH= 2.7), one mild (Clearfil^{TM} Universal bond quick, Kuraray Noritake; pH= 2.3) and one strong universal adhesive (iBond® Universal Adhesive, Heraeus Kulzer; pH= 1.6-1.8) to compare the effect of different application protocols on different pH values.

Since chemistries of all bonding agents can deteriorate over time especially with variations in storage temperatures, only single dose Lolli-pops of the adhesives were used[4]. Also, adhesives were stored in the refrigerator and warmed up only 30 minutes before their application as per the recommendations of Alex G, 2015, to decrease the effect of temperature on adhesives' deterioration [3].

A universal microhybrid composite resin restorative material (Filtek™ Z250 Universal Restorative System, 3M™ Oral Care) was chosen for this study with wide range of indications such as: direct anterior/posterior restorations, core build-ups, splinting, and indirect restorations. The selection of the shade A3 was to be able to compare our data with the existing calibrated operators.

6.1.2 Teeth collection and storage

Although bond strength studies on bovine teeth are common [53], we have chosen to conduct our study on human molars to ensure results that are close as possible to the real-life situation. Our study was conducted on primary molars with at least one sound buccal or lingual surface to ensure the reproducibility of results. [5]

As per Armstrong et al, teeth were disinfected upon their collection in 0.5 % Chloramine-T-solution [10], then they were further stored in distilled water at a temperature of -22 ˚C.

Storage solutions are important since they can prevent dehydration of teeth and their cross contamination. Unlike dentin, there are limited studies that tested the effect of storage solutions on the properties of enamel. In this study, the choice of distilled water was based on a previous study that found no effect of the storage medium whether formalin, distilled water or thymol on the microshear bond strength values on enamel. [115] Although distilled water has no antibacterial properties it was still used in the study due to its availability, lack of scientific proof that it affects the bond strength values on enamel, and since teeth were previously disinfected in Chloramine-T-solution. The freezing effect on enamel of cryopreserved teeth for autogenous tooth transplantation was tested in one study and it was found out that freezing enamel to degrees below -80 ˚ C and -196 ˚ C did not affect the failure load of the specimens [8], therefore, it was concluded that a temperature below -22 ˚ C has no effect on the enamel.

6.1.3 Study design

For this study, it was planned to collect a total of 72 primary molars (n= 6/ subgroup). Teeth were meant to be divided into two halves in a split technique with one half to be tested after 24 h storage (phase I) and the other after 6 months (phase II) in distilled water. Due to difficulties in finding caries free teeth on both buccal and lingual surfaces, added to the dropouts on tooth level presented in small total bonding areas, the study design was modified to fit the number of teeth that can be collected.

Due to the considerable amount of tooth halves with clinical signs of dental caries, those had to be excluded from the study because the inclusion criterion of having a caries-free enamel surface was not met, the number of collected teeth was not exactly double the number of collected tooth halves.

At first a baseline group with SU for phase I was tested to identify the differences to be expected in μ-TBS between the subgroups. It was shown through the results that subgroup D (active rubbing of the adhesive material) was associated with high SD, probably due to inability to stabilize the hand pressure and rubbing motion all over the tested surface. Accordingly, subgroup D was excluded from further groups. Furthermore, subgroups B (S30s) and C (S15s) showed no significant differences between them. Therefore, subgroup C was chosen in phase II to save time during the restorative procedure in children and was compared to subgroup A (SE) as a control group. For this new study design, the study group managed to collect 132 teeth halves out of 119 freshly extracted primary molars.

Those collected teeth halves varied in the total bonding area, yielding different number of sticks per tooth half. In this study, sticks were considered the experimental unit therefore, it was so important to equalize the number of sticks used per subgroup. That's why, different number of tooth halves were used in each subgroup in order to bring the total number of sticks tested per subgroup as close as possible to each other.

A block randomization process with a block size of 10 teeth was chosen to compensate for the dropouts on the tooth level. 10 teeth were randomly chosen and sequentially numbered for each subgroup. In case both tooth halves of one tooth met the inclusion criteria; the tooth halves were assigned to two consecutive blocks to make sure that only one tooth half per tooth was assigned to the same experimental subgroup.

6.1.4 Tooth preparation

Several challenges were faced in the tooth preparation phase, that affected the process and had to be adjusted. Among which was the debonding of the sticks due to short tooth half of the stick. So, pulp chambers were filled with composite resin directly after removing the roots, to elongate the resulting sticks. Filling the pulp chambers helped elongate the sticks in the middle of the tooth with at least 1.5-2 mm rendering them more stable during testing. To test the effect of pulp chamber filling on the μ-TBS value, 5 teeth with pulp chamber filling were compared to 5 teeth without any pulp chamber filling for the SU-30s-24h, and it was found out that the μ-TBS values were not significantly different from each other ($p = 0.53$). Yet, it was difficult to find teeth with long enough tooth structure to be fixed properly on both sides without debonding.

Moreover, it was decided to split teeth into buccal and lingual halves, and to treat each half as a separate sample, first to increase the sample size, and second to create a flat surface on one end of the sample, which helped fix the samples properly during applying the tested materials and sawing.

Furthermore, variations in sizes of the tested tooth halves were compensated through measuring the total bonding area and excluding teeth with bonding area less than 4x 4, making the most common bonding area that we worked upon between 25 to 30 mm. Trying to standardize the total bonding area, decreased the variations in the number of sticks and teeth tested per subgroup.

A cross sectional area of 0.7 x 0.7 mm was chosen for our sticks, based on the study of Shono et al., who found out that the best tensile bond strength results for enamel are achieved with bonded areas less than 1 mm. Smaller specimens with smaller flaws fail at high stresses when compared to large bonded areas with bigger flaws and defects that can act as stress raisers. Added to that, they found out that failures resulting from smaller surface areas where predominantly adhesive or cohesive in the resin indicating that the bond strength was approaching to the cohesive strength of the resin. [105]

6.1.5 Enamel surface preparation

Aprismatic enamel existence was reported in literature in both primary and permanent teeth [96]. This layer is less permeable to bonding and must be ground to expose

the underlying prismatic enamel. [80] Several studies have tried to penetrate the aprismatic layer with acids such as ethylenediamine tetra acetic acid (EDTA) [27], phosphoric acid and polyalkenoic acid in different concentrations and time [103], yet the infiltration did not extend beyond the aprismatic layer. Therefore, it was decided in this study to ground the aprismatic enamel before applying the tested materials [85].

In this study, we examined 4 enamel surface preparation methods under SEM (4.2.4, page 25) and the results were as follows:

1. Silicon carbide paper did not remove enough aprismatic enamel and the etch pattern was not regular and not deep enough.

2.,3. Yellow ring and red ring finishing stones showed almost the same intensity and distribution, but yellow ring was chosen because it was believed to cause less surface cracks than the red one. Yet, both methods didn't create a flat buccal surface for the application of composite.

4. Yellow ring finishing stone followed by Silicon carbide paper was used, because it produced satisfactory etching pattern together with a flat surface that enabled good layering of the composite resin without its easy breakage during sawing.

This step did not resemble the real situation in patient's mouth yet was inevitable for the technicalities of the test, so we made sure through measuring the remaining enamel thickness that we are removing a maximum of 50 µm, so that all the aprismatic enamel is removed without decreasing enamel thickness.

Earlier, the hybrid layer was a term that was restricted to dentin, and it meant resin-impregnation and combination with the collagen layer of dentin creating a transitional layer of resin and tooth structure. This layer seals the surface against leakage and improves acid resistance.[79] Yet, SEM and transmission electron microscope (TEM) studies have found a new structure that was formed by the penetration of resin into the porous enamel which consists partly of enamel and partly of resin, and it was also named the hybrid layer, concluding that hybrid layers do not need to contain collagen fibrils.[85] Therefore, we have chosen to refer to it as a hybrid layer throughout our whole study.

This study was conducted on ground enamel. Grounding aimed mainly at creating a flat surface as well as at removing the impervious aprismatic layer, hence enhancing the

penetration of the acidic monomer. There are several studies that compared ground and unground enamel behaviour during bonding in primary teeth, among which were studies that discovered that ground enamel was a better substrate for effective bonding of SE adhesives [104], and others that showed that 37% phosphoric acid etching had the same effect on both ground and intact enamel [90]. In a study evaluating self-etching priming agents in relation to their etching effect when applied to ground and intact enamel under SEM, it was found out that a more extensive adhesive penetration was apparent into ground enamel, forming a 2 μm thick hybrid layer. Although the correlation between morphological aspects and bond strength values is difficult, it was claimed that the bond strength achieved on intact enamel using mild self-etching systems (e.g. Clearfil SE Bond), is relatively low (approximately 11-20 MPa) when compared to ground enamel. [41]

6.1.6 Application of the tested materials

Since manufacturers of universal adhesives claim that they can be used in all application modes (self-etch, etch-and-rinse and selective-etch) [44], this study focused on the effect of the application mode and the phosphoric acid etching time in relation to μ-TBS. Passive application of the adhesive was chosen for all groups of this study, yet it is not recommended in clinical settings since, active rubbing helps evaporate the water solvent and enhances the penetration of acidic monomers into enamel and dentin increasing the substrate/adhesive interaction, hence improving demineralization and increasing bond strength. [64]

Active rubbing of the bonding agent was excluded following a pilot study that the authors performed to the baseline group (SU). The study aimed to compare the μ-TBS values as well as the etching patterns of active vs. passive application of the adhesive in the self-etch mode (SG1). SEM images of active application of the adhesive did not provide a satisfactory etching pattern and μ-TBS values of active versus passive application were not significantly different from each other (15 and 12 MPa respectively; $p = 1.000$). Additionally, the active rubbing group showed higher standard deviation, which was claimed to be due to lack of standardisation of hand pressure during its application. A study evaluated 3 self-etching adhesives in regards to their shear bond strength on dentin and enamel with and without agitation at 3 different application times in vitro. And, it was shown that active application

did not improve the enamel bond strength for any of the materials tested [117]. The self-etch group was also added passively, to act as a negative control for the other two groups.

6.1.7 Microtensile bond strength (μ-TBS) test

Internal calibration was done to make sure that the operator can master the application techniques of etching, bonding, and composite application, and can use the instruments and materials available in the laboratory, thus preventing the effect of systematic errors on the process. During the internal calibration process SD was kept constant between 35-50% and parameters were accordingly enhanced.

The alternation of the fixing materials between flowable compomer and SU adhesive during fixation of sticks for μ-TBS testing was meant to eliminate the dropouts resulting from debonding of the sticks before the test was completed.

During the calibration process, the standard deviation between sticks of the same tooth was kept lower than 50% of the mean value, yet this was not adopted as a criterion in the main experiment since groups which were etched for 30s and 15s were compliant with this standard, while groups with no etching showed higher SD, due to increase ptf, which were given a zero value.

Bond strength evaluation methods should be repeatable, easy to perform, and should provide predictable clinical outcomes. Microshear bond strength test was recommended for brittle structures such as glass ionomer cement and enamel based on the fact, that it generates less damage during specimen preparation. Yet, the tensile stresses generated within, are responsible for crack initiation and a measured bond strength value less than the true one due to non-uniform stress distribution. [94] Therefore, the authors have chosen the μ-TBS testing method of this study based on the recommendation of Armstrong et al, who considered the μ-TBS for testing dental composites on both dentin and enamel the best surrogate method [10]. Technique sensitivity of the μ-TBS test was avoided through the following steps: 1- choosing to cut our specimens in the form of sticks, since they present less stress distribution when compared to the hour glass appearance [97], 2- choosing a suitable specimen size (0.7 x 0.7 mm), 3- limiting the stresses resulting from the specimen preparation, through using a low speed in the microtome, 4- setting strict exclusion criteria for specimens with morphological defects resulting from manipulation errors, 5- adjusting the test speed to

1mm/min crosshead speed, which was reportedly recommended because of a more uniform stress-time pattern. [94]

6.1.8 Aging methods

As per Armstrong et al., aging can be classified into short term (< 1 month), medium term (1–6 months) and long-term (6 months or longer). This study has chosen to perform the long-term aging for 6 months. [10]

During the aging process it was chosen to section the specimens into rods after the 6 months incubation period and not before storage, since water storage of the rods can cause hydrolytic degradation, which in turn increase the aging effects on the adhesive area creating conditions that don't mimic the clinical situation. [63]

Distilled water was chosen as our storage medium based on a study by Gomes et al., which tested four different aging protocols (Distilled water, Artificial saliva, Thermocycling, and Citric acid) on the effect of μ-TBS of different adhesives on dentin. The study suggested that storing samples for 180 days in distilled water or artificial saliva did not promote a pronounced aggression of the bond interface. (35) Furthermore, our samples were stored in an incubator in a temperature of 37˚C to mimic the oral conditions.

The storage solution was not changed in phase II due to the fear of contamination of the samples and further premature failures resulting from temperature changes during refreshing the solution. Similarly, a study that tested the effect of different storage media and the regular change of the storage solution on the μ-SBS of resin cements to dentin found that changing the storage medium induced a calcium loss from dentin, collagen exposure, and decreased bond strength. Therefore, it was recommended to leave the storage solution unchanged. [49]

6.2 Discussion of results

6.2.1 Microtensile bond strength (μ-TBS) test

24 hours

Selective etching of enamel with phosphoric acid was proven to increase the μ-TBS values significantly. [9, 30, 72, 91] Similarly, our study showed a significant difference in all tested adhesives between the subgroups etched with phosphoric acid prior to the UAs

application and subgroups that were self-etched in both phases regardless of the etching time ($p < 0.001$). This can be explained by the fact that enamel bonding, on contrary to dentin and against the claims of the manufacturers, still relies mainly on the micromechanical retention of the resin in the enamel porosities rather than the chemical interaction. Those microporosities result mainly from the dissolution of the hydroxyapatite crystals, leading to increase in the surface area of the substrate, thus forming prism-like resin tags after polymerization. Additionally, etching increases the surface energy of enamel, hence providing better wettability of the substrate. [30]

Although, iBU (pH = 1.6-1.8) is an intermediately strong adhesive with strong acidic potentials when compared to SU (pH = 2.7) and CU (pH = 2.3), it presented only a significant difference in the self-etch mode with CU (Sidak, $p = 0.029$) and not with SU, indicating that selective acid etching of enamel prior to UAs application is a must regardless the pH value of the adhesive used. [30, 70, 93, 95]

The authors claim that the first reason why iBU showed μ-TBS values lower than expected was the mix between the 10-MDP functional monomers with 4-META, decreasing the weight % of 10-MDP monomers.[3, 124] A study by Nikaido et al., tested the acid base resistant zone (ABRZ) in both enamel and dentin after application of 3 functional monomers namely 10-MDP, 4-META, and 3D-SR, and they found out that ABRZ formed from 10-MDP was thicker than the other two monomers and that among the three monomers used, a funnel shaped erosion at the junction of the bonding interface between enamel and the outer demineralized layer was observed only with 4-META.[81]

Furthermore, the combined effect of the acidic pH of iBU and the phosphoric acid etch in subgroups B (30s) &C (15s) did not show any increase in the μ-TBS results, since the etching capacity cannot fully explain the mechanism of adhesion of the monomers to the HAp and is influenced by the dissolution rate of the calcium salts formed with each acidic monomer in the acid solution. [124] This is believed to be the second reason of iBU's lower results. SU and CU like most of the adhesives nowadays are methacrylate resin-based adhesives, which employ the combination of 2-bis[4-(2-hydroxy3-methacryl-oxypropoxy)-phenyl]-propane (Bis-GMA) as a base monomer and the low-viscosity 2-hydroxyethyl-methacrylate (HEMA) as a diluent monomer to enhance the handling properties of the adhesives and their dentin infiltration. This Bis-GMA/HEMA combination had several disadvantages, among which is the high-water sorption of the

hydrophilic HEMA leading to failure in the adhesive/tooth interface and subsequently decreasing the bond durability, also, health problems associated with the leach of unreacted HEMA and degradation products of Bis-GMA such as bisphenol A were reported. The above-mentioned concerns lead to the replacement of this combination in the iBU with a UDMA/TEGDMA hydrophobic resin network which was thought to be less likely to absorb water, decreasing the hydrolysis of collagen at the composite/tooth interface and increasing the durability of the restoration. [122] Yet, it was proven that as the fraction of the TEGDMA increased, the mean shear bond strength on dentin seemed to decrease [84]. Unfortunately, there was no similar studies conducted on enamel to know if this was the reason why iBU showed less than expected results. Added to that, a study has tested the effect of different concentrations of HEMA monomer alone on the μ-TBS of composite to enamel and dentin, and it found out that it had no significant effect on the bond strength of enamel, yet its effect on dentin bond strength was pronounced due to its hydrophilic wetting effect. [58]

The last compositional difference in iBU and the reason secondly responsible for our results according to the authors, is its solvent. iBU used acetone/water combination instead of ethanol/water in both SU and CU. A study evaluated different solvents (ethanol alone, acetone, and ethanol/water) effects on the shear bond strength of composite to dry and wet enamel and they concluded that adhesives with ethanol/water-based solvent showed higher bond strength values, followed by acetone then ethanol on dry enamel. On the other hand, acetone-based adhesives exhibited better performance when applied on wet enamel, which indicates that the acetone based iBU solvent was a weak point in the adhesion in this study as our enamel surface was always dry before the adhesive application. [116]

The use of phosphoric acid etching should modify the enamel surface to enhance microporosities without changing the substrates morphologies. Therefore, the idea of shortening the etching time was introduced in research [48]. Our results for comparing the phosphoric acid etching time of the tested adhesives conformed to the results of one study which compared the effect of etching acid concentrations and timing on the tensile bond strength of enamel of primary teeth and they observed that, both factors didn't affect their values. [37] This phenomenon can be explained by similar etching depths irrespective of the etching time and hence similar resin tag lengths. [48] It is recommended for paediatric patients to use the least phosphoric acid etching time that produces a satisfactory bond strength to limit the chair time. However more research regarding the effect of shorter etching time on the microhardness, marginal leakage, resin tags length and other

mechanical properties is required to reach a conclusion regarding the optimal etching time for primary enamel.

The effect of active rubbing of the adhesive agent on dentin was proven to improve both immediate and aged bond strength, since it helps the impregnation of monomers inside the smear layer, hence decreasing the degradation of the hybrid layer and enhancing the adhesive interface quality.[40] Yet in our study, its effect on enamel was found to yield results similar to the self-etch group, indicating that active rubbing alone is not a sufficient adhesive strategy to be adopted when bonding to enamel. Therefore, our third null hypothesis was rejected.

6-months

Long-term bonding stability is considered a desirable clinical success measure when compared to immediate bond strength values [30]. In this study, aging for 6 months in distilled water showed a decrease on the μ-TBS values of all subgroups in all adhesives, yet this was only significant in SU when etched for 15 s. These results contradict, the results of a study by Makishi et al., which found out that the aging process had affected the bond strength results significantly for both dentin and etched enamel [67]. Yet, both studies can hardly be compared due to differences in aging time and storage conditions. Also, it contradicts the results of a systematic review and meta-analysis, which showed that aging of mild universal adhesives, irrespective of their application technique, had no influence on the μ-TBS values owing to their strong bonding that can seal off the water diffusion pathway between the tooth and the restoration thereby limiting hydrolysis and degradation of the components [30].

Yet, our results support the results of a study which tested the effect of 12 months aging in deionized water of 15 s phosphoric acid etched and non-etched enamel on the μ-TBS of SU adhesive to composite resin. The results of this study showed that the bond strength values decreased significantly after aging. As seen in (**Table 1**, page 19), SU consists of 10-MDP, 2-hydroxyethyl methacrylate (HEMA) and Vitrebond copolymer (VCP) (a polyalkenoic acid copolymer). The chemical interaction between MDP and the calcium, released from the partial dissolution of the hydroxyapatite crystals, form a water-stable nanolayered MDP-Ca salt structure which favours the adhesive stability. However, HEMA slows the production of this salt in the enamel and VCP bonds to calcium of the hydroxyapatite crystals competing with the last two, therefore, compromising the

nanolayered structures, impairing the resistance to biodegradation, and decreasing the bond stability. [119] Therefore, it was assumed that the results were material dependant, since particular structures and concentrations of different functional monomers can result in different adhesive interface and different bond effectiveness. However, it is recommended to undergo SEM studies for SU adhesive after aging to study the nature of bond degradation and microleakage.

Controversies in the bond strength results between the studies can be attributed to factors such as age and type of the tested tooth, bonded surface area, degree of mineralization, bond strength test used, storage medium, the operator's skills/knowledge, and the testing conditions. [86]

6.2.2 Analysis of failure patterns

In phase I, failure modes of all adhesives were mainly adhesive followed by a mixed failure in the enamel-adhesive interface, while after aging there was a shift of the failure modes to preferentially mixed fractures in enamel-adhesive interface. This can be due to hydrolysis of the filler matrix interface of the resin due to reduction in the frictional forces between the polymer chains, which may lead to decreasing the mechanical properties, as well as shifting the failures to mixed failures in composite resin-adhesive-enamel interface as well as cohesive failures in composite.[73] The fact that premature failures of both phases occurred only when the adhesives were used in the self-etching mode (SGA) supports the importance of selective acid etching of enamel to achieve better bond stability.

Furthermore, the increase in the pre-test failures after aging is a thorough indication of bond degradation during storage, disregarding the μ-TBS values.

6.2.3 Scanning electron microscopic analysis

The challenge of bonding to enamel is determined by the imperviousness of enamel to acid attack. Thus, the inability of some adhesive systems to produce satisfactory and durable bonding to enamel may be attributed to their inability to produce an acidic environment. [41] Bearing in mind that little is known about the etching patterns of enamel produced on primary teeth with different application modes, our study attempted to study this through three different methods. (4.2.7, page 32)

It was suggested that both the crystallites orientation in relation to the acid-attack direction, as well as the compositional differences between the peripheral and central enamel prisms' parts can contribute to the different etching patterns produced with phosphoric acid etching. [106]

SEM samples in this study were exemplary samples to understand the effect of different application modes on the etching pattern, yet a clear correlation between a definite etching pattern and the microtensile bond strength values cannot be concluded here.

Examination of the enamel-resin interface of groups treated with selective-etching under SEM showed well-formed resin tags, a thick hybrid layer, and confirmed the deep demineralization of the enamel surface seen in the indirect etching pattern images. The main reason for this is the high acidity of phosphoric acid combined with the acidity produced by the low pH value of the adhesives themselves, irrelevant of whether the adhesive mild or strongly acidic, [99] while groups not treated with phosphoric acid showed scarce resin tags and low to no enamel demineralization. One suggestion to this may be that the adhesives themselves interact superficially with enamel creating a lower potential for micromechanical interlocking when compared to phosphoric acid despite their acidity. [41]

Hanning et al., found out that the remaining residues of primers of self-etch adhesive systems as well as calcium phosphate resulting from phosphoric acid etching on enamel surface can mask the alterations on the surface produced by the acid. That's why it was recommended, to indirectly observe the etching pattern remaining on the resin production after complete dissolution of enamel. [38] So, it is to be concluded that, more studies are needed to link the SEM analysis with bond strength values of the different adhesives.

6.2.4 Discussion of null hypotheses (H_o)

Null hypothesis 1: Selective acid etching has no effect on the μ-TBS of universal adhesives to enamel of primary teeth.

The first null hypothesis was rejected for all the tested adhesives, since all adhesives have shown a significant difference in the μ-TBS values between subgroups that were self-etched and subgroups that were selectively etched irrespective to the etching time (LMM, REML, $p < 0.001$).

Null hypothesis 2: Acid etching time has no effect on the μ-TBS of universal adhesives to enamel of primary teeth.

The second null hypothesis was accepted, since it was proven in this study that etching times of 15 s and 30 s did not affect the μ-TBS values within the same adhesive. The difference between 15 s and 30 s selective etching of all adhesives was not significant, it was (Sidak, $p < 0.576$) in SU, (Sidak, $p = 0.692$) in CU, and (Sidak, $p < 1.000$) in iBU, therefore 15 s etching time was recommended by the study group, to decrease the chair time in Paediatric patients, thus increasing their cooperation and compliance.

Null hypothesis 3: Active application of the universal adhesive has no effect on the μ-TBS to enamel of primary teeth.

This null hypothesis was accepted. Active application of the adhesive was tested only for the SU adhesive, due to limited number of teeth, hence this hypothesis can't be generalized to all other adhesives. This application mode has shown no significant difference for the SU adhesive in comparison to the subgroup which was self-etched and applied passively (Sidak, $p < 1.000$).

Null hypothesis 4: Artificial aging of primary enamel treated with acid etching in different etching times for 6 months in an aqueous solution has no effect on its μ-TBS.

The fourth null hypothesis was partially rejected since aging for 6 months in distilled water had no significant effect on the μ-TBS values of all subgroups of all tested adhesives except the SU when etched for 15 s (Sidak, $p = 0.014$).

6.3 Recommendations

6.3.1 Strengths of the study

Among the strengths of this study, is that it was conducted on sound human primary molars to mimic the situation in the oral cavity. It is one of the few studies that has evaluated bonding of UAs to enamel rather than dentin, added to the aging effect on the bond strength of UAs on primary enamel. In this study, we have studied the effect of different application modes not only on the bond strength values but also on the etching pattern and on the nature of the adhesive bond using SEM.

6.3.2 Limitations of the study

The main limitation of this study was the low number of extracted primary molars gathered which led to the inability to implement the split tooth technique, prevented the testing of all subgroups in phase II, and led to the examination of only exemplary samples under the SEM preventing the correlation between the histological appearance and the bond strength value. Added to that, proper block randomization could not be implemented here due to the increased number of drop outs on the tooth level.

6.3.3 Implications for future research

From our observations we recommend the conduction of more clinical studies testing the effect of different etching times, use of various etching acids, and application protocols of universal adhesives to primary enamel. More laboratory studies are needed to measure the μ-TBS of other universal adhesives available in the market in different application modes on primary enamel. As well as, more SEM studies to examine the relationship between different etching patterns and the bond strength values of universal adhesives to composite on enamel.

6.4 Conclusions

From the results of this study, it is to be concluded that:

1. Etching with phosphoric acid remains the gold standard for bonding universal adhesives to primary enamel.
2. Etching time showed no significant effect on μ-TBS of universal adhesives to primary enamel.
3. Artificial aging for 6 months in distilled water can affect the μ-TBS values, which was only shown for SU in 15 s etching mode in this study.

7 SUMMARY

The increased demand for simple and efficient adhesive systems made current multimode universal adhesives of great interest in pediatric dentistry. These are mainly characterized by a reduction in the number of application steps, hence limiting the duration of treatment during restorative procedures, together with achieving good dentin adhesion. However, application protocols for universal adhesives have not been defined precisely for primary enamel. Furthermore, the effect of aging was ignored, which lead to reporting higher bond strength values than the real ones due to overlooking the effect of factors, such as thermal stresses and normal daily functions on the bond strength.

Thus, the aim of this study was to evaluate the effect of selective acid etching, different etching times, and active rubbing of universal adhesives on the microtensile bond strength to the enamel of primary teeth and to determine the stability of selective acid etching after 6 months aging in an aqueous solution.

Sound human primary molars were sectioned mesiodistally. Tooth halves (n=113) were randomized into 3 groups based on the adhesive used: SU: Scotchbond Universal (3M); CU: Clearfil Universal Bond Quick (Kuraray Noritake); iBU: iBond Universal (Heraeus Kulzer). Aprismatic enamel was removed, then groups were subdivided according to application mode into 4 subgroups (SG): SGA: self-etch mode, SGB: 30s-selective etching, SGC: 15s-selective etching, SGD: active rubbing of the adhesive. Adhesives and composite were added (Filtek Z250, 3M). Samples were incubated in distilled water at +37°C for 24h, while separate samples for SGA& SGC were aged for 6 months. Specimens were dissected into rods (0.7 mm × 0.7 mm; IsoMet Highspeed Pro, Buehler), then tested for microtensile bond strength (μTBS; TC-550, Syndicad). Failure patterns were evaluated under light microscope (AZ100M, Nikon). Data were analysed with linear mixed effects model (restricted maximum likelihood, REML) and Sidak post-hoc-tests using SPSS 26.0 (Statistical Packages for Social Sciences, IBM Statistics, Armonk, NY, USA) with significance level set at $\alpha < 0.05$.

Our results showed that selective-etching increased μ-TBS of universal adhesives to primary enamel ($p < 0.001$) without significance between enamel etching for 15 s and 30 s ($p > 0.05$). Six-month aging significantly reduced the μ-TBS of SU in SGC ($p = 0.014$) compared to μ-TBS after 24h incubation.

So, it was concluded that, selective etching with phosphoric acid remains the gold standard for bonding universal adhesives to primary enamel. Etching time showed no significant effect on μ-TBS. Aging may affect the μ-TBS of SU applied on 15 s phosphoric acid etched primary enamel.

8 ZUSAMMENFASSUNG

Aufgrund der steigenden Nachfrage nach einfachen und effektiven Adhäsivsystemen für die Kinderzahnheilkunde sind auch die aktuellen multimodalen Universaladhäsive von Interesse. Diese sind unter anderem durch eine Reduktion der Applikationsschritte bei gleichzeitig guter Dentinhaftung gekennzeichnet. Die verkürzte Behandlungszeit hat den Vorteil der Zeitersparnis während der Füllungstherapie. Die Verwendungsprotokolle der Universaladhäsive sind jedoch für den Milchzahnschmelz nicht exakt definiert. Außerdem wurden die Auswirkungen der Alterung bisher nicht berücksichtigt, was dazu führte, dass die Haftfestigkeitswerte höher als die tatsächlichen Werte angegeben wurden, weil die Auswirkungen von Faktoren wie thermische Belastungen und normale tägliche Funktionen auf die Haftfestigkeit dabei nicht berücksichtigt wurden.

Ziel war es daher, die Wirkung des selektiven Säureätzens, verschiedener Ätzzeiten und des aktiven Reibens von Universaladhäsiven auf die Mikrozugfestigkeit auf dem Zahnschmelz von Milchzähnen zu untersuchen und die Stabilität des selektiven Säureätzens 6-monatiger Alterung in einer wässrigen Lösung zu bestimmen.

Extrahierte, mindestens einflächig kariesfreie Milchmolaren wurden in mesio-distaler Richtung geteilt. Die Zahnhälften (n = 113) wurden je nach Art des verwendeten Adhäsivs zufällig in 3 Gruppen eingeteilt und mit folgenden Adhäsiven behandelt: SU: Scotchbond Universal (3M™); CU: Clearfil Universal Bond Quick (Kuraray Noritake Dental); iBU: iBond Universal (Heraeus Kulzer). Der prismenlose Schmelz wurde mit einem Feinkorndiamanten abgetragen. Die Gruppen wurden je nach Applikationsmodus des adhäsives in 4 Untergruppen (UG) unterteilt: UGA: Self-Etch-Modus, UGB: 30s-selektives Ätzen, UGC: 15s-selektives Ätzen, UGD: aktives Reiben des Adhäsiven. Anschließend wurde das Adhäsiv aufgetragen, polymerisiert und Komposit (Filtek™ Z250, 3M™) aufgeschichtet. Die Proben wurden 24 h bei 37°C in Aqua dest. gelagert, während separate Proben für UGA und UGC 6 Monate lang gealtert wurden. Proben wurden dann gesägt (0,7 mm × 0,7 mm; IsoMet Highspeed Pro, Bühler) und gezogen (µ-Tensile Bond Strength [µ-TBS]; TC-550, Syndicad). Die Bruchanalyse wurde mit einem Lichtmikroskop (AZ100M, Nikon) unter vierfacher Vergrößerung durchgeführt. Mittels linearen gemischten Modells (Restricted Maximum Likelihood, REML) und post-hoc-Tests wurden die Daten auf statistisch signifikante Unterschiede ($\alpha < 0,05$) mithilfe von SPSS Statistics 26.0 (Statistical Packages for Social Sciences, IBM Statistics, Armonk, NY, USA) überprüft.

Unsere Ergebnisse zeigten, dass die Phosphorsäurekonditionierung die Verbundfestigkeit zwischen Milchzahnschmelz und Komposit für alle untersuchten Universaladhäsive signifikant erhöhte ($p < 0{,}001$). Der µ-TBS-Wert unterschied sich nach 15 s Ätzen bei allen Gruppen nicht im Vergleich zur Ätzzeit von 30 s ($p > 0{,}05$). Die sechsmonatige Alterung reduzierte die µ-TBS von SU in SGC signifikant ($p < 0{,}014$) im Vergleich zur µ-TBS nach 24 h Inkubation.

Daraus wurde gefolgert, dass die Phosphorsäurekonditionierung der Goldstandard für die Schmelzkonditionierung bei Milchmolaren bleibt, wobei eine Ätzzeit von 15 s beziehungsweise 30 s die Verbundfestigkeit nicht nachteilig beeinflusst. Die Alterung kann die µ-TBS von SU, wenn dieses auf für 15 s geätzten Primärschmelz aufgetragen wird, beeinträchtigen.

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10 APPENDIX

10.1 Abbreviations

µ-SBS	**:**	Microshear bond strength
µ-TBS	**:**	Microtensile bond strength
10-MDP	**:**	10- methacryloyloxydecyl-dihydrogen-phosphate
ABRZ	**:**	Acid base resistance zone
ANOVA	**:**	One-way analysis of variance
ART	**:**	Atraumatic restorative technique
Bis-GMA	**:**	Bisphenol A- glycidylmethacrylate
BPA	**:**	Bisphenol A
CI	**:**	Confidence interval
CU	**:**	Clearfil Universal Bond Quick
ECC	**:**	Early childhood caries
EDTA	**:**	Ethylenediaminetetraacetic acid
ER	**:**	Etch and rinse
FM	**:**	Florescence microscope
HAp	**:**	Hydroxyapatite
HEMA	**:**	2-Hydroxyethyl Methacrylate
iBU	**:**	iBond Universal
LM	**:**	Light microscope
LMM	**:**	Linear mixed models
MID	**:**	Minimal invasive dentistry
NRCT	**:**	Non-restorative cavity treatment
REML	**:**	Restricted maximum likelihood
SE	**:**	Self-etch
S-ECC	**:**	Severe early childhood caries
SEM	**:**	Scanning electron microscope
SPSS	**:**	Statistical Packages for Social Sciences
SU	**:**	Scotchbond Universal
TEM	**:**	Transmission electron microscope
UA	**:**	Universal adhesive

10.2 List of Tables

10.3 List of figures

10.4 Publications

The results of the study were published as follows:

10.4.1 Scientific papers

i. Short communication: Hamdy D, Amend S, Lücker S, Krämer N (2023) Effect of conditioning on microtensile bond strength of universal adhesives to primary enamel. OP & KzH 45:20-22· https://doi.org/10.1007/s44190-023-0640-y.

ii. Hamdy D, Amend S, Lücker S, Frankenberger R, Krämer N (2024) Effect of Application Mode and Aging on Microtensile Bond Strength of Universal Adhesives to Enamel of Primary Teeth. IJPD 0:1-14. https://doi.org/10.1111/ipd.13293.

10.4.2 Scientific award

The Award of the best poster at the 29. DGKiZ-annual conference (Hamburg, 22-24 September 2022).

10.4.3 Posters and presentations

i. A poster at the 29. DGKiZ-annual conference (Hamburg, 22-24 September 2022).

ii. An oral presentation at the JLU-Career-Retreat, Faculty of Medicine, Justus-Liebig-University, Giessen (Giessen, 25 October 2022).

iii. A poster at the Science Day, Faculty of Medicine, Justus-Liebig-University, Giessen (Giessen, 11 November 2022).

iv. A poster at the conference of the American Academy of Pediatric Dentistry (AAPD), USA (Orlando, Florida, 23 May 2023).

10.5 Scholarships

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11 EHRENWÖRTLICHE ERKLÄRUNG

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_____ _____

Ort, Datum Unterschrift

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