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Effect of Chlorhexidine and/or ethanol pre-bonding treatment on the shear bond strength of resin composite to dentin

(A Comparative in Vitro Study)

A Thesis
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Dedication

To my dear mother and the spirit of my father

To my wonderful wife who supported me during my study

To my lovely daughter who made my life happy and full of fun

To my supervisor, friend and brother dr. Abdulla Alshamma

Ahmed

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Abstract

Deterioration at the dentin-composite interface is a major defect in the tooth coloured restorative dentistry. This study was conducted to evaluate the effect of 2% chlorhexidine, 100% ethanol or both of them on the shear bond strength of composite to dentin when tested after 24 hours, 3 months and 6 months of aging.

Ninety-six sound human maxillary premolars were included in this study. Teeth roots were embedded in acrylic blocks and the occlusal surfaces were cut horizontally to gain a flat dentin surface free of enamel. Teeth were randomly assigned into four groups of 24 teeth according to the adhesive procedure as follows: Group A (the control group), in which teeth were etched, rinsed, blot dried, bonded and restored with composite (Filtek Z 350). Group B, in which teeth were etched, rinsed, blot dried, treated with 2% CHX for 60 seconds, blot dried, bonded and restored with composite. Group C, in which teeth were etched, rinsed, blot dried, treated with 100 % ethanol for 60 seconds, blot dried, bonded and restored with composite, and Group D, in which teeth were etched, rinsed, blot dried, treated with 2% CHX for 60 seconds, blot dried, treated with 100% ethanol for 60 seconds, blot dried, bonded and restored with composite.

Each group was further subdivided according to the time of SBS testing into three subgroups (n=8): subgroup 1 was tested after 24 hrs, subgroup 2 was tested after 3 months and subgroup 3 tested after 6 months of aging.

Composite resin application was done using a specially designed and fabricated teflon mold. All samples had been stored in distilled water till testing for shear bond strength using universal testing machine at cross head speed 1mm/min.

The gathered data were statistically analysed by one-way ANOVA test and Tukey’s test. ANOVA test results showed non-significant difference among groups after 24 hrs and 3 months with highly significant difference only after 6 months. Tukey’s test showed non-significant difference among treated groups B, C and D but with highly significant difference between group A and other groups. ANOVA tests results also showed non-significant difference within each group except for group A where highly significant difference was found, Tukey’s test showed non-significant difference between subgroup A1 and A2, a significant difference was found between A2 and A3 and highly significant difference was found between A1 and A3.

Concerning the mode of failure, all of the groups tested after 24 hours showed predominantly mixed failure. However, the percentage of adhesive failure increased over time with different levels among groups. Adhesive failures were highest in group A and lowest in group D after 6 months.

In conclusion, the control group showed highly significant reduction in SBS values, while 2% CHX or 100% ethanol were able to improve the durability of the bond after six months. However, no significant difference was found when CHX and ethanol were used separately or together raising question about their ability to act synergistically to improve the durability of the bond.

List of Contents

List of Tables

List of Figures

List of Abbreviations

Symbols	Abbreviation
µm	micrometer
BisEMA	Ethoxylated Bis-Phenol A methacrylate
Bis-GMA	Bisphenol A glycol dimethacrylate
S	Significant
NS	Non-significant
HS	Highly-significant
CEJ	Cementoenamel Junction
CHX	Chlorhexidine
DEJ	Dentinoenamel junction
HEMA	Hydroxyethyl methacrylate
MMPs	Matrix metalloproteinase
MPa	Megapascal
SBS	Shear bond strength
TEGDMA	Triethylene glycol dimethacrylate
UDMA	Urethane dimethacrylate

List of Appendices

Introduction

Introduction

Composite is the material of choice in direct esthetic dentistry because of its high esthetic quality that can mimic numerous shades and in different translucencies, conservative tooth preparation and reinforcement of remaining tooth structure due to adhesion (Ferracane, 2011). Despite the advancement that eliminate many of composite disadvantages; composite still has some shortcomings like polymerization shrinkage, technique sensitivity and lack of durable bond (Spencer *et al.*, 2014).

In adhesive dentinal bonding agents with etch and rinse technique, the minerals of dentin are removed leaving the organic matrix exposed which should be infiltrated completely with resin to form the hybrid layer after polymerization. Hybrid layer anchors firmly to dentin and adhere to the subsequent layer of composite (Tjaderhane *et al.*, 2012).

In reality, resin monomer doesn’t infiltrate the exposed organic matrix completely leaving the deepest part of demineralized matrix partially or totally exposed and subjected to nanoleakage later (Pashley *et al.*, 2011b).

Enzymatic degradation of the organic matrix by matrix metalloproteinases (MMPs) together with the hydrolytic degradation of the hydrophilic resin components in the hybrid layer leads to the deterioration of the hybrid layer and the failure of the composite restoration later (Breschi *et al.*, 2008).

Many strategies were suggested to counteract the degradation in the hybrid layer to improve the lifespan of the restorations. One of these strategies was the use of different materials that act as MMP inhibitors like chlorhexidine, EDTA (ethylene diamine tetra acetic acid), Galardin, tetracycline and green tea extract (Pashley *et al.*, 2004).

The study of Tekce *et al.* (2016) showed that the use of chlorhexidine as an intermediate agent between etching and bonding were able to reduce the bond strength reduction of composite to dentin using microtensile test after one year of storage.

Another strategy focuses on improving the infiltration of resin monomer into demineralized dentin for coating exposed collagen fibrils more efficiently thus reducing the nanoleakage and the hydrolysis of the hybrid layer, an example for this strategy is the use of ethanol after dentin demineralization. Ethanol is a solvent for hydrophilic and hydrophobic resin monomers that is able to expel water remnant from dentin and keeping collagen network distended to provide an easier path for resin infiltration (Pashley *et al.*, 2007; Hosaka *et al.*, 2009).

Aim of the study

The aim of this study was to evaluate effect of Chlorhexidine and/or ethanol pre-bonding treatment on the shear bond strength of resin composite to dentin using etch and rinse adhesive system after 24 hrs, 3 months and 6 months of aging.

Chapter One

Review of Literature

Review of literature

1.1 Dentin

The dentin is a mineralized connective tissue that makes the major portion of the tooth structure. Dentin is a vital tissue, continues to change through the life by physiological and pathological stimulus (Goldberg *et al.*, 2011).

Dentin provides support for enamel and act as shock absorber as dentin is tougher and can dissipate forces better than enamel which is hard but brittle. Dentin has also an important role in pulp protection from external stimuli (Eick *et al.*, 1997).

Dentin is composed of 50% by volume of inorganic material, 30% organic matrix and the remaining is water. The organic matrix is formed almost of type I collagen fibrils that constitutes 90% by volume and only 10% of non-collagenous protein and these collagen fibrils are arranged to form a mesh for the crystals of the inorganic material. Dentin is less mineralized than enamel but more mineralized than bone and cementum. Dentin minerals are mainly hydroxyapatite crystallites having a length of 20-100nm and a width of 3nm similar in dimensions to the hydroxyapatite crystallites found in cementum and bone but smaller than that in enamel (Teruel Jde *et al.*, 2015).

Dentin is slightly yellow and becomes darker with age, harder than bone and cementum but softer than enamel. Dentin hardness near the pulp is one third of its hardness near the dentinoenamel junction (DEJ). Dentin becomes harder with aging due to mineral deposition, even though it is flexible with a modulus of elasticity about 18 gigapascals (MPa) (Goldberg *et al.*, 2011).

1.1.1 Structure of dentin

1.1.1.1 Dentinal tubules

Dentinal tubules are small canals that run in a wavy pattern across the whole thickness of dentin from the pulp towards the DEJ in the crown and to the cementodentinal junction in the root (Eick *et al.*, 1997).

Each tubule contains a cytoplasmic process of the odontoblastic cell. The diameter of the dentinal tubule is about 0.63µm near the DEJ and increases towards the pulp to reach 2.37µm near the pulp. The tubules are sparsely distributed near the DEJ and densely packed near the pulp and their numbers range from 15000-20000/mm^2 near the DEJ to 45000-65000/mm^2 near the pulp (Trowbridge *et al.*, 2002).

The increased number of tubules and the diameter of the tubules in approximation to the pulp increases the dentin permeability as we go deeper in dentin and the remaining dentin is not as an effective barrier as it is close to the DEJ (Kinney *et al.*, 2003).

1.1.1.2 Peritubular and intertubular dentin

Peritubular dentin is the dentin that lines the tubule and forms its wall and surrounded by the intertubular dentin, the intertubular dentin forms the greatest portion of the dentin and lies between the tubules (Goldberg *et al.*, 2011).

1.1.1.3 Predentin

The odontoblast firstly forms predentin, which consists of organic matrix only and undergoes mineralization later, it lies close to the pulp and its width is about 2 µm or more depending on the activity of the odontoblast (Sturdevant, 2014).

1.1.2 Types of dentin

According to Bhaskar (1997) there are three types of dentin; primary, secondary and tertiary that form in different developmental stages.

1.1.2.1 Primary dentin

Primary dentin forms the primary shape of the tooth and it is completed three years after tooth eruption. It starts from the point of the future cusp tip or the incisal edge and continue until complete root formation in the case of permanent teeth (Bhaskar, 1997).

1.1.2.2 Secondary dentin

After the formation of primary dentin, secondary dentin starts to form throughout the life in a very slow manner even without an external stimulus. Dentinal tubules are less regular in secondary dentin but usually continuous with those of the primary dentin. Secondary dentin deposits on the periphery of the pulp but in multi-rooted teeth they tend to deposit on the floor and roof rather than the walls (Bhaskar, 1997).

1.1.2.3 Tertiary dentin

When the tooth is subjected to a harmful stimulus like caries or abrasion, odontoblast forms tertiary dentin as a reparative process in response to this stimulus and it is characterized by fewer dentinal tubules with more twisted form (Bhaskar, 1997).

1.1.3 Collagen fibrils

Collagen is family of ten correlated proteins, type I collagen is the primary type that predominates in dentin. The fibril’s length is 300nm consists of three polypeptide chains each one of 1056 amino acid units (Prati *et al.*, 1999).

Collagen fibrils form a complex 3-dimensional network that become mineralized by nanoscopic apatite crystallites during the process of dentinogenesis (Veis, 2003)**.**

Dentinal collagen does not metabolically turn over, meaning that it is not easily degraded, but once it is, it cannot be replaced (Breschi *et al.*, 2018)**.**

1.1.4 Smear layer

After tooth preparation, a layer of debris is formed on the dentin surface and termed “smear layer”. Smear layer may vary in its thickness, roughness or adhesion to the dentin depending on the cutting procedure of the dentin. This layer plays an important role in adhesive dentistry because it must be removed or modified to achieve a successful bond between the dentin and the restoration (Oliveira *et al.*, 2004).

Smear layer thickness is 1-10 µm and consists of two zones, the inner zone has a disordered crystalline arrangement while the outer zone contains destructed collagen fibrils and appetite crystallites formed during mechanical cutting and the heat of friction during the preparation (Pashley and Carvalho, 1997).

1.2 Adhesive systems

Adhesive systems can be considered revolutionary in many aspects of conservative dentistry, making possible previously inconceivable clinical maneuvers. Current adhesive systems allow clinicians to bond to tooth structure without the need of a retentive cavity since they provide immediate bond strength (Breschi *et al.*, 2018)**.**

1.2.1 Classification of adhesive systems

1.2.1.1 Three step etch and rinse adhesive

In three step etch and rinse a(Perdigão, 2001 #156)(Perdigao, 1999 #64)dhesive system, the smear layer is totally removed by acid etchant to permit resin bonding to the underlying dentin substrate (Van Meerbeek *et al*, 1992).

Acids demineralize intertubular and peritubular dentin, open the dentin tubules, and expose a dense filigree of collagen fibrils, increasing the microporosity of the intertubular dentin. Dentin is demineralized by up to approximately 7.5 µm, depending on the type of acid, application time, and concentration (Perdigao *et al*, 2001).

This bonding system consists of three essential components that are applied sequentially, the three essential components are (1) a phosphoric acid–etching gel that is rinsed off; (2) a primer containing reactive hydrophilic monomers in ethanol, acetone, or water; and (3) an unfilled or filled resin bonding agent. Some authors refer to this third step as *adhesive*. It contains hydrophobic monomers such as Bis-GMA, frequently combined with hydrophilic molecules such as HEMA (Sturdevant, 2014).

The acid-etching step not only alters the mineral content of the dentin substrate but also changes its surface free energy. The latter is an undesirable effect because for good interfacial contact, any adhesive must have a low surface tension, and the substrate must have a high surface free energy (Tsujimoto *et al*, 2017).

After etching, the dense web of exposed collagen is a low surface energy substrate. The primer in a three-step system is designed to increase the critical surface tension of dentin, and a direct correlation between surface energy of dentin and shear bond strengths has been shown (Erickson *et al*, 1992).

When primer and bonding resin are applied to etched dentin, they penetrate the intertubular dentin, forming a resin–dentin interdiffusion zone, or hybrid layer. They also penetrate and polymerize in the open dentinal tubules, forming resin tags. For most etch-and-rinse adhesives, the ultramorphologic characterization of the transition between the hybrid layer and the unaffected dentin suggests that an abrupt shift from hybrid tissue to mineralized tissue occurs, without any empty space or pathway that could result in leakage. The demarcation line seems to consist of hydroxyapatite crystals embedded in the resin from the hybrid layer. For self-etch systems, the transition is more gradual, with a superficial zone of resin-impregnated smear residues and a deeper zone, close to the unaffected dentin, rich in hydroxyapatite crystals (Sturdevant, 2014).

1.2.1.2 Two-step etch and rinse adhesive system

In vitro dentin bond strengths have improved so much that they approach the level of enamel bonding. Therefore, much of the research has focused on the simplification of the bonding procedure by reducing number of steps into two-step etch-and-rinse adhesive system. They are sometimes called “one-bottle” systems because they combine the primer and bonding agent into a single solution and a separate etching step still is required (Swift *et al*, 2001).

With this system, primer and adhesive are mixed in one bottle and the application of two coats are recommended. The first coat applied on etched dentin works as a primer-it increases the surface free energy of dentin The second coat acts as the bonding agent used in three-step systems-it fills the spaces between the dense network of collagen fibers (Freedman and Goldstep, 1997).

1.2.1.3 Two-step self-etch adhesive system

An alternative bonding strategy is the self-etch approach. a type of acidic conditioner was introduced -the self-etching primers (SEPs)- and has proved to be successful. These acidic primers include a phosphonated resin molecule that performs two functions simultaneously; etching and priming of dentin and enamel (Perdigao and Lopes, 1999).

In contrast to conventional etchants, SEPs are not rinsed off. The bonding mechanism of SEPs is based on the simultaneous etching and priming of enamel and dentin, forming a continuum in the substrate and incorporating smear plugs into the resin tags. In addition to simplifying the bonding technique, the elimination of rinsing and drying steps reduces the possibility of over-wetting or over-drying, either of which can affect adhesion adversely. Also, water is always a component of SEPs because it is needed for the acidic monomers to ionize and trigger demineralization of hard dental tissues; this makes SEPs less susceptible to variations in the degree of substrate moisture but more susceptible to chemical instability due to hydrolytic degradation (Fukuoka *et al*, 2011).

One disadvantage of SEPs that are currently available is that they do not etch enamel as well as phosphoric acid, particularly if the enamel has not been instrumented. The seal of enamel margins in vivo might be compromised (Patil *et al*, 2013).

When enamel bonds are stressed in the laboratory by thermal cycling, SEPs are more likely than etch-and-rinse systems to undergo deterioration. This decrease in bond strengths with thermal fatigue might be a sign that a potential exists for enamel microleakage when SEPs are employed to bond to enamel (Sturdevant, 2014).

SEPs have been classified in three categories: mild, moderate, and aggressive. Mild SEPs tend to provide excellent dentin bond strengths and poorer enamel bonds, whereas more aggressive self-etch systems provide the reverse (Ermis *et al.,* 2009).

SEPs are less technique sensitive than are etch-and-rinse adhesives. Additionally, SEPs are less likely to result in a discrepancy between the depth of demineralization and the depth of resin infiltration because SEPs demineralize and infiltrate dentin simultaneously. SEPs do not remove the smear layer from dentin completely, which is the main reason that they might result in less postoperative sensitivity compared with etch-and-rinse adhesives (Christensen, 2002).

1.2.1.4 One-Step Self-Etch Adhesives

Continuing the trend toward simplification, no-rinse, self-etching materials that incorporate the fundamental steps of etching, priming, and bonding into one solution have become increasingly popular. these one-step self-etch or “all-in-one” adhesives contain uncured ionic monomers that contact the composite restorative material directly. Their acidic unreacted monomers are responsible, in part, for the incompatibility between these all-in-one adhesives and self-cured composites. Additionally, one-step adhesives tend to behave as semi-permeable membranes, resulting in a hydrolytic degradation of the resin-dentin interface (Tay *et al*, 2004).

Because these adhesives must be acidic enough to be able to demineralize enamel and penetrate dentin smear layers, the hydrophilicity of their resin monomers, usually organophosphates and carboxylates, also is high. Some of these resin monomers are too hydrophilic, which makes them liable to water degradation (Tay and Pashley, 2003).

1.2.2 Mechanism of dentin bonding

1.2.2.1 Dentin conditioning

Two mechanisms for dentin conditioning are available, in the fourth and fifth generation of adhesives which are termed ‘etch and rinse adhesive systems’ the smear layer is completely removed and the dentinal tubules are opened by acid etchants. In the sixth and seventh generations which are termed ‘self-etch adhesive systems’ the smear layer is incorporated in the hybrid layer by the acidic primer which partially demineralize and infiltrate it simultaneously (Breschi *et al.*, 2004; Pashley *et al.*, 2011a).

The demineralization effect of acid etchant used in etch and rinse adhesive systems depends on its type, concentration, time of application, wettability, viscosity, water content and PH (Perdigao, 1995; Scheffel *et al.*, 2012). Time of acid application is not proportional with the degree of penetration into dentin due to the buffering action of dentin that prevents acid infiltration to deep layers during dentin demineralization (Hashimoto *et al.*, 2002).

Acid etchant also affects surface free energy, proteins exposure after dentin conditioning leads to a drop in surface free energy and low wettability that is difficult to provide good adherence to its surface (Yesilyurt and Bulucu, 2006).

After acid etching with the etch and rinse technique; a collagen network will be exposed as shown in **Figure (1-1)** and must be infiltrated by resin to form the hybrid layer. If dentin is air dried before bonding, collagen network will collapse and prevent the infiltration of the bonding resin and poor quality hybrid layer will be formed (Nishitani *et al.*, 2006a).

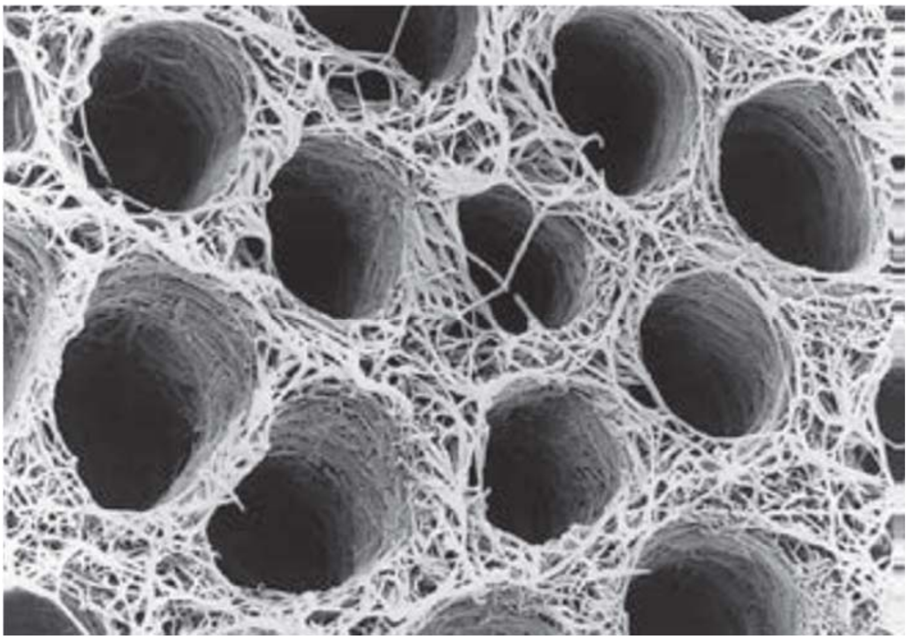


Figure (1-1): Scanning electron micrograph of etched dentin showing exposed collagen fibrils (Sturdevant, 2014).

When dentin is kept moist, collagen network will be distended and allow easy infiltration of the resin monomer and high quality hybrid layer will be formed with higher bond strength of the restoration as shown in **Figure (1-2)** (Cardoso *et al.*, 2005).

With air drying of the etched dentin, evaporation of the water inside he collagen fibrils will happened and rewetting can expand these fibres again and a bond strength will be restored to normal levels (Perdigao *et al.*, 2002).

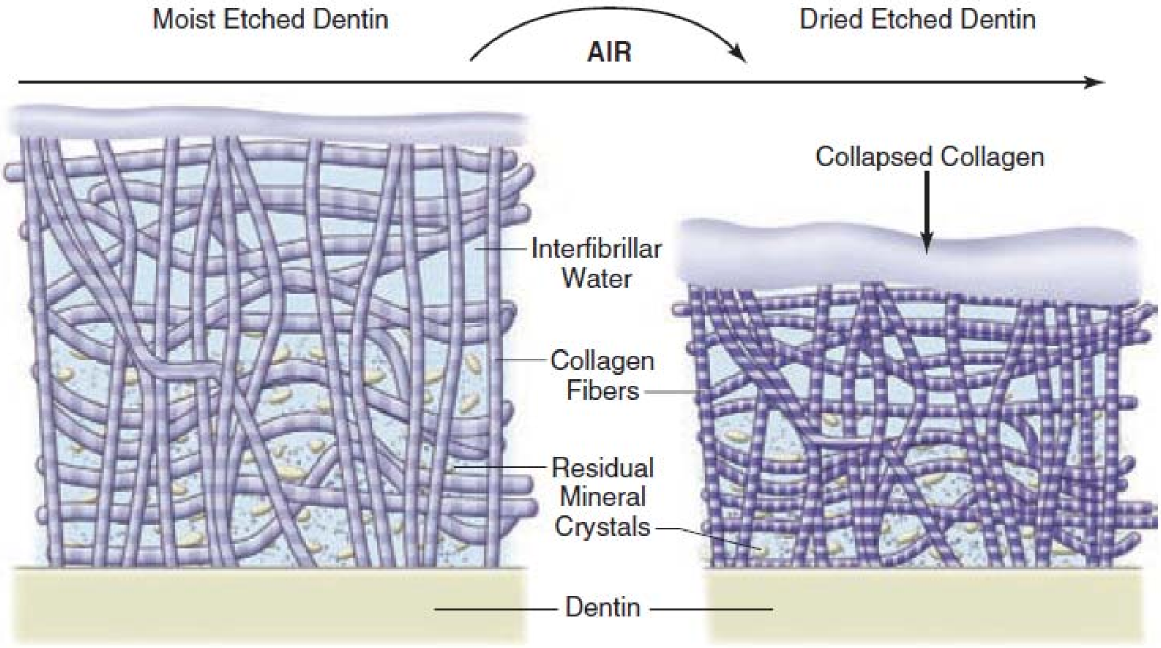


Figure (1-2): The difference between moist and dry dentin (Sturdevant, 2014)**.**

1.2.2.2 Priming of dentin

Primer is the intermediate agent that is applied between conditioning and bonding. After demineralization of dentin; a protein layer is exposed which reduce surface free energy and hence wettability. The primer raises the free energy and increase affinity of dentin to resin infiltration efficiently and form a hybrid layer for bonding. Primer consists of bifunctional molecules, each molecule has two functional groups, one of them is hydrophilic and has affinity for dentin while the other is hydrophobic with affinity for the adhesive resin (Van Meerbeek *et al.*, 1998).

A main constituent of the primer in many adhesive systems is hydroxyethyl methacrylate (HEMA). HEMA molecule resembles methyl methacrylate except that the methyl ester group was replaced with ethoxy ester group to make it hydrophilic (Carvalho *et al.*, 2003).

Modern adhesive systems are hydrophilic and contain resin monomers dissolved in acetone, ethanol, water or combination of these with organic solvents and termed as water chasers. They expel water from dentin and replace it with resin monomer (Reis *et al.*, 2004).

1.2.2.3 Bonding resin application

After the priming of dentin, an adhesive resin based on Bisphenol A glycol dimethacrylate (Bis-GMA) is applied which will infiltrate between collagen fibrils and into open dentinal tubules to form the hybrid layer and the resin tags which are the micromechanical retentive mean for the restoration (Van Meerbeek *et al.*, 1998).

1.2.2.3.1 Hybrid layer

Polymerization of infiltrated resin into collagen fibrils form the hybrid layer as mentioned previously and along with the resin tags form a hermetic seal on the opened dentinal tubules (Swift *et al.*, 1995).

There are three different zones within the hybrid layer. The top of the hybrid layer consists of an amorphous electro-dense phase, which has been ascribed to denatured collagen. At the middle of the hybrid layer there are cross sectioned and longitudinally sectioned collagen fibrils which separated by electro-lucent spaces, these spaces represent areas where hydroxyapatite crystals had been removed and replaced by resin as a result of the process of hybridization. The base of the hybrid layer represents the gradual transition to the underlying intact dentin with partially demineralized zone of dentin that contain hydroxyapatite crystals enveloped by resin (Perdigao, 1995).

The ideal percentage of resin in the hybrid layer is 70% by weigh which is difficult to achieve in most situations due to collapse in the collagen fibrils, incomplete resin infiltration and/or polymerization and water remnant that interfere with resin infiltration (Eick *et al.*, 1997).

1.2.2.3.2 Resin tags

Resin infiltration inside dentinal tubules and polymerization in situ leads to the formation of resin tags. Resin can penetrate to 2-4 µm distance inside the tubules, these tags increase area available for retention to 30-40% (Prati *et al.*, 1998).

Resin tags are important to get a good seal to prevent pulpal irritation and improve retention of the restoration. The percentage of the strength added by the resin tags to the total bond strength depends on the diameter of the tags and the cohesive forces binding the resin molecules (Pashley and Carvalho, 1997).

Micromechanical retention in superficial layers of dentin occur primarily by the hybrid layer with little participation of the resin tags because of the low number of resin tags in the superficial dentin. In deep layers of dentin, the number of tubules are increased with larger diameter and limited amount of intertubular dentin; for these reasons the retention will be majorly by resin tags and minimally by intertubular dentin (Pashley and Carvalho, 1997).

1.3 Dental composite

Resin based composite had evolved greatly since its introduction. The evolution of resin based composite was toward reducing the particle size of the filler to produce a material with high wear resistance and high polishability, then the focus was shifted toward improving the matrix of the composite to reduce the polymerization shrinkage and more importantly polymerization shrinkage stress and to make it self-adhesive to tooth structure (Ferracane, 2011).

1.3.1 Composition

Composite consist of three major components that are resin matrix (organic phase), filler (inorganic phase) and the coupling agent (silane), it also contains minor constituents like initiator, inhibitor, stabilizer, pigments and other components (Zimmerli *et al.*, 2010).

1.3.1.1 Organic phase

1.3.1.1.1 Methacrylate organic phase

The major monomers used with composite are bisphenol A-glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA) and ethoxylated bisphenol-A dimethacrylate (BisEMA) (Lempel *et al.*, 2016).

Bis-GMA is highly viscous because of its long chain and can’t be used alone, it is used with another shorter chain monomer like TEGDMA, increasing the percentage of TEGDMA while reducing Bis-GMA will increase the polymerization shrinkage, replacing Bis-GMA totally by TEGDA will reduce the flexural strength of the composite but increase the tensile strength. Although UDMA has a molecular weight close to Bis-GMA (470g/mol), it has lower viscosity and can be used solely or combined with other monomers like Bis-GMA or UDMA, it also has greater flexibility thanks to its urethane linkage that can increase composite toughness when incorporated in its composition (Peutzfeldt, 1997).

BisEMA is another monomer that can be added to the composite, it is less viscous than Bis-GMA due to the lack of the pendant hydroxyl group responsible for the hydrogen bonds among molecules which raise viscosity (Schneider *et al.*, 2010).

1.3.1.1.2 Non-methacrylate organic phase

Silorane-based resin was introduced in a category of a material commercially marketed as a low-shrinkage composite. Its organic matrix consists of oxirane and siloxane polymer with no methacrylate in its composition. It uses a cationic form of polymerization which reduces shrinkage and polymerization stress. Some laboratory studies show that silorane-based resins have low bond strength and greater nanoleakage (Goncalves *et al.*, 2012).

1.3.1.2 Inorganic phase

Inorganic phase is made of fillers. These fillers affect the mechanical properties of the composite. Increasing filler content can reduce polymerization shrinkage, coefficient of thermal expansion and contraction and water sorption. In the same time; strength, elastic modulus and wear resistance will be increased (Mahmoud *et al.*, 2008).

Many types of inorganic fillers are available like quartz, silica glass, colloidal silica, lithium aluminium silicate glass, zirconium silica nanoclusters, silica nanoparticles and sialorane (Yu, 2009).

Composites are usually classified according to the size and amount of the inorganic filler, they are broadly divided into macrofill composite, microfill composite, hybrid composite that include many types under this name and nanofill composite (Ferracane, 2011).

Macrofill composite has a large filler particle that give strength to the composite but the polymeric resin wears and produces a rough surface overtime, in contrast; microfill composite can maintain a very smooth surface over time but lack strength and may fracture under occlusal loading, nanofill composite has fillers in the form of clusters that are made by nanotechnology, these clusters resemble microfillers which give the material high polishability and simultaneously act like a large filler particle that give strength to the material (Ferracane, 2011).

1.3.2 Classification of resin composites

Composites are usually classified primarily on the basis of the size, amount, and composition of the inorganic filler. Different types of composite used since its introduction include macrofill composites (also called *conventional composites*), microfill composites, hybrid composites (including traditional hybrid, microhybrid, and nanohybrid composites), and nanofill composites. Composites also have been classified on the basis of their handling characteristics, for example, as flowable and packable composites (Randolph *et al.*, 2016).

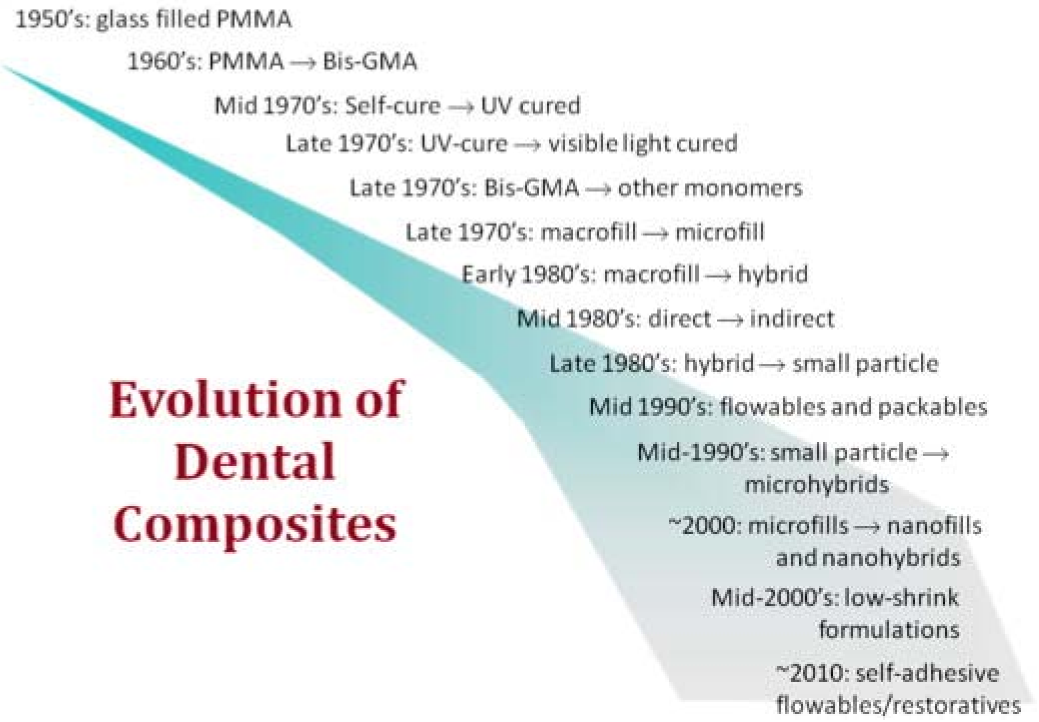


Figure (1-3): Evolution of dental composite (Randolph *et al.*, 2016).

1.4 Bond strength

The bond strength is defined as the force per unit area needed to break up two bonded surfaces at or close to the adhesive interface (Versluis *et al.*, 1997).

Longevity of a restoration is predicted to some extent by its adhesive ability, and this in turn can be measured by bond strength testing. Though the validity of bond strength tests to predict clinical performance of dental adhesives is questionable, existing evidence shows that clinical performance can be predicted by appropriate types of laboratory study results (Sirisha *et al.*, 2014).

1.4.1 Factors affecting bond strength

1.4.1.1 Factors related to bonding substrate

1.4.1.1.1 Enamel prism orientation

Composite resin restorations will have a longer life span if the enamel walls are given marginal forms to produce transverse sections of enamel prisms rather than longitudinal sections (Munechika *et al.*, 1984).

1.4.1.1.2 Dentin depth and permeability

The dentinal tubules are fluid filled from the pulp to the DEJ. If the dentin is exposed to the external surface for any reason, fluid will move inside the tubules and activate pulpal nerves which cause pain (Pashley *et al.*, 1993).

The diameter and number of tubules per square millimetre (density of tubules) increase with increasing depth in dentin while approaching the pulp, this lead to an increase in fluid movement through the tubules and increase in permeability. permeability differs from tooth to another and from age to age (Ulu Guzel *et al.*, 2018).

Dentin permeability increases almost logarithmically with cavity depth. In majority of studies, lower shear bond strengths were reported with increased dentin depth and permeability, and this permeability is less when smear layer was retained (Tagami *et al.*, 1990; Prati and Pashley, 1992).

Dentin permeability sophisticates the bonding process which is more difficult than enamel, after acid etching; the smear layer and plugs are removed which increase the permeability (Soderholm, 1991).

To optimize dentin bonding, resin must infiltrate dentin in both intratubular and intertubular way to form a continuous layer of hybrid layer with resin tags. In superficial dentin which contains fewer tubules; intertubular resin infiltration will be responsible of the bonding process (Nakabayashi *et al.,* 1991).

In the deep layers of dentin where the density of dentinal tubules is high, intratubular infiltration of resin will be responsible of a large part of the bond strength, intratubular infiltration may encounter difficulties in the deep layers due to pulpal pressure that push the fluids away from the pulp and reduce resin infiltration (Pashley and Carvalho, 1997).

Dentin affected by caries has low permeability due to bacterial presence and calcium deposits within dentinal tubules, therefore a low bond strength will be achieved by bonding to carious dentin. Sclerotic wedge shaped defects in the cervical dentin is another example of a low bond strength substrate due to minerals deposition inside the tubules that reduce resin infiltration (Eick *et al.*, 1997).

1.4.1.1.3 Dentinal tubules orientation

Dentinal tubules that run parallel to the bonded surface has greater amount of peritubular dentin than dentin with tubules running perpendicularly to the bonded surface providing more surface area for conditioning and a thicker hybrid layer will be formed resulting in higher bond strength values (Sattabanasuk *et al.*, 2004).

1.4.1.1.4 Degree of dentin mineralization

Degree of mineralization may be increased in dentin in response to physiological or pathological stimulus, if the dentin is exposed to the oral cavity in non-carious cervical lesions or if it is close to caries for a prolonged period of time; tricalcium phosphate deposits and obliterates dentinal tubules. Hypermineralized dentin is termed “sclerotic dentin” and it interferes with resin infiltration and produce a weaker bond strength than with normal dentin (Hosoya, 2006).

1.4.1.1.5 Surface area of the bonded interface

Studies had demonstrated that there is no linear correlation between the bond strength and the dentinal surface area at the bonded interface. In other words, doubling the interface available for bonding will not double the force needed to break the bond and this can be explained by increase in surface defects and flaws with increase in surface area available for bonding (Phrukkanon *et al.*, 1998)**.**

1.4.2 Factors related to adhesive material and technique

1.4.2.1 Surface wetness

Studies had shown that wet bonding, in which, dentin is dried but left visibly moist gives bond strength greater than dry bonding technique (Irmak *et al.*, 2016).

Pooled moisture should not remain on the tooth because excess water can dilute the primer and render it less effective (Van Dijken, 2000)**.**

1.4.2.2 Etching time

When etching time is prolonged a wide zone of demineralized dentin will be created and it is difficult to be infiltrated completely by resin, thus a resin free zone will be crated between the hybrid layer and the intact dentin and this zone will create a pathway for nanoleakage and bond degradation (Perdigão and Lopes, 2001).

Studies had shown that prolonged etching time (over etching) did not increase bond strength but actually reduce it and specimens fractured from the demineralized zone that was not infiltrated by resin (Zafar and Ahmed, 2015).

1.4.2.3 Number of adhesive resin application

An important way to improve bond strength is by increasing the number of adhesive resin application to improve resin infiltration and creating a high quality hybrid layer. However, there is no consensus about the number of applications needed to achieve good results (D'Arcangelo *et al.*, 2009). Some studies found that two applications are enough to produce a good bond strength while other studies found that several applications lead to better results (Carvalho *et al.*, 2017).

1.4.2.4 Use of hydrophobic coating

Since the incorporation of hydrophilic monomer blends in simplified adhesives (two-step etch-and-rinse and one-step self-etch adhesives) dramatically reduced bond longevity, the need of a hydrophobic coating with a not-solvated bonding layer seems to be pivotal to reduce water sorption and stabilize the hybrid layer over time, i.e., etch-and-rinse three steps and self-etch two-step adhesives should be preferred to simplified ones. Also applying a hydrophobic layer on one-step self-etching adhesives could improve bond strength and durability (Reis *et al*, 2008).

1.4.2.5 Extended polymerization time

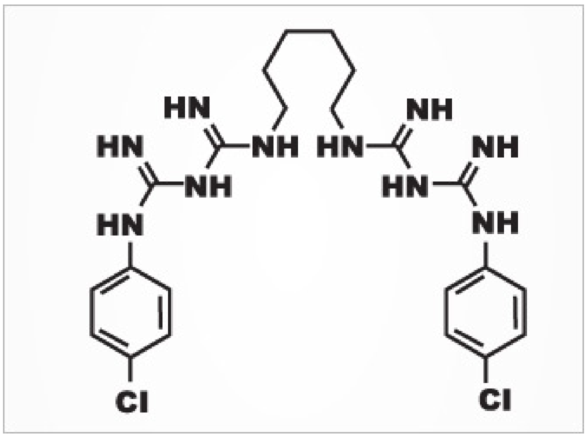
Extending the curing times of simplified adhesives beyond those recommended by the manufacturers resulted in improved polymerization and reduced permeability and appears to be a possible means for improving the performance of these adhesives (Samimi *et al*, 2017).

1.4.2.6 Use of MMPs inhibitors

The use of MMPs inhibitors as additional primer has been claimed to reduce interfacial aging over time by inhibiting the activation of endogenous dentin enzymes which are responsible for the degradation of collagen fibrils in the absence of bacterial contamination (Li *et al*, 2018).

1.4.2.6.1 Chlorhexidine

Chlorhexidine is a bisbiguanide antiseptic consisting of two biguanide groups and four chlorophenyl rings. The biguanide groups are bonded by a central hexamethylene bridge **(Figure (1-4)).** Chlorhexidine is a strong base with two positive charges (cations) on both sides of the hexamethylene bridge (Albert and Serjeant, 1962).



Figure(1-4): Chlorhexidine molecule (Albert and Serjeant, 1962).

1.4.2.6.1.1 Dental applications of chlorhexidine

Chlorhexidine is a broad spectrum antimicrobial agent with antiplaque inhibitory effect and for this reason it is used as mouth rinse, it retains in the oral cavity for up to 24 hrs by binding to phosphate, sulphate and carboxyl group of the bacteria, saliva and enamel surface. Chlorhexidine is also effective as a disinfectant for dentin after tooth preparation to decrease the number of bacteria inside dentinal tubules when applied for 10 min with 2% concentration (Rosenthal *et al.*, 2004)**.**

Chlorhexidine can also be used as endodontic irrigant from 0.12-2 % concentration with residual antimicrobial effect last for 3 days (Al Ubaidi and Al Rawi, 2007).

1.4.2.6.1.2 Mechanism of action of chlorhexidine

Chlorhexidine is a broad spectrum antibacterial agent which is effective against Gram-positive and negative bacteria but mainly on positive group. Its mechanism of action is related to the cationic action of the molecule; when the molecule is adsorbed to the negatively charged inner cell membrane, this will disrupt the integrity of the membrane and increase its permeability (Noites *et al.*, 2014).

At low concentrations, chlorhexidine exerts a bacteriostatic action only as a result of the efflux of low molecular weight substances like potassium and phosphorous from bacteria specially *S.mutans*. It is also effective against some viruses and fungi but not against spores. At high concentration, chlorhexidine exerts a bactericidal effect by coagulating or precipitating of cytoplasm by interaction with phosphate entities like adenosine triphosphate (ATP) and nucleic acids within the cytoplasm of bacteria (Paulson, 1993).

1.4.2.6.1.3 Chlorhexidine as a matrix metalloproteinase inhibitor

CHX has a potential MMPs inhibitory effect by calcium and zinc-chelation mechanism (Gendron *et al.*, 1999).

Application of 2% chlorhexidine for 1 minute between etching and bonding steps inhibits hybrid layer degradation for at least 14 months. CHX was also found to inhibit cysteine cathepsins enzyme that presents in mineralized and non-mineralized dentin which is responsible for MMPs activation and extracellular matrix degradation (Nascimento *et al.*, 2011).

1.4.2.7 Improved impregnation

Various methods have been recently proposed to enhance dentin impregnation, i.e., prolonged application time, vigorous brushing technique, and electric impulse assisted adhesive application. The latter technique recently revealed increased bond strength and reduced nanoleakage expression if adhesives are applied under the effects of an electric signal. Junior et al (2008) improved impregnation of dentinal collagen by adhesives via the evaporation of adhesive solvent by a stream of warm air. The use of a warm air-dry stream to evaporate the solvent of adhesives seems to be a clinical tool to improve the bond strength and the quality of the hybrid layer (less nanoleakage infiltration).

Another approach to improve impregnation of collagen by the adhesive after acid etching was the simultaneous acid etching and deproteinization suggested by Nassif and El Korashy. The simultaneous etching and deproteinization by NaOCl/phosphoric acid for 15 s showed a hybrid layer with improved bond strength. This was attributed to removal of shredded collagen found in the smear layer that could not be removed by acid etching only. Removal of this disorganized collagen would give more open structure to the collagen network and improve its impregnation by the adhesive (Nassif and El-Korashy, 2009).

1.4.2.8 Wet ethanol bonding approach

Ethanol is used to replace water just prior to bonding, thus avoiding the collapse of the collagen matrix. Ethanol-wet dentin may permit the infiltration of hydrophobic monomers to disperse into the demineralized dentin, creating a hydrophobic hybrid layer (Tay *et al*, 2003).

1.4.2.8.1 Ethanol

Ethanol is a clear, colorless liquid. It has bactericidal activity and is used often as a topical disinfectant. It is widely used as a solvent and preservative in pharmaceutical preparations as well as serving as the primary ingredient in alcohol beverages**.** Ethanol is naturally produced by the fermentation of sugars by yeasts or via petrochemical processes and has the chemical formula C2H5OH **(Figure (1-5))** (Saiz *et al.*, 1997).

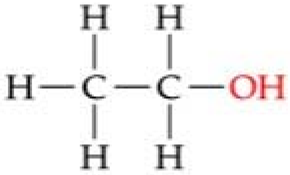


Figure (1-3): Structure of ethanol (Saiz *et al.*, 1997).

1.4.2.8.2 Mechanism of action of ethanol wet bonding

The adhesive resin used with the dry bonding was generally hydrophobic which is not miscible with water. After the introduction of wet bonding systems, hydrophilic resins were added to the adhesive resin to increase its miscibility with the intrinsically moist dentin. However, the use of increasing concentrations of hydrophilic resins raises concern that such adhesives have become too hydrophilic (Tay and Pashley, 2003). The incorporation of hydrophilic monomers results in increased water sorption that expedites hydrolysis and decreases mechanical properties (Yiu *et al.*, 2004; Nishiyama *et al.*, 2006). Bonding to wet dentin has also been shown to be challenging even with the use of hydrophilic adhesives. The surface moisture required for collagen expansion may also cause phase separation of some etch-and-rinse adhesive systems (Reis *et al.*, 2004), thus resulting in poor resin infiltration to the deepest regions of the demineralized dentin (Spencer and Wang, 2002). The exposed, uninfiltrated collagen fibrils are then susceptible to the enzymatic action of host metalloproteinases (MMPs) (Mazzoni *et al.*, 2007) that ultimately results in deterioration of the bond over time (Hebling *et al.*, 2005).

The ethanol wet-bonding concept has been presented as an alternative technique to overcome problems associated with the collapse of the collagen matrix if water is removed from the surface (Pashley *et al.*, 2007; Tay *et al.*, 2007). As ethanol has been shown to be able to expand and maintain collagen fibrils apart, it can be used to replace water, leaving demineralized dentin saturated with ethanol. This concept has been proved successful when used with experimental adhesive resins (Sadek *et al.*, 2008; Shin *et al.*, 2009) or commercial etch-and-rinse adhesives (Sadek *et al.*, 2010; Sauro *et al.*, 2011).

Ethanol is used to chemically dehydrate acid-etched demineralized dentin matrices (Nishitani *et al.*, 2006a). This results in a lateral shrinkage of collagen fibrils, causing an increase in the width of their fibrillar spaces and a reduction in the hydrophilicity of the collagen matrix allowing infiltration of the hydrophobic resin (Tay *et al.*, 2007).

1.4.2.9 Enhancing biomimetic remineralization

Biomimetic remineralization is a process that allows remineralization of dentinal collagen fibrils around and within collagen that still have intermolecular cross-links, like collagen fibrils in caries-affected dentin and phosphoric acid demineralized dentin. Biomimetic remineralization helps the rebuilding of dentin minerals in the same hierarchical pattern of apatite nanocrystals deposition both intrafibrillar and interfibrillar (Tay and Pashley, 2008).

Tay and Pashly (2008) suggested a guided remineralization of partially demineralized human dentin where they used set white Portland cement as a source of Ca ions in a phosphate-containing fluid to precipitate apatite nanocrystals around demineralized collagen. When polyvinyl phosphonic acid and PAA were included, these nanoprecursors were attracted to the acid-demineralized collagen matrix and transformed into polyelectrolyte-stabilized apatite nanocrystals that assembled along the microfibrils (intrafibrillar remineralization) and surface of the collagen fibrils (interfibrillar remineralization). Transition from nanocrystals to larger apatite platelets probably occurred via the formation of mesocrystal intermediates. Guided tissue remineralization is potentially useful in the remineralization of acid-etched dentin that is incompletely infiltrated by dentin adhesives as well as partially demineralized caries-affected dentin. Another approach is the use of bioactive “smart” composites containing reactive calcium-silicate Portland-derived mineral powder as tailored filler. This innovative method for the biomimetic remineralization of apatite-depleted dentin surfaces and for preventing the demineralization of hypomineralized/carious dentin could be potentially great advantage in clinical applications (Gandolfi *et al*, 2011).

1.4.2.10 Filler contents in the adhesive products

Characteristics of fillers like shape, size, type and interaction with matrix and solvents may affect bond strength. Filled adhesives may act as a shock absorber that dissipates functional forces thus increasing bond strength of composite to dentin (Swift *et al.*, 2001).

The loading of adhesive resin with fillers and nanoparticles has led to a significant reinforcement effect of the adhesive. Carboxylic acid functionalized titanium dioxide (Sun *et al.*, 2017), copper (Gutierrez *et al.*, 2017), silver (Torres-Mendez *et al.*, 2017), and zinc oxide (Barcellos *et al.*, 2016) nanoparticles have been used to reinforce the organic matrix of resin adhesives, thus improving physicomechanical properties of the material, and, consequently, bond strength between the restoratives and dental substrates**.** In another study by (Lohbauer *et al.*, 2010), zirconia nanoparticles were incorporated into the primer or adhesive of a commercial three-step etch-and-rinse adhesive system (SBMP, Scotchbond™ Multipurpose™; 3M ESPE, St. Paul, MN, USA) resulting in increased dentin bond strength. The formation of a strong adhesive interface is usually associated with a higher resistance to hydrolytic phenomenon, which may enhance bond durability. Once the hybrid layer is strong, water uptake is reduced, hydrolysis is diminished, and proteases activity is retarded, thereby reducing the rate of bond degradation over time (Carvalho *et al.*, 2016).

1.4.2.11 Type of organic solvent

Contemporary total-etch adhesives mainly contain ethanol/water or acetone as a solvent, in which resin monomers are dissolved (Van Landuyt *et al.*, 2007). Solvents are responsible for water displacement from collagen network and infiltration of resin monomers into spaces previously occupied by water (Kanca, 1992). Composition of the adhesive and solvent type requires different moisture spectrums (Tay *et al.*, 1998). Acetone based systems evaporate much residual water than ethanol/water based systems; however, they are more sensitive to air-drying as they cannot re-expand the shrunken collagen fibrils (Pashley *et al.*, 2002). Ethanol/water based systems are less moisture sensitive and good at re-expanding collagen matrix and produce higher bond strengths in dried dentin (Reis *et al.*, 2003). Wet bonding may be the ideal technique for current adhesives; however, this moisture concept varies widely among clinicians and manufacturers. Drying time and air-syringe distance, air pressure are variables that also have an effect on bond strengths and not easy to control (Kanca, 1996).

An adhesive containing a different solvent, tertiary butanol is on the market. According to its manufacturer, this adhesive system has a high degree of technique robustness due to chemical composition of tertiary butanol (XP BOND. Konstanz, Germany: Dentsply DeTrey, 2006) (Irmak *et al*., 2016).

1.5 Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are zinc and calcium dependent endopeptidases that can degrade extracellular matrix proteins, growth factors, lipoproteins and cell adhesion molecules (Osorio *et al.*, 2011).

They are cell derived proteolytic enzymes that are secreted as inactive form (proenzyme) which don’t cleave extracellular matrix proteins. The MMPs are trapped in the dentin during its formation (Mazzoni *et al.*, 2007).

MMPs consist of a prodomain, a catalytic domain with a highly conserved zinc binding site, a hinge region and a hemopexin domain **(Figure (1-4)).** The catalytic domain contains cysteine rich repeats that are necessary for the binding and cleaving activities of these proteolytic enzymes (Van Wart and Birkedal-Hansen, 1990; Visse and Nagase, 2003).

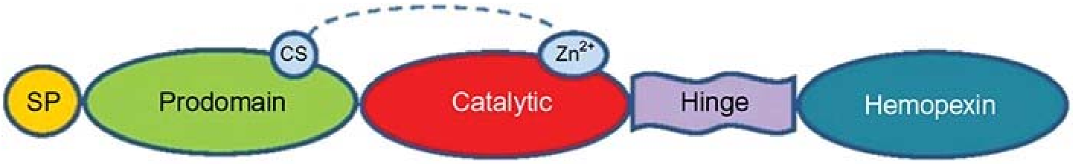


Figure (1- 6): Structure of MMP (Visse and Nagase, 2003).

Studies have demonstrated the presence of at least gelatinases MMP-2 and -9 and collagenase MMP-8 in human dentin (Martin-De Las Heras *et al.*, 2000; Sulkala *et al.*, 2007; Mazzoni *et al.*, 2011).

The main function attributed to MMPs is the degradation of the extracellular matrix (Visse and Nagase, 2003). However, it became widely acknowledged that MMPs play an important role in cell signaling by generating peptides with specific biological activities (Page-McCaw *et al.*, 2007).

1.5.1 Potential mechanism(s) of MMPs activation

MMPs are secreted into the extracellular matrix as a proenzyme and require activation in order to be able to degrade the matrix components. The activation is a complex process for which several mechanisms had been suggested. PH alterations can change the conformation of the propeptide which induces the cysteine switch and represents a key step in the activation of the enzyme (Van Wart and Birkedal-Hansen, 1990). Even though MMPs require low PH for the activation; it can work only in a neutral PH that is achieved by the dentin buffering mechanisms, etch and rinse adhesives and water (Haapasalo *et al.*, 2007).

Acid etch in etch and rinse adhesives (Mazzoni *et al.*, 2006) and self-etching primers in self-etch adhesives (Tay *et al.*, 2006) had been shown to increase the collagenolytic and gelatinolytic activity of MMPs trapped in dentin.

Three non-collagen proteins found in dentin are released by acid attacks from etching step or by the carious lesion and can bind to MMPs causing their activation (Chaussain-Miller *et al.*, 2006), they are part of the Small Integrin Binding Ligand N-linked Glycoproteins (SIBLING) gene family (Fedarko *et al.*, 2004), the three binding proteins are Bone Sialoprotein (BSP) which binds only with MMP-2, Osteopontin (OPN) which binds only with MMP-3 and Dentin Matrix Protein1 (DMP1) which binds only with MMP-9.

Another enzyme family which is the cysteine cathepsins was identified in dentin and was suggested that when they are in active form can activate pro-MMPs (Tersariol *et al.*, 2010), a study revealed a strong immunostaining of cathepsins B in carious dentin compared to sound with sound dentin (Nascimento *et al.*, 2011).

1.5.2 Durability of bond strength with etch and rinse adhesives

Even though water is necessary at the beginning of resin infiltration, it must be eliminated from dentin after serving its purpose otherwise it will cause hydrolytic degradation of the resin matrix by esterase and degradation of collagen by the collagenolytic and the gelatinolytic enzymes (Liu *et al.*, 2011).

Another problem related to the etch and rinse technique is that resin concentration is reduced as we go deeper towards the bottom of the hybrid layer, this phenomenon is more prevalent in carious dentin than sound dentin which is the usual cause for tooth restoration. When resin infiltration is not complete, denuded collagen fibrils will be the result which are susceptible to enzymatic attack and fatigue failure (Wang *et al.*, 2007).

In vitro researches had shown that resin dentin interface deteriorates with time due to degradation of the collagen fibrils at the interface (De Munck *et al.*, 2009); in addition to the hydrolytic degradation of the adhesive resin (Hashimoto *et al.*, 2000). Proteolytic degradation of collagen fibrils may also be responsible for the diminished dentin bond strength over time (Hashimoto, 2010). *In vivo* studies have also found that collagenous part of the hybrid layer undergoes a gradual hydrolytic degradation (Hashimoto *et al.*, 2003a).

1.6 Bond strength measurement methods

The tests of bond strength are classified into:

1.6.1 Tensile bond strength test

When two forces with opposite directions but within the same straight line are applied on a subject, tension will result and the forces are termed as tensile forces (Sano *et al.*, 1994).

In a tensile bond test, load will be exerted on either sides of the test specimen. The specimen can be held by active or passive gripping methods. Active gripping method involves mechanical fastening of specimen to gripping device, such as glue or clamps, whereas in passive gripping method, specimen is placed in a testing device without the aid of glue or mechanical gripping (Armstrong *et al.*, 2010). It can be used to measure the bond strength of cements to hard materials such as ceramics and metal alloys (Van Meerbeek *et al.*, 2010).

1.6.2 Microtensile bond strength test

It is a newer method for testing materials in dentistry. It allows testing subjects with less than 1 mm^2 cross-section (Sano *et al.*, 1994).

Microtensile bond strength test has the advantage over tensile and shear bond strength test in that many samples can be fabricated only from one tooth with fewer defects to avoid scatter in results (Escribano *et al.*, 2003).

The major disadvantage of this test is that it is technique demanding with higher cost than other tests. Another disadvantage is that the cutting procedure to produce the samples may reduce the bond strength (Sano *et al.*, 1994).

1.6.3 Shear bond strength test

When two parallel forces directed towards each other in different planes shear will result, shear strength refers to the maximum stress the material can take before failing by shear mode. This test is important to study interfaces between different materials (Burke *et al.*, 2008).

It was found that force distribution in SBS-test is more uniform than TBS-test and the latter must be carefully conducted otherwise specimens will undergo torque stress which reduce bond strength values (Sano *et al.*, 1994).

1.6.4 Micro shear bond strength test

It was developed as an alternative to the Microtensile bond strength test, although it is considered more useful for testing bond strength to enamel, it’s a difficult method with high probability of enamel chipping off from the underlying dentin governed by its brittle nature (Shimada, 1999).

Chapter Two

Materials & Method

Materials and Method

2.1 Materials and Equipment

The armamentarium for the study includes the followings **(Figure (2-1))**:

2.1.1 Materials

1. Chlorhexidine digluconate 2% solution (Cerkamed, Poland) (LOT 311151. EXP. DATE 2020-11).
2. Deionized distilled water (Almansur factory, Iraq).
3. Disposable nitrile gloves (Broche, Turkey) (LOT 2887. EXP.DATE 2019-01).
4. Ethanol alcohol 100% (Scharlab, Spain) (LOT ET00162500. EXP. DATE 2018-07).
5. Filtek Z350 composite restorative material (shade A2) (3M ESPE, USA) (LOT N777652. EXP. DATE 2019-04).
6. Flexible applicators for bond application (Kerr, Germany).
7. Glass slide (Swan, England).
8. Ninety-six newly extracted sound human maxillary premolar teeth.
9. Phosphoric acid gel 37% (Alphadent, USA) (LOT Y215HA. EXP.DATE 2019-02).
10. Sandpaper (Al-Ugaily trading company, China).
11. Self-cured Acrylic resin (Dt, Czech-Republic) (LOT. 2378779. EXP. Date 2019-05).
12. Single Bond Universal (3M ESPE, USA) (LOT 634192.EXP.DATE 2018-06).

13. Sponge for blot drying (Diadent, China).

2.1.2 Equipment

1. Custom made rubber mold for acrylic block construction.
2. Dental microscope (Zumax, China).
3. Dental surveyor (Kavo, Germany).
4. Electrical diamond saw (Gamberini, Italy).
5. LED wireless light cure device (SDI, Australia) (light intensity: 600mW/cm2)
6. Teflon mold designed and fabricated for the study.
7. Universal testing machine (Tinius Olsen, Germany)

2.1.3 Instruments

1. Dental probe (Medesy, Italy).
2. Digital stop watch.
3. Gold plated ash 49 for composite application (Garrison, USA).
4. Periodontal probe (Towne, Pakistan).
5. Screw driver (China).
6. Vernier calliper (China).

Figure (2-1): Some of the materials and equipment used in the study.

2.2 Methods

2.2.1 Teeth Collection

Ninety-six sound human maxillary premolar teeth extracted for orthodontic demand with age range between 14-30 years collected from multiple health centres in Baghdad were used in this study. Teeth collected for the study were crack-free and sound when examined by light curing device and dental microscope (5X). The teeth were cleaned with rubber cup ad pumice then washed with distilled water (Hamouda and Shehata, 2011).

Teeth were stored in thymol solution (0.1%) for about two months till the time of the study to prevent bacterial and fungal growth (Simoes *et al.*, 2014b).

2.2.2 Construction of acrylic block

A specially designed cubic silicone mold with internal dimensions 1.5 cm x 1.5 cm x 1.5 cm was used for the construction of acrylic blocks as shown in **Figure (2-2 A)**.

CEJ was marked and another marking 2mm apical to the CEJ was made which represent the level of seating the teeth inside the acrylic. Each tooth was positioned with the aid of a dental surveyor and a sticky wax was used to attach the tooth to the rod of the surveyor so that the long axis of the tooth and the rod are parallel (Ayad *et al*., 1997) as shown in **Figure (2-2 B)** .

A cold cure acrylic resin was mixed according to manufacturer's instructions and poured inside the silicon mold then the tooth was inserted inside the acrylic 2 mm apical to the CEJ until complete polymerization as shown in **Figure (2-2 C)**.

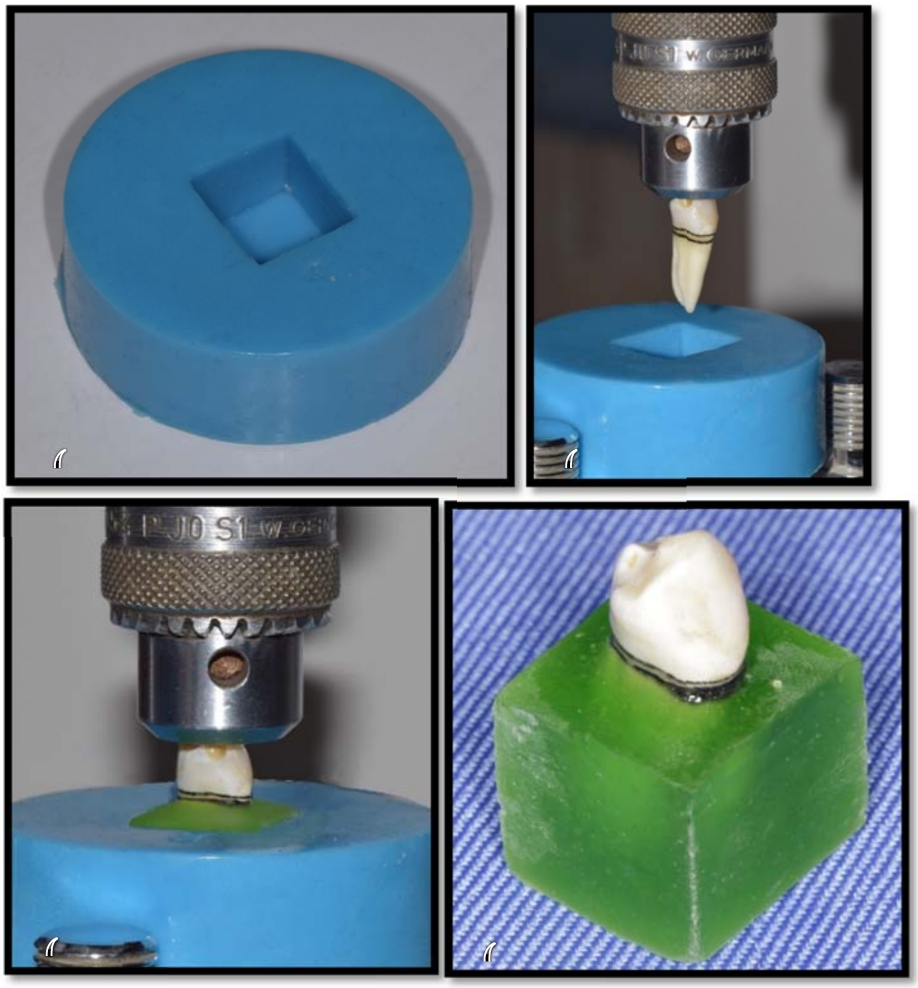


Figure (2-2): Acrylic block construction (A) rubber mold for acrylic block construction, (B) attaching the tooth to a dental surveyor to aid in positioning, (C) inserting the tooth in the acrylic resin during dough stage, (D) tooth with acrylic block after complete polymerization.

2.2.3 Teeth preparation

In order to provide exposed dentin surface for the study, both buccal and palatal cusps were sectioned using electric saw, the cutting was one and a half millimeter cervical to the mesial pit **(Figure (2-3))**. A periodontal probe was used to measure the depth of the pit to the mesial marginal ridge to estimate the height of the ridge and one and a half millimeter was added to this estimation and marked on the mesial side of the tooth to be cut later by the saw under running water to gain a flat horizontal surface (Khalil and Al-Shamma, 2015) **Figure (2-4 A)**.

The cut surface was checked by dental microscope (5x) for any enamel remnants then smoothed with sand paper (600 grit) by moving the dentin surface 4 times against the sandpaper of 10 cm length and washed to remove debris (Khalil and Al-Shamma, 2015) as shown in **Figure (2-4 B).**

Figure (2-3): Determination of sectioning level (A) measurement of mesial pit, (B) determination of sectioning line, (C) drawing of sectioning level.

Figure (2-4): Creating a flat surface of dentin (A) tooth sectioning, (B) sectioned tooth after smoothening.

2.2.4 Sample grouping

The ninety-six teeth were divided into four main groups of twenty-four teeth each as shown in **Figure (2-5)**:

Group A (control group): teeth were acid etched, rinsed, blot dried, bonded and restored with composite.

Group B (CHX group): teeth were acid etched, rinsed, blot dried, rewetted with 2% CHX for 60 seconds, blot dried, bonded and restored with composite.

Group C (EWB group): teeth were acid etched, rinsed, blot dried, rewetted with 100 % ethanol for 60 seconds, blot dried, bonded and restored with composite.

Group D (Mixed group): teeth were acid etched, rinsed, blot dried, rewetted with 2% CHX for 60 seconds, blot dried, rewetted with 100% ethanol for 60 seconds, blot dried, bonded and restored with composite.

Each group was further subdivided into three subgroups (n=8), subgroups 1 were subjected to shear bond strength test after 24 hrs, subgroups 2 after three months and subgroups 3 after six months.

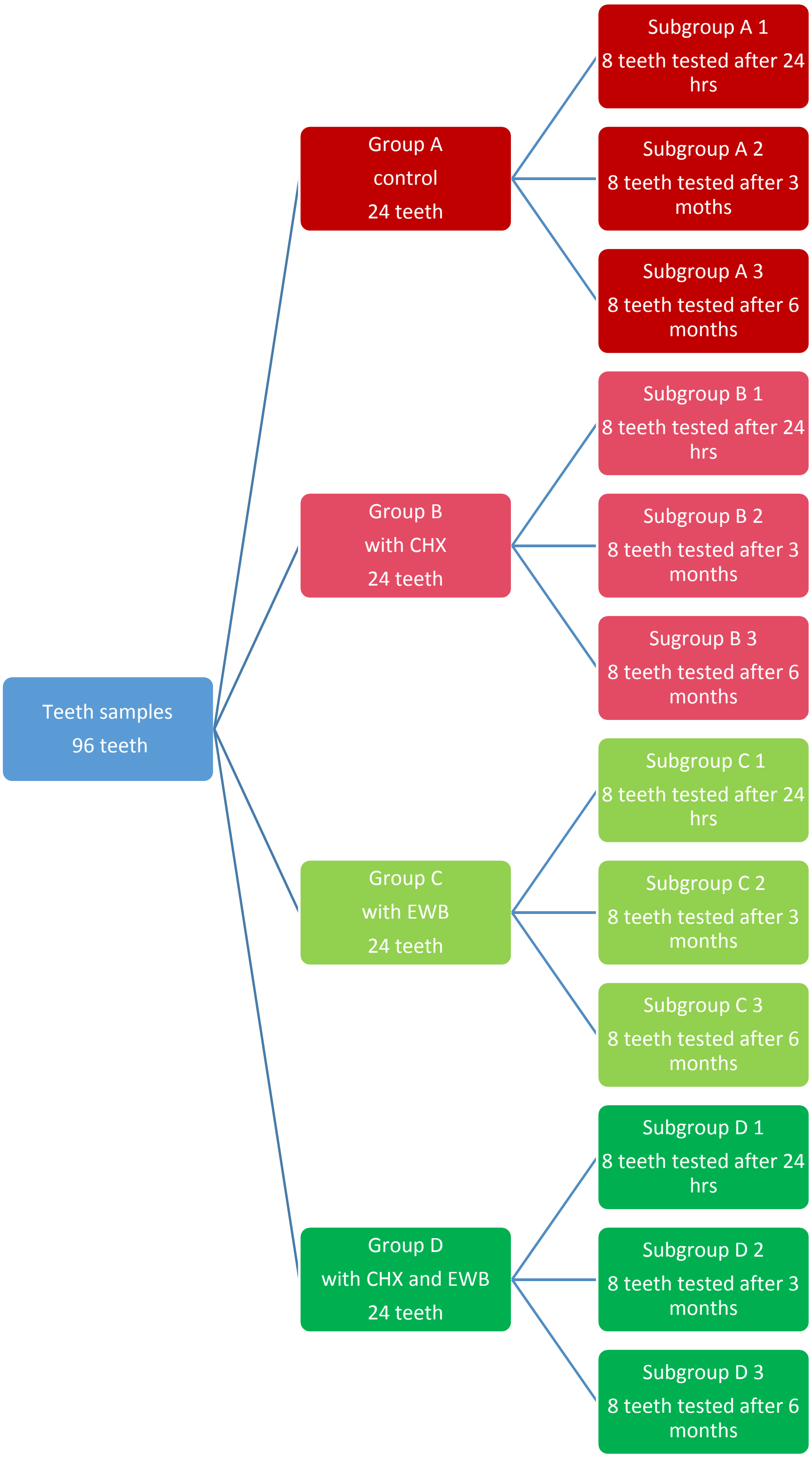


Figure (2-5): Sample grouping.

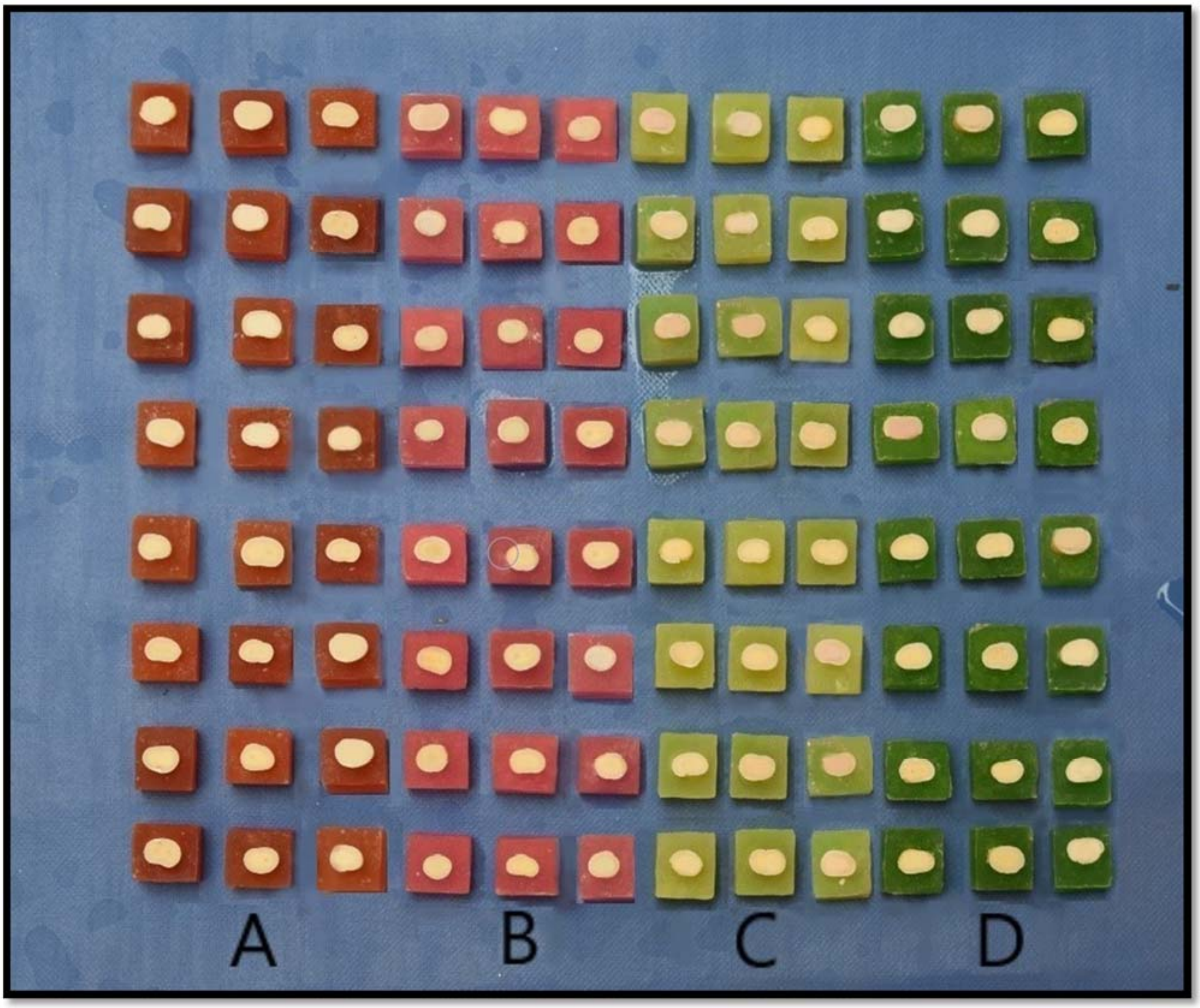


Figure (2-6): Study samples.

2.2.5 Adhesive procedure

2.2.5.1 Etching step

The exposed dentin surface was etched with 37% phosphoric acid (Alpha dent, USA) for 15 seconds and rinsed with water for 15 seconds **(Figure (2-7))**, excess water was removed by a sponge leaving the surface visibly moist (Irmak *et al.,* 2016).

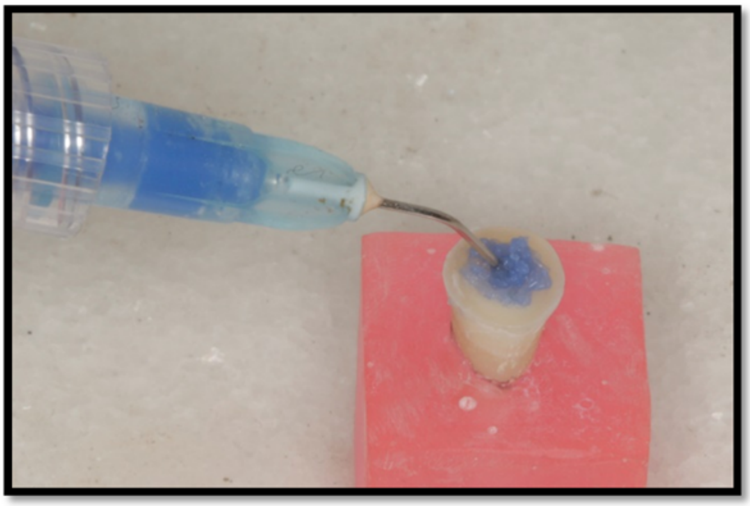


Figure (2-7): Application of acid etch.

2.2.5.2 Adhesive application step

2.2.5.2.1 Composition of single bond universal

1-MDP phosphate monomer

2-Dimethacrylate resin

3-HEMA

4-Vitrebond TM copolymer

5-Filler

6-Ethanol

7-Water

8-Initiators, Silane

Vitrebond copolymer was first introduced with Scotchbond Multi-Purpose adhesive. During the development of the Single Bond Multi-Purpose adhesive system, it was noted that the bond strengths of other dental adhesive systems tended to decrease when under varying humidity conditions. Also during this period, it was noted that Vitrebond™ Light Cure Glass Ionomer Liner/Base bond strengths did not seem to vary to the same degree. It was determined that by incorporating the proprietary copolymer used in Vitrebond liner/base (now termed the Vitrebond Copolymer) into the Single Bond Multi-

Purpose Primer yielded a system that was very resistant to the detrimental effects of varying humidity (Fundingsland et al. 1992).

Single Bond Universal adhesives differ from Adper Single Bond 2 adhesive primarily in the partial replacement of the methacrylate monomers (UDMA and GDMA) with the phosphorylated methacrylate monomers (MHP or MDP) to allow the acidity for the self-etching capability.

The MDP allows for the self-etching properties and good adhesion performance to enamel, great product stability, and adhesion to metal and non-glass ceramic substrates. The addition of the silane allows for adhesion to glass-ceramic surfaces without the need for a separate primer.

2.2.5.2.2 Group A (control)

Teeth belong to this group were coated with Single Bond Universal using disposable applicator with rubbing action for 20 seconds followed by gentle air blow for about 5 seconds with triple syringe until it no longer moved and the solvent was completely evaporated (Munoz *et al.*, 2015; Irmak *et al.*, 2016).

Adhesive agent was then light cured with LED light curing device (SDI, Australia) for 10 seconds according to manufacturer’s instruction. A specially designed handle was used to direct the light 2 mm away from the dentin surface in a vertical direction. Radiometer was used to measure power intensity before using the light curing device which was 600mw/cm^2 (Khalil and Al-Shamma, 2015).

2.2.5.2.3 Group B (CHX)

Following acid etching, teeth of this group were rewetted with 2% CHX for 60 seconds using rubbing action. Teeth were then blot dried and the adhesive agent was applied and light cured as for the control group (Khalil and Al-Shamma, 2015).

2.2.5.2.4 Group C (EWB)

Following acid etching, teeth were rewetted with 100% ethanol for 60 seconds using rubbing action (Ekambaram *et al.*, 2014). Teeth were then blot dried and the adhesive agent was applied as mentioned in the control group.

2.2.5.2.5 Group D (CHX and EWB)

Following acid etching, teeth belong to this group were rewetted first with 2% CHX for 60 seconds using rubbing action, blot dried then rewetted with 100% ethanol for another 60 seconds, blot dried and then adhesive agent was applied as for the control group.

2.2.6 Placement of composite restoration

A special device was designed and fabricated from teflon to standardize the manner of composite application to the dentin surface. This device consists of a cylinder to accommodate the acrylic block, from top of this cylindrical mold, there is a removable translucent teflon cover of two half circles of 2mm thickness attached to the cylinder by four screws while from the bottom of the cylinder there is a removable metal bar with a screw in its centre to fix the acrylic block towards the teflon cover, around the cylinder there are four screws positioned against each other to stabilize the acrylic block and direct the dentin surface to a hole of 4 mm diameter in the teflon cover for composite placement **(Figure (2-8))**.

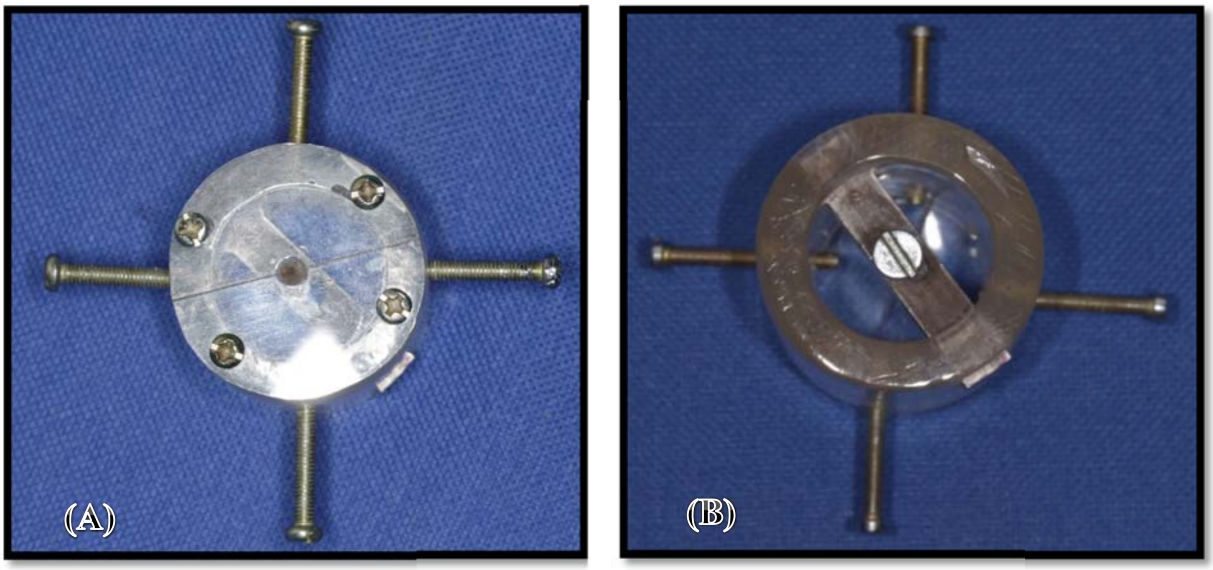


Figure (2-8): The teflon mold. (A) Top view. (B) Bottom view.

Through the aforementioned hole in the cover, composite (Filtek Z350) was applied in a single increment by a plastic instrument and a celluloid strip was placed over it. A glass slide with 200 gm load is placed over the celluloid strip for 1 minute, after that the load and the glass slide are removed and excess composite is removed before light curing for 40 seconds (Ciccone-Nogueira *et al.*, 2007), the light curing device was in intimate contact with the celluloid strip as shown in **Figure (2-9)** (Loguercio *et al.*, 2009).

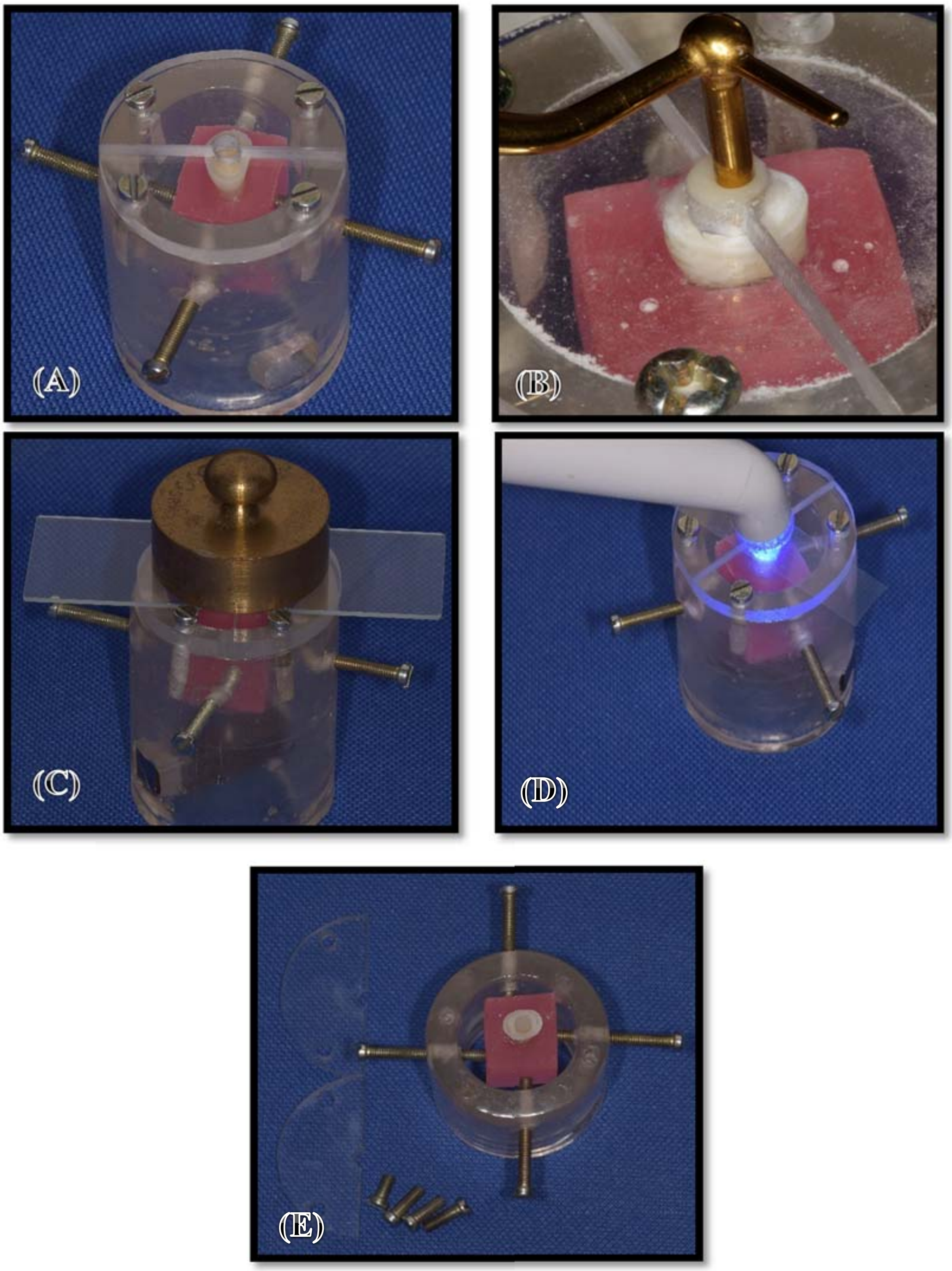


Figure (2-9): Standardization for restorative material application. (A) placing the acrylic block with the dentin surface beneath the hole. (B) placement of composite inside the hole. (C) 200 mg load application after composite placement. (D) curing of composite with the light curing device in intimate contact with the celluloid strip. (E) Study sample after light curing.

2.2.7 Storage

Samples of all groups were stored in dark containers filled with distilled water and incubated in incubator at 37℃ for 24 hrs for subgroup 1, and for three and six months for subgroup 2 and 3, respectively.

2.2.8 Shear bond strength testing

Shear bond strength of composite to dentin was tested using universal testing machine (Tinius Olsen, Germany) at a cross head speed of 1mm/min. The acrylic blocks were fixed to the lower jaw of the testing machine in such a way that the chisel rod was held perpendicular to the composite tooth interface from the buccal aspect as shown in **Figure (2-10)** and the test continued until failure (Boruziniat *et al.*, 2013).

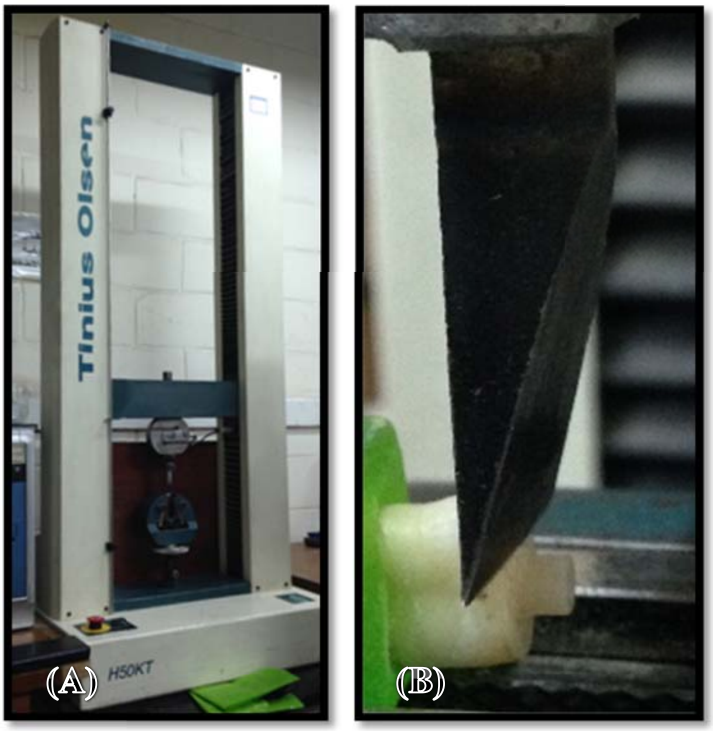


Figure (2-10): (A) Univesal testing machine (B) Sample in lower jaw of the machine.

The force was calculated in Newton and divided by the surface area of the dentin-composite interface (12.56mm^2) to get shear bond strength in MPa (N/mm^2).

$$SBS = \frac{Force(Newton)}{Surface\ area(\mathrm{mm2})}$$

Surface area=πr^2, π=3.14 ➡ $3.14 * 2^2 = 12.56$ mm^2

r=radius=2mm

2.2.9 Mode of failure

After shear bond strength testing, the tested samples were checked by naked eye for any composite remnants (Manso *et al.*, 2014). Findings were supported by dying the samples using methylene blue dye and taking photos for the samples using DSLR camera with macro-lens. Photos were collected and analysed accurately by image software in the computer (JPG viewer). Teeth were analysed to define whether failure was adhesive, mixed (partially adhesive/partially cohesive) or cohesive failure within composite or dentin.

2.2.10 Statistical analysis

Methods used for statistical analysis and assessment of results include:

A. Descriptive Statistics

1. Arithmetic means.
2. Standard deviation.
3. Statistical tables.
4. Graphical presentation by bar charts.

B. Inferential statistics

One-way Analysis of Variance test (ANOVA) was used for testing any statistically significant differences among groups with different adhesion protocols and also was used for testing each three subgroups within the same group for any significant difference.

In the aforementioned tests, p value equal or more than 0.05 was considered as statistically non-significant (NS), p value between 0.05 and 0.01 as statistically significant (S), and p-value less than 0.01 as highly significant (HS) as follows:

- $P \geq 0.05$ (Non-significant).
- $0.05 < P \leq 0.01$ (Significant).
- $P < 0.01$ (Highly significant).

Chapter Three

Results

Results

3.1 Descriptive statistics

Data of the SBS in MPa for all groups are presented in (Appendix I). Means and standard deviations of SBS with minimum and maximum values which were calculated for each group are shown in table (3-1).

Table (3-1): Descriptive statistics of shear bond strength for all four groups

Storage period	Groups	N	Mean	±S.D.	Min.	Max.
Immediate	A1	8	8.384	1.343	6.37	11.03
	B1	8	8.425	1.157	6.1	9.95
	C1	8	8.660	0.960	7.5	10.74
	D1	8	8.815	1.530	6.74	10.62
After 3 months	A2	8	7.151	0.903	5.71	8.63
	B2	8	8.006	1.570	6.28	10.85
	C2	8	7.707	1.562	6.30	10.95
	D2	8	8.202	0.714	7.11	9.44
After 6 months	A3	8	5.885	0.623	5.11	6.9
	B3	8	7.429	0.777	6.45	8.86
	C3	8	7.431	1.080	6.19	9.07
	D3	8	7.965	0.735	6.79	9.23

From this table we can see that the higher mean value among the groups tested immediately, after 3 months and after 6 months was for group D and the lowest mean value was for the group A.

The mean values of shear bond strength of all four groups are shown in **Figure (3-1)**.

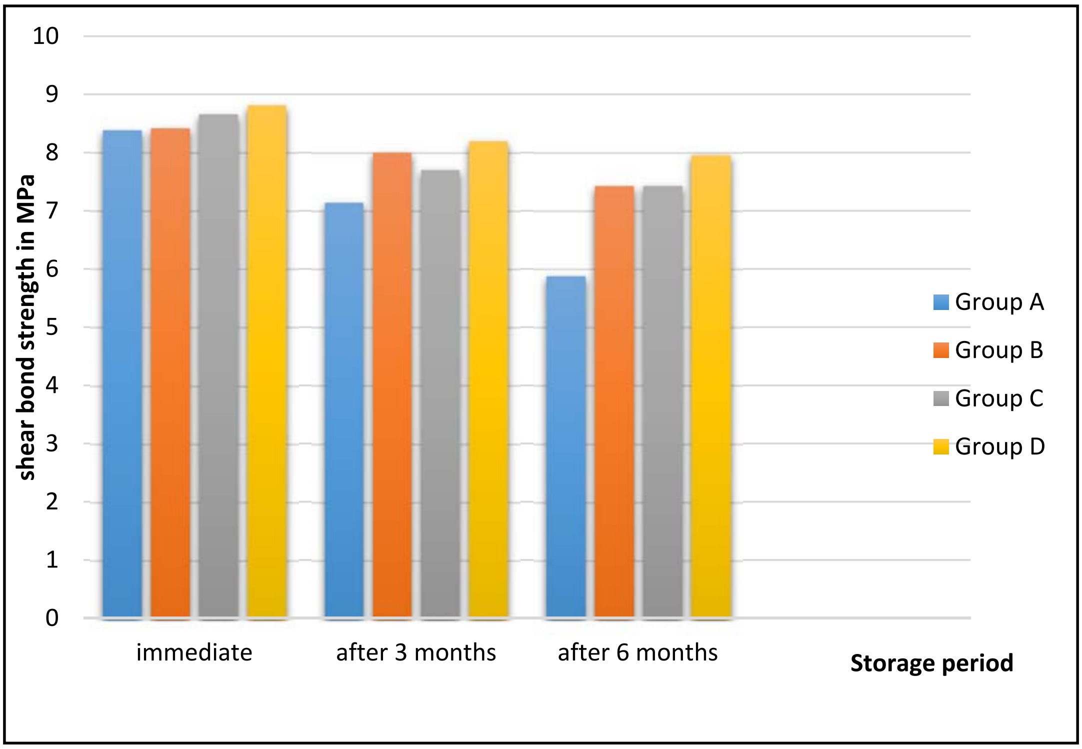


Figure (3-1): Bar-chart showing the mean SBS values of the four groups at different durations.

From this bar chart, it can be seen that for all time intervals, Group D showed the highest shear bond strength mean values, while Group A showed the lowest. Also it can be seen that the shear bond strength mean values decreased with tine for all groups.

3.2 Inferential statistics

3.2.1 Effect of storage period

To see whether storage period has an impact on the mean value of SBS for all groups, one-way ANOVA was used as seen in **Table (3-2)**.

Table 3-2: One way-ANOVA within groups for testing the effect of aging.

Groups	Storage period	Comparison (d.f.=23)	
		F-test	p-value
A	Immediate	12.457	0.000 (HS)
	After 3 months		
	After 6 months		
B	Immediate	1.362	0.278 (NS)
	After 3 months		
	After 6 months		
C	Immediate	2.202	0.136 (NS)
	After 3 months		
	After 6 months		
D	Immediate	1.362	0.278 (NS)
	After 3 months		
	After 6 months		

No significant differences were found among the different subgroups of group B, C and D except for group A as highly significant difference was found among its subgroups. Tukey-test was used as shown in **table (3-3)** to find the subgroup responsible for the high difference.

Table (3-3): Tukey's HSD for control group.

Storage period		Mean difference	*P*-value
A1	A2	1.232	0.056 (NS)
	A3	2.498	0.000 (HS)
A2	A3	1.266	0.049 (S)

No significant difference was found after three months when compared with immediate. A significant difference was found between the 3 months and 6 months' period and highly significant difference was found between the immediate and six months' period.

3.2.2 Effect of adhesive protocol

To examine the effect of different adhesive protocols, one-way ANOVA test was also used as shown in **table (3-4)**.

Table (3-4): The effect of different adhesive protocols

Durations	Groups	Comparison (d.f.=31)	
		F-test	*p*-value
Immediate	**Control** **CHX** **Ethanol** **Mixed**	0.206	0.891 (NS)
After 3 months	**Control** **CHX** **Ethanol** **Mixed**	1.007	0.375 (NS)
After 6 months	**Control** **CHX** **Ethanol** **Mixed**	9.563	0.000 (HS)

As highly significant difference was found among subgroups only after 6 months and non-significant difference was found among subgroups immediately and after 3 months, Tukey –test was used for further analysis as shown in **Table (3-5)**.

Table 3-5: Tukey's HSD test after ANOVA test

Duration	Groups		Mean Difference	p-value
After 6 months	**Control**	**CHX**	-1.543	0.004 (HS)
		Ethanol	-1.546	0.004 (HS)
		Mixed	-2.080	0.000 (HS)
	CHX	**Ethanol**	-0.002	1.000 (NS)
		Mixed	-0.536	0.567 (NS)
	Ethanol	**Mixed**	-0.533	0.571 (NS)

A highly significant difference was found between the control and other groups and non-significant difference was found among other experimental groups.

3.3 Mode of failure

After careful examination of the photos of the samples after testing, it had been shown that failures fallen in one of two categories:

1. Adhesive failure that occurred between composite and dentin.
2. Mixed failure that involved failure within composite and adhesive layer.

However, no sample showed any failure within dentin as shown in **Table (3-6)** and **figure (3-2)** and **(3-3)**.

Table (3-6): Modes of failure of the different groups.

	24 hrs		**3 months**		**6 months**	
	Mixed	**Adhesive**	**Mixed**	**Adhesive**	**Mixed**	**Adhesive**
A	6	2	5	3	3	5
B	7	1	7	1	5	3
C	6	2	5	3	4	4
D	8	0	7	1	6	2

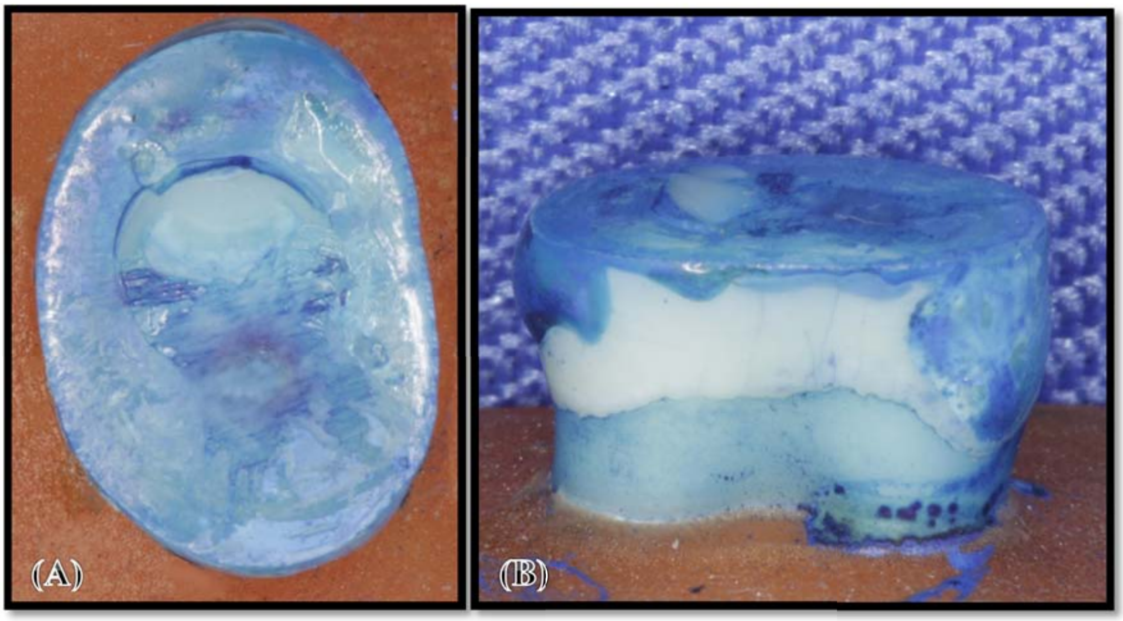


Figure (3-2): Mixed failure. (A) occlusal view (B) lateral view.

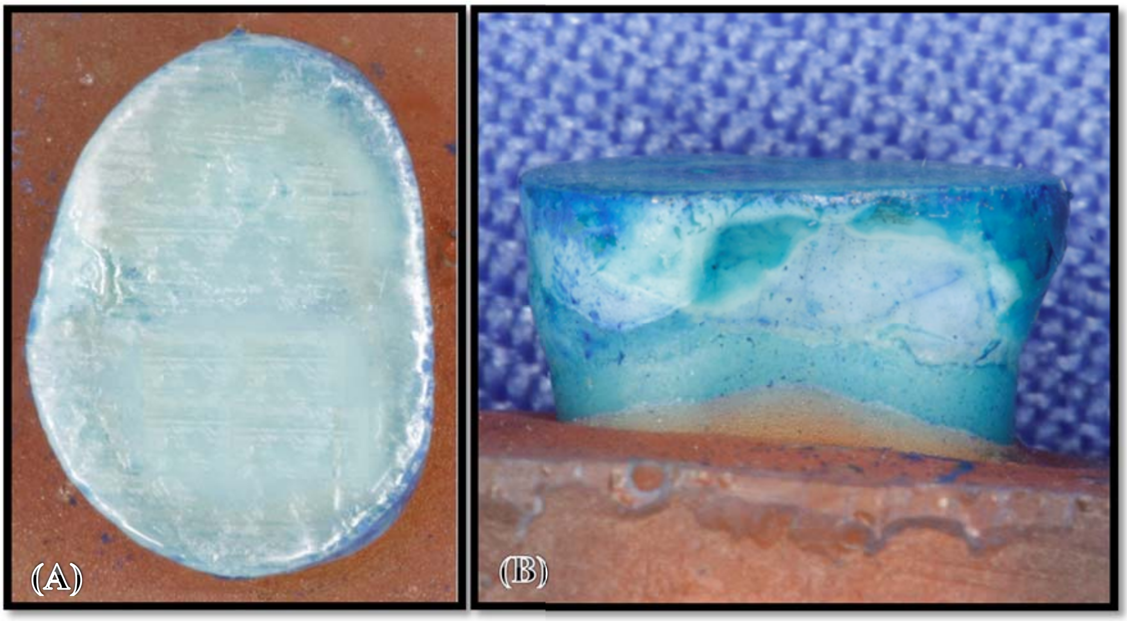


Figure (3-3): Adhesive failure (A) occlusal view (B) lateral view**.**

Chapter Four

Discussion

Discussion

4.1 Teeth collection

Sound maxillary premolars extracted for orthodontic demand were included in this study. Patients' ages were between 14 and 30 years to minimize the effect of reparative dentin on the topographical details of the dentin surface and permeability of dentinal tubules which are two factors that could affect the degree of resin penetration inside the tubules (Khouri *et al.*, 1997).

4.1.1 Chlorhexidine

Several *in vitro* and *in vivo* studies suggested the use of CHX solution to acid-etched dentin for 60 seconds could maintain the stability of adhesion to dentin over time while bond strength significantly decrease in teeth without CHX application (Brackett *et al.*, 2007; Carrilho *et al.*, 2007).

The concentration of CHX for this study was 2% resembling with what was stated in many previous studies (de Castro *et al.*, 2003; Carrilho *et al.*, 2007; Loguercio *et al.*, 2009; Boruziniat *et al.*, 2013).

It has been stated that higher concentration can completely stop MMPs 2,8 and 9 activity by protein denaturation (Loguercio *et al.*, 2009), while low concentrations like 0.0001%, 0.02% and 0.002 can reduce protease activity by acting as chelators for zinc and calcium ions essential for MMPs activation (Gendron *et al.*, 1999), However, a study found that concentration of CHX below 2% is unable to preserve the bond strength and may reduce it immediately (Campos *et al.*, 2009).

4.1.2 Ethanol

The ethanol wet-bonding concept has been suggested as a technique to overcome problems associated with the collapse of the collagen matrix if water is removed from the surface (Pashley *et al.*, 2007). 100% ethanol has been shown to be able to expand and maintain collagen fibrils apart, it can be used to replace water, leaving demineralized dentin saturated with ethanol promoting resin infiltration (Hosaka *et al.*, 2009).

4.2 Discussion of results

4.2.1 Effect of storage period

4.2.1.1 Control groups

The results of this study revealed that there is no statistically significant difference in SBS means between subgroup A1 and A2. This result can be explained by that the action of MMPs in the three months may be insufficient for producing significant bond strength reduction and this finding is supported by De Munk *et al* study (2009) who found non-significant difference between samples tested immediately and after 3 months of storage using microtensile bond strength test.

However, after six months of aging, statistically significant and highly significant differences were found as compared with the control groupwhich indicates the continuous reduction of bond strength and loss of durability over time.

The loss of SBS could be due to the following:

a. Human dentin contains collagenase (MMP-8), gelatinase (MMP-2) and (MMP-9), which are zinc-calcium endopeptidase trapped within the mineralized dentin matrix. When dentin is etched, these peptidases are activated allowing slow degradation of the unprotected collagen fibrils at the bottom of the hybrid layer (Hashimoto *et al.*, 2003a; Chaussain-Miller *et al.*, 2006).

b. Inability to expel water completely from the expanded collagen fibrils may result in breakdown of uncovered collagen fibrils (De Munck *et al.*, 2003). Water is considered as an obligatory requirement for the action of MMPs, as no loss of bond strength over time was observed if mineral oil was used as a storage medium instead of water. Water is used in etch and rinse adhesive system to remove phosphoric acid and keeping collagen fibrils distended for resin (Chaussain-Miller *et al.*, 2006; Sulkala *et al.*, 2007).

c. Plasticization of adhesive which may happen with time because of water sorption by the hydrophilic resins in the hybrid layer which cause hydrolytic degradation of unreacted adhesive resin with reduction of bond strength over time. After hydrolysis, resin elutes through nano-channels exposing collagen fibrils and enhancing the loss of bond durability over time (Wang and Spencer, 2003).

These results are supported by the studies of (Munoz *et al.*, 2015; Nagi, 2015) who found significant difference between the control samples tested immediately and after six months using microtensile bond strength test.

4.2.1.2 Chlorhexidine groups

Comparing group B, no statistically significant differences were found among them. The most reasonable explanation for this finding would be the ability of CHX to inhibit matrix metalloproteinase firstly by chelating calcium and zinc ions responsible for MMPs activation resulting in decreased degradation of the collagen fibrils over time (Gendron *et al.*, 1999) and secondly by protein denaturation at higher concentration (Loguercio *et al.*, 2009).

This result is supported by the studies of (Carrilho *et al.*, 2007; Loguercio *et al.*, 2009; Loguercio *et al.*, 2016) who used micro tensile bond strength tests of composite to dentin and found non-significant difference between samples tested immediately and after the storage period. The study of (Shafiei *et al.*, 2017) was also supporting this study by using push out bond strength test on fibre posts cemented to root dentin using total etch technique with CHX after two years of storage, when comparing the control group with group treated with CHX; a statistically significant higher reduction in bond strength could be seen in the control group.

The *in vivo* study of (Brackett *et al.*, 2007) partially supports the result of this study. Transmission electron microscope was used to examine the degradation of hybrid layer after six months retention inside patients' mouth. A slight degradation in the hybrid layer was observed in the control group with no degradation in the CHX group. However, microtensile bond strength was also tested and no significant difference was found between the groups and this could be attributed to the different environments of these two studies.

Although non-significant difference was found among subgroups, subgroup B3 was lower than B2 and the latter was lower than B1 in SBS and this finding could be explained by that collagenolytic degradation was reduced but did not stop completely to prevent any loss in bond strength. Also water can leach out uncured and hydrophilic monomer in adhesive systems resulting in loss of bond strength over time as modern adhesive systems contain high percentage of hydrophilic monomers to enable bonding to wet dentin substrate, but this may produce permeable unstable resin matrices that are liable to water sorption, resin leaching and hydrolysis over time (Malacarne *et al.*, 2006; Zhang and Kern, 2009; Boruziniat *et al.*, 2013).

4.2.1.3 Ethanol groups

Concerning Group C, results showed no statistically significant difference among its subgroups. This may indicate the ability of ethanol to preserve bond strength and improve the durability. Ethanol can expel water from dentin providing better medium for resin infiltration and enveloping more exposed collagen fibrils and preventing their degradation by MMPs (Nishitani *et al.*, 2006b; Sadek *et al.*, 2010), it also creates a more hydrophobic environment reducing water sorption over time which is a key factor in bond degradation (Tay and Pashley, 2003; Malacarne *et al.*, 2006; Zhang and Kern, 2009) .

This finding is supported by the studies of Sadek *et al* (2010), Ekambaram *et al* (2014), Simoes *et al* (2014b), Venigalla *et al* (2016) who studied the microtensile bond strength of ethanol wet bonding in different periods of time. And found a significant difference between the control group and the group treated with ethanol wet bonding.

However, this finding disagrees with the results of Ayar (2015) who found no statistically significant difference between ethanol and control group after six months of storage. The reason behind this contrary in results could be attributed to the use of bovine incisors as a substrate for bonding which differ from human teeth and do not simulate natural situation.

Even though non-significant; time dependent reduction among subgroups could be seen when looking at the results and this could be explained by the same causes mentioned for the CHX groups.

4.2.1.4 Ethanol and Chlorhexidine groups

Comparing the three subgroups of group D showed preservation of bond strength over time. The reason for this finding must be clear as this technique might combine the positives of CHX and ethanol, as ethanol expel water thus improving resin infiltration and creates a more hydrophobic hybrid layer which reduce water sorption (Tay and Pashley, 2003; Malacarne *et al.*, 2006; Nishitani *et al.*, 2006b; Zhang and Kern, 2009; Sadek *et al.*, 2010) while CHX act as an MMP inhibitor reducing hydrolysis of collagen fibrils thus improving the durability of the bond (Gendron *et al.*, 1999; Brackett *et al.*, 2007; Breschi *et al.*, 2009; De Munck *et al.*, 2009).

This result is supported by the study of (Ekambaram *et al.*, 2014). Another study by (Manso *et al.*, 2014) used two types of bonds, Excite (Ivoclar Vivadent) and All bond 3 (Bisco) and their effect on microtensile bond strength when used with CHX and ethanol. Adhesion with Excite stabilized the bond after 15 months while All bond 3 did not, the reason behind that may be the higher ethanol content of All bond than Excite (ethanol content for AB3 49%, Excite 2%), this high content may interfere with the bonding procedure by diluting resin excessively and preventing the formation of good quality hybrid layer.

4.2.2 Effect of adhesive procedure on shear bond strength

4.2.2.1 Immediate shear bond strength

In this study, statistically non-significant difference was found between the four groups tested immediately, and this means that neither positive nor negative influence of the different procedures could be seen when compared to control group and this agree with (Khalil and Al-Shamma, 2015) and supported by the study of (Loguercio *et al.*, 2009; Manso *et al.*, 2014). It is also supported by the study of (Guimaraes *et al.*, 2012) which was a microtensile bond strength test study for ethanol assessment and no significant difference was found between the control and ethanol group.

A deeper look in the results may reveal few facts about the effect of those different treatment procedures. Even though non-significant difference was found among groups, ethanol showed slightly higher immediate bond strength and this can be attributed to the fact that ethanol is a solvent that can dissolve hydrophilic and hydrophobic resin monomers and load collagen fibrils with these monomers more efficiently improving the quality of the hybrid layer (Pashley *et al.*, 2007; Cadenaro *et al.*, 2009).

This finding is supported by the studies of (Ekambaram *et al.*, 2014; Venigalla *et al.*, 2016).

CHX was also able to improve immediate bond strength non-significantly in this study and this agree with (Perdigao *et al.*, 1994; de Castro *et al.*, 2003; Khalil and Al-Shamma, 2015) and this finding could be attributed to certain CHX properties, including strong positive ionic charge, ready binding to phosphate groups, strong affinity to the tooth surface which is increased by the acid etching and finally an increase in surface free energy of dentin are the likely reasons responsible for the good resin-dentin bond strengths obtained when CHX was applied after acid etching (Perdigao *et al.*, 2002).

The combined use of CHX and ethanol showed non-significant increase in bond strength immediately compared to other groups. Ethanol and CHX might act synergistically to improve the bond. This result is supported by (Ekambaram *et al.*, 2014; Manso *et al.*, 2014).

4.2.2.2 Shear bond strength after three months

Results showed non-significant difference between the four groups after three months of storage and this can be explained as mentioned previously that three months’ period may be insufficient for occurrence of severe drop in bond strength allowing significant difference among groups. This finding is supported by the study of (De Munck *et al.*, 2009) who found non-significant difference between control and chlorhexidine group after three months using microtensile strength tests. The results of (Khalil and Al-Shamma, 2015) disagree with this study as they found significant difference between control and chlorhexidine group after three months and this could be attributed to the different adhesive agents and composite materials used in these two studies.

4.2.2.3 Shear bond strength after six months

When comparing the four groups after six months, a highly significant difference was found between the control and the other groups in mean SBS.

4.2.2.3.1 Control group vs CHX group

The control group showed a highly significant lower SBS after six months when compared with CHX group and this may reveal the role of CHX as MMPs inhibitor to prevent collagen degradation thus improving the durability of bond strength, this finding is supported by the studies of (Brackett *et al.*, 2007; Carrilho *et al.*, 2007; Loguercio *et al.*, 2009; Boruziniat *et al.*, 2013; Loguercio *et al.*, 2016; Shafiei *et al.*, 2017).

4.2.2.3.2 Control group vs ethanol group

The control group also showed a highly significant lower SBS than the ethanol group after six months which indicates the preservation of bond strength over time in the ethanol group. It can be explained as mentioned previously that ethanol acts as a solvent for both hydrophilic and hydrophobic resins found in the adhesive systems thus improving resin infiltration and coating the exposed collagen fibrils protecting them from MMPs activity and improving the bond durability (Pashley *et al.*, 2007; Cadenaro *et al.*, 2009).This result is supported by (Sadek *et al.*, 2010; Ekambaram *et al.*, 2014; Simoes *et al.*, 2014a; Venigalla *et al.*, 2016).

4.2.2.3.3 Control group vs CHX and ethanol (mixed)

A high significant difference was found between the control and the mixed group after six months due to the high drop in the control group and this can be explained by the anti-degradation properties related to CHX and ethanol as mentioned previously, this finding is supported by the study of (Ekambaram *et al.*, 2014).

4.2.2.3.4 CHX group vs ethanol group

Non-significant difference could be seen between these two groups after six months of storage. Even though ethanol and CHX act by different mechanisms to protect the hybrid layer, results showed that both of them are equally active after six months. This finding is supported by the study of (Ekambaram *et al.*, 2014).

In contrast, Sadek *et* al (2010) found a significant difference between ethanol and CHX. The microtensile bond strength results was higher for the ethanol group after 18 months of storage. The reason behind that could be due to the use of experimental hydrophobic adhesive resin with the ethanol group which creates a better hydrophobic environment reducing water sorption and bond degradation upon storage.

4.2.2.3.5 CHX and ethanol groups vs mixed group

No significant difference was found between CHX and mixed group from one side and also no significant difference was found between ethanol and mixed groups from the other side. This finding may indicate the poor synergistic effect between CHX and ethanol when mixed together to produce a more durable bond with significant difference. This finding is supported by the study of (Sadek *et al.*, 2010) as they applied ethanol after CHX application in one group and no significant difference could be seen from the group with the sole ethanol application.

The study of (Ekambaram *et al.*, 2014) is also supporting this study as they found non-significant difference when they add CHX to ethanol and compare it to ethanol group only.

4.2.3 Mode of failure

The mode of failure found in this in vitro study was either adhesive or mixed failure with no cohesive failure in dentin or composite.

The majority of failures were mixed type of failure and this can be explained as no degradation in the hybrid layer occurred in this short period results in higher SBS values and failure was partially in composite and partially in the hybrid layer. The highest subgroup with mixed failures was subgroup D1 while other subgroups showed less mixed failures reflecting lower SBS values. This finding agrees with (Khalil and Al-Shamma, 2015).

More adhesive failures could be seen after three months indicating the continuous degradation seen in most of groups. subgroup B2 an D3 were higher than A2 and C2 in numbers of cohesive failures and this finding also agrees with (Khalil and Al-Shamma, 2015).

After six months, all of the four groups showed increase in numbers of adhesive failures and this may indicate the degradation of the hybrid layer which became the weakest chain where the fracture occurred. The subgroup with the highest number of adhesive failure was the control subgroup A3 and this coincide with the fact that this group was the lowest in SBS mean value after six months. The lowest group with adhesive failure was group D3 and this coincides with the finding that this group showed the highest mean value of SBS.

Chapter Five

Conclusions & Suggestions

Conclusions and Suggestions

5.1 Conclusions

Within the limitations of this study, the following conclusions could be stated:

1- At 24 hrs and after three months, there is no significant difference in shear bond strength of composite to dentin between the control group and all experimental groups in which dentin surface was treated with 2% CHX and/or 100% ethanol.

2- After six months, deterioration of the shear bond strength of composite to dentin was seen in the control group.

3- After six months, dentin surface treatment with 2% CHX and/or 100% ethanol for 60 sec. after acid etching and prior to adhesive application was effective in preventing the deterioration in shear bond strength of composite to dentin.

4- No statistically significant difference was seen between the use of 2% CHX alone, 100% ethanol alone or their successive application even though the latter showed higher mean value of shear bon strength of composite to dentin

Depending on the results of the study, we recommend the combined use of 2% CHX and 100% ethanol after acid etching as a pre-adhesive application step or at least the use of one them to reduce the deterioration in bond strength and improve the longevity of the composite restoration.

5.2 Suggestions

For future studies, the following ideas are suggested:

1. Studying the effect of thermocycling and mechanical loading on the long term effect of chlorhexidine and/or ethanol on the shear bond strength of composite to dentin.
2. Study of the dentin composite interface pre-treated with chlorhexidine and/or ethanol under scanning electron microscope.
3. Immediate and long term effects of chlorhexidine, ethanol and their combination on the microtensile bond strength of composite to dentin.
4. Studying the effect of other MMPs inhibitors like Galardin or green tea extract on shear bond strength of composite to dentin.
5. Effect of chlorhexidine, ethanol and their combination on microleakage at the composite to dentin interface.

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Appendices

Appendix 1: The shear bond strength values for all of the tested groups in MPa

24 hrs				3 months				6 months			
A1	**B1**	**C1**	**D1**	**A2**	**B2**	**C2**	**D2**	**A3**	**B3**	**C3**	**D3**
7.96	9.15	10.74	10.62	7.21	7.83	6.34	8.65	6.01	7.17	7.84	8.44
11.03	8.22	9.03	8.08	6.76	9.68	6.65	8.05	5.47	6.56	6.76	7.84
6.37	8.87	8.22	10.57	8.63	6.28	10.95	8.29	5.11	6.45	8.63	8.08
7.64	9.95	8.48	8.48	7.3	7.45	6.3	7.46	6.9	7.23	9.07	7.25
8.87	6.1	8.08	8.29	7.45	10.85	8.74	8.17	6.14	8.86	6.19	7.95
9.03	9.13	7.5	7.32	7.84	8.16	7.72	7.11	6.49	7.46	6.9	6.79
8.12	7.95	8.81	10.42	5.71	7.44	7.95	9.44	5.22	7.89	7.83	9.23
8.05	8.03	8.42	6.74	6.31	6.36	7.01	8.45	5.74	7.81	6.23	8.14

الخلاصة

يعتبر فقدان قوة الارتباط بين راتنج الكمبوزت و العاج من أهم المشاكل في الحشوات التجميلية.

أجريت هذه الدراسة لتقييم تأثير 2% كلورهكسدين, 100 % إيثانول أو الإثنين معا على قوة الربط القصي للكمبوزت مع العاج عند اختبارها بعد 24 ساعة, ثلاثة أشهر أو ستة أشهر.

تم جمع ستة و تسعين سن من الضواحك العلوية لهذه الدراسة المختبرية. تم غمر جذور الأسنان بواسطة مادة الأكريلك و تم قص السطح العلوي للسن لإزالة طبقة المينا. تم توزيع الأسنان بصورة عشوائية على أربعة مجاميعمتكونة من 24 سن لكل مجموعة كما يلي:

المجموعة أ: 24 سن تم تخريشها بحامض الفسفوريك ثم غسلها و تنشيفها بصورة متوسطة ثم نضيف المادة اللاصقة و إضافة حشوة الكمبوزت.

المجموعة ب: 24 سن تم تخريشها بحامض الفسفوريك ثم غسلها و تنشيفها بصورة متوسطة ثم ترطيبها بواسطة 2% كلورهكسدين لمدة ستين ثانية ثم نضيف المادة اللاصقة و إضافة حشوة الكمبوزت.

المجموعة ج: 24 سن تم تخريشها بحامض الفسفوريك ثم غسلها و تنشيفها بصورة متوسطة ثم ترطيبها بواسطة 100% إيثانول لمدة ستين ثانية ثم نضيف المادة اللاصقة و إضافة حشوة الكمبوزت.

المجموعة د: 24 سن تم تخريشها بحامض الفسفوريك ثم غسلها و تنشيفها بصورة متوسطة ثم ترطيبها بواسطة 2% كلورهكسدين لمدة ستين ثانية ثم 100% إيثانول لمدة ستين ثانية ثم نضيف المادة اللاصقة و إضافة حشوة الكمبوزت.

كل مجموعة تم تقسيمها لمجموعات أصغر متكونة من 8 أسنان, مجموعة 1 تم إختبارها بعد 24 ساعة, مجموعة 2 بعد 3 أشهر و مجموعة 3 بعد ستة أشهر.

تم وضع راتنج الكمبوزت على العاج بمساعدة قالب خاص للحصول على طريقة مثالية و قابلة للتكرار في وضع الكمبوزت. جميع الأسنان تم حفظها في الماء المقطر حتى وقت البحث. تم إختبار الأسنان بواسطة الجهاز المعروف ب (universal testing machine).

النتائج المحصلة تم تحليلها إحصائيا بواسطة إختبار تحليل التباين بإتجاه واحد (one way ANOVA) و الإختبار الإحصائي المعروف ب (Tukey test) و تم إثبات وجود فوارق ذات دلائل إحصائية عالية بين المجاميع و داخل المجموعة أ.

آلية فشل العينات تم تحليلها بإستخدام صبغة المثلين الزرقاء مع كاميرا دجيتال و عدسة تكبير عالية. كل المجاميع التي تم إختبارها بعد 24 ساعة أظهرت فشلا مختلطا, بينما أظهرت زيادة في نسبة فشل الإلتصاق بعد 3 و 6 أشهر و كانت أعلى نسبة في الفشل الإلتصاقي في المجموعة أ و أقلها في المجموعة د.

بالنتيجة, 2% كلورهكسدين أو 100% إيثانول أظهرا القدرة على الحفاظ على قوة اللصق بعد ستة أشهر من الخزن و لكن لم يوجد فرق إحصائي بين الكلورهكسدين و الإيثانول عندما تم إستعمالهم كل على حدة أو عند مزجهما مما يثير التساؤل حول فائدة مزجهما لإطالة عمر قوة الإلتصاق.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد
كلية طب ألاسنان

التأثير الأولي و الطويل الأمد للكلورهكسدين, الإيثانول و الإثنين معا على قوة الربط القصي لراتنج الكمبوزت مع العاج

(دراسة مختبرية)

رسالة مقدمة إلى

مجلس كلية طب الأسنان \جامعة بغداد كجزء من متطلبات نيل شهادة الماجستير في معالجة الأسنان

من قبل

أحمد عبد الرسول عبد الحسين

بكلوريوس طب و جراحة الفم و الأسنان

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دكتوراه معالجة الأسنان

2018 م **1438 هـ**