Shear Bond Strength of Composite Resin to dentin using a Vth Generation Bonding Agent Optibond Solo before and after dentine deproteinization –An In vitro study and SEM analysis

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ABSTRACT- This in vitro study is aimed to evaluate the influence of collagen removal with 5% sodium hypochlorite on the shear bond strength of composite resin to dentin before and after removal of collagen layer following acid conditioning using a Vth generation bonding agent. Twenty recently extracted premolar teeth were selected for the study. The samples were divided into two main groups of ten specimens each according to the dentin surface pre-treatment .The enamel on the occlusal surface of teeth was ground to expose dentine and the surface was made flat. . Each of the prepared 20 teeth were individually embedded in rectangular blocks of self cure acrylic resin. In one group Optibond Solo adhesive(Kerr manufacturing Co) was used to bond the composite specimen after acid conditioning of the dentinal surface . In the other group acid conditioning was followed by Sodium hypochlorite treatment of the dentinal surface using the same adhesive. The test specimens were subjected to Hounsefield tensometer, type W horizontal 2 ton to test the shear bond strength.. The present study concludes that dentin deproteinization treatment with 5% sodium hypochlorite enhanced the shear bond strength of composite resin when used along with a Vth generation bonding agent., Optibond Solo adhesive (Kerr manufacturing Co).

Index Terms— Deproteinization, Hybrid Layer ,Optibond Solo, Reverse Hybrid Layer ,Shear Bond Strength, Sodium Hypochlorite

1. INTRODUCTION

Adhesion of restorative materials to enamel has become routine and reliable aspect of modern adhesive restorations. But dentinal adhesion has proved to be more difficult and less predictable. Advancements in dentine bonding agents have helped achieve optimal bond strength but degradation of this bond over time remains an issue till date. Much of the difficulty in bonding to dentine is the result of the complex histologic structure and variable composition of dentine itself. Moreover dentine is intimately connected to the pulpal tissues and numerous fluid filled channels or tubules traverse through dentin from pulp to the dentino enamel junction. This makes dentine an inherently wet substrate on which bonding is unpredictable due to poor surface wettability of the bonding agents [1].

Current theories on dentine bonding suggest that the mineral phase should be removed without damaging the collagen matrix with the help of acid conditioning. Microscopic spaces created by removal of inorganic minerals must be filled with an adhesive resin that penetrates the exposed collagen fibril network extending into the partially demineralized dentin which is later polymerized hard. This resin infiltrated dentin layer is called the Hybrid Layer. Formation of this hybrid layer was first described by Nakabayashi et al in 1982 and is thought to be the primary bonding mechanism of most current adhesive systems[2].

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Complete resin penetration of chemically altered dentine is paramount in achieving optimal bond strength. But it is found that dentine bonding agents do not fully diffuse through the collagen network that remains after acid conditioning of dentine. Failure to adequately penetrate the collagen network into the partially demineralised dentine may produce a weak porous layer of collagen protected neither by hydroxyapatite nor encapsulated by resin. Subsequent hydrolysis of exposed collagen fibrils would lead to degradation of the bond resulting in decreased bond strength and increased micro leakage in the long run.[3] Removal of organic collagen following acid conditioning and subsequent bonding onto partially demineralized dentine layer may produce more durable adhesion to the hydroxy apatite component of the dentine substrate. Sodium hypochlorite is a non-specific proteolytic agent that effectively removes organic compounds at room temperature.[4]

This in vitro study is aimed to evaluate the influence of collagen removal with 5% sodium hypochlorite on the shear bond strength of composite resin to dentin after following acid conditioning using Optibond Solo(Kerr manufacturing Co) a Vth generation bonding agent and also to examine the interdiffusion zone under SEM."

2 REVIEW OF LITERATURE

Many studies have shown that the deproteinization achieved by removal of collagen fibrils with sodium hypochlorite have yielded better bond strength.[5] The longevity of hybrid layer within esthetic restorations is critical as this layer undergoes hydrolysis overtime due to the presence of collagen fibrils[6].A study conducted on shear bond strength on demineralized and remineralized dentine after hypochlorite treatment of acid etched dentine showed that NaoCI treatment of the dentin yielded better bond strength [7]. The decalcification protocol of dentin surface led to a more porous dentin, while the application of NaOCI removes the exposed collagen fibrils.[8] The action of NaOCI on collagen removal is well determined in the literature due to its known nonspecific proteolytic activity. Studies done on deproteinization of collagen layer after acid etching showed increased bond strength to conventional hybrid layer, Many studies conducted on the effect of sodium hypochlorite have shown that NaOCl is believed to improve the longevity of the bonding interface .[9]

3 MATERIALS AND METHODS

Twenty recently extracted premolar teeth for orthodontic purpose were selected for the study. A loop made of stainless steel wire of gauge 23 mm and a rectangular mould of dimension 4 cm x 2 cm x 0.6 cm made out of mild steel was employed for the present study. The teeth were thoroughly cleaned and used within one month of extraction and storage. A circular Teflon mold of height 2 mm on which two circular holes of diameter 3.4 mm punched was prepared for the study. Hounsefield Universal Testing machine was used for testing the shear bond strength. Manually operated dynamic load generation system was used with cross head speed of 0.5 mm/minute and a load range of 0-30 kg.

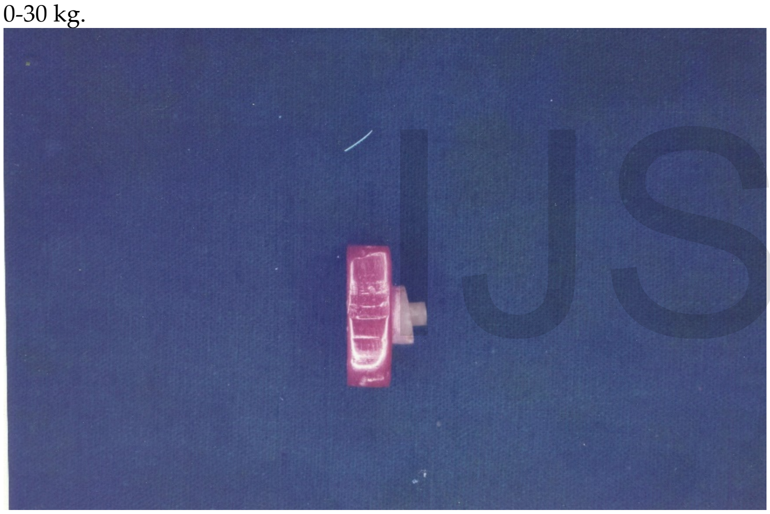

Fig-1 Specimen showing composite specimen bonded to conditioned dentine

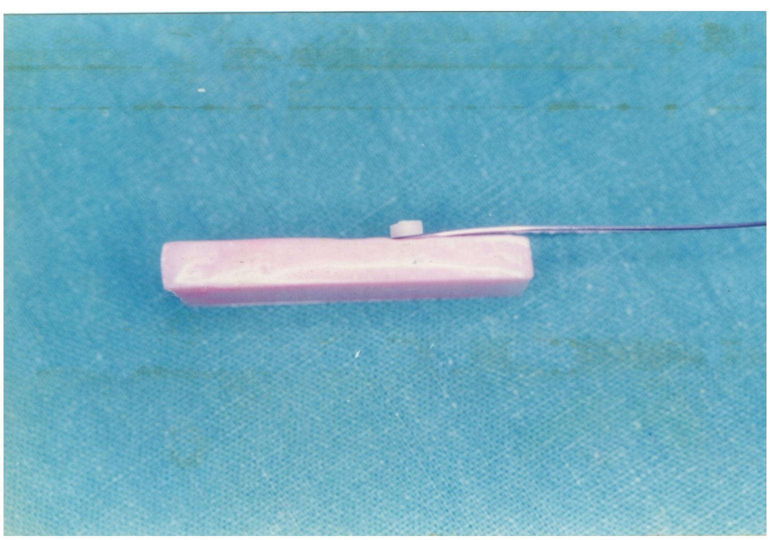

Fig-2 Wire loop contoured around the base of cylinder

3.1 Preparation of Specimens

20 Teeth were divided into 2 groups of 10 teeth each.

Group I OptibondSolo Adhesive (Kerr Manufacturing Co) adhesive was used to bond the composite specimen after acid conditioning of the dentinal surface

Group II OptibondSolo Adhesive(Kerr Manufacturing Co) adhesive was used to bond the composite specimens after acid conditioning followed by 5% Sodium hypochlorite treatment of the dentinal surface for 2 minutes.

The enamel on the occlusal surface of the experimental teeth in group I and II were ground to expose dentine. The exposed dentine was made into a flat surface using standard diamond disks and points uniformly. The teeth were sectioned below the cervical line .A rectangular mould of dimension 4 cm x 0.6 cm was utilized to embed the prepared tooth in self cure acrylic resin. Each of the prepared 20 teeth were individually embedded in rectangular blocks of self cure acrylic resin with the dentine on the occlusal surfaces exposed. All specimens were stored in buffered saline solution.

3.2 Method

The stored specimens were retrieved and the dentinal surfaces were cleaned with slurry of pumice using a rubber cup. All the 20 specimens were then washed with water under air pressure and then dried with air from an oil free air source.

In Group I the dried dentin surface was treated with phosphoric acid gel etchant (37%) for 15 seconds, rinsed with water for 30 seconds and dried optimally to remove excess water leaving back a moist surface. OptibondSolo adhesive was applied according to the manufacturer's instructions. The excess solvent was removed with air syringe and light cured for 10 seconds. For optimal results a second layer was applied and solvent removed immediately and light cured for another 10 seconds. The Teflon mould was located at the predesigned location. With the help of a cement carrier composite resin (Z 100 composite resin) was artfully teased into the circular punch hole to obtain a cylinder of diameter 3.4 mm and height 2 mm. The composite was then light cured for 40 seconds. Once the composite was cured the Teflon mould was gently lifted out.

In Group II the dried dentine surface was treated with 37% phosphoric acid gel for 15 seconds. After etching these specimens were treated with 5% sodium hypochlorite for 2 minutes, then rinsed and dried to remove excess water. After this OptibondSolo Adhesive bonding agent was applied on to the dentinal surface. Oil free air was blown gently to remove excess solvent. This layer was polymerized for 10 seconds. For optimal bonding a second layer of bonding agent was applied, excess solvent removed quickly and polymerized for another 10 seconds. Following this the Teflon mould was located at the pre designed location. With the help of a cement carrier composite resin was artfully teased into the punch hole of the above mentioned dimension. The composite resin was cured for 40 seconds. After polymerization the Teflon mould was gently lifted out. The teeth in the different groups were labelled and acrylic blocks colored for reference.

All the 20 test specimens were stored under humid conditions and kept in an incubator at 37° C for 24 hours.

3.3 Testing Shear Bond Strength

The mounted prepared tooth was held in one grip of the shear chuck available especially for testing shear bond strength. The inner surface of configuration of the shear chuck is so contoured as to hold firmly the acrylic block in which the test specimen is embedded. The wire loop was contoured to hold the base of the cylinder. The two parallel arms of the wire loop were then held in the other grip of testing shear chuck. Care was taken not to unnecessarily tense the wire. The loading was done incrementally until the shearing of composite cylinder from the dentinal surfaces occurred.

3.4 Equipment Operation Details

The equipment was adjusted to operate on a load range of 0-30 kgs. A cross head speed of 0.5 mm/minute was used. The breaking load was measured by tabulating the increase in mercury column provided by the mercury scale. The breaking shear loads in kilograms were then converted into bond strength in mega pascals.

3.5 Specimen Preparation for SEM Study

SEM study was carried out to confirm the effect of 5% NaOCI exposure on acid conditioned dentinal surface. Specimens both with 5% NaOCI wash and without 5% NaOCI wash were prepared and bonded with OptibondSolo (Kerr Manufacturing Co) adhesives . Two specimens from each group (same as previously mentioned) were prepared and evaluated under SEM.

Freshly extracted premolar teeth were ground to expose dentine. The exposed tooth surface was cleaned with pumice slurry and dried later. Acid etching was carried out using 37% phosphoric acid gel for 15 seconds. In group I - the acid conditioned dentinal surface was rinsed with water for 30 seconds and air dried optimally for 15 seconds to leave back a moist surface. OptibondSolo Adhesive (Kerr Manufacturing Co) was applied and light polymerized for 20 seconds. In group II NaoCI was used after acid conditioning for 2 minutes. Composite resin was applied flat over the entire dentinal surface alone and cured for 40 seconds.

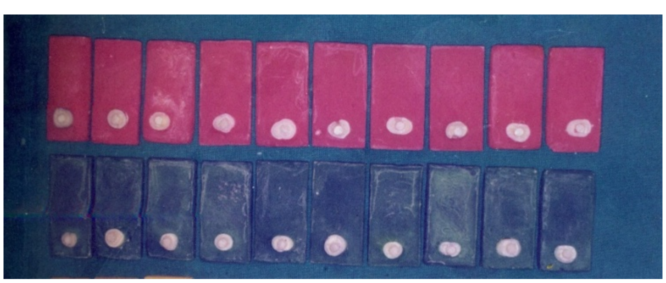

Fig-3 -Prepared specimens

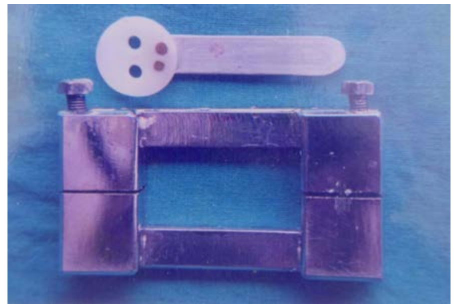

Fig-4 -Teflon dye

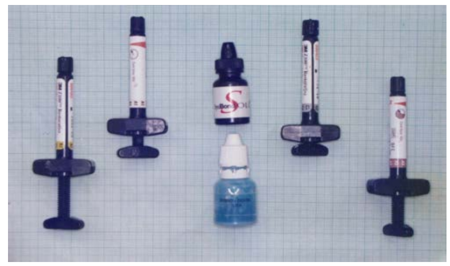

Fig-5-Optibond solo bonding agent & Z 100 composite

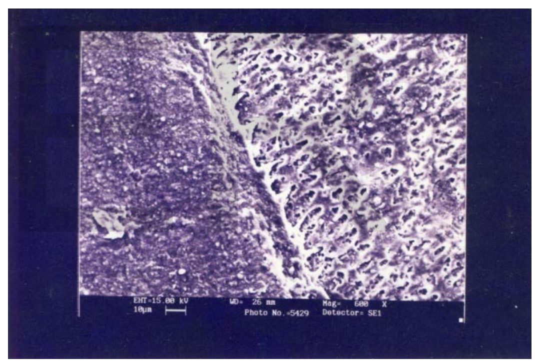

Fig-6- Resin tag penetration after NaoCl Wash

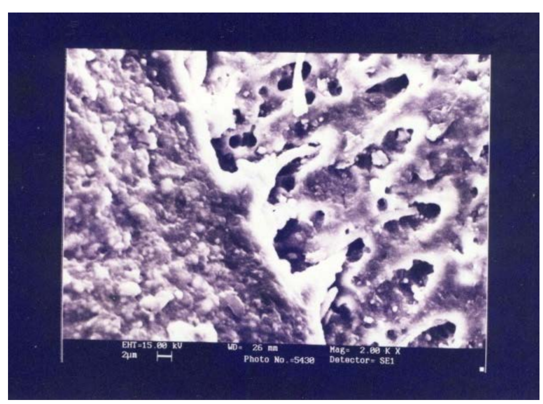

Fig-7- Resin tag penetration after higher magnification

4. RESULT INTERPRETATION

The shear bond strength of composite resin to exposed dentinal surface with and without hybrid layer using Optibond Solo (Kerr manufacturing Co) adhesive was evaluated. 20 specimens were collected and grouped into 2 groups for the purpose of this study. Table I - Represents shear bond strengths in MPa. Graph I - Shows comparison of shear bond strength among the groups. According to the results summarized, Group I (Optibond Solo without NaOCI treatment) showed the less bond strength, 18.65Mpa. when compared to that after NaoCI wash, 21.13 Mpa. Students t test was carried out to find out the standard deviation and significance of the study. The study was statistically significant(p<0.005)

TABLE -1
SHEAR BOND STRENGTH IN MPA

SL. NO	OPTIBOND	OPTIBOND (Naocl wash)
1	16.41	20.02
2	16.20	19.3
3	17.26	15.3
4	17.41	20.4
5	16.20	25.6
6	17.50	18.2
7	18.60	16.9
8	17.72	23.2
9	18.61	15.8
10	15.91	21.0

TABLE-2
INDEPENDENT SAMPLE TEST

	Levenes test for equality of variance		T test for equality of variance		
	F	Sig	T	df	sig
SBS equal variances assumed	6.497	.020	-2.1356	18	.047
equal variances not assumed			-2.136	10.888	.056

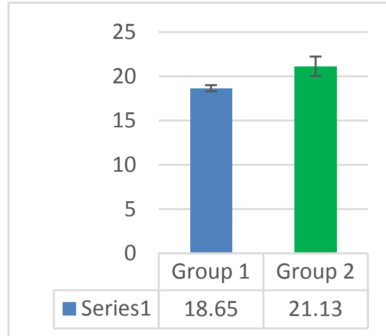

Fig-7 -Graph showing comparison of shear Bond strength between Group I and Group II

DISCUSSION

Unlike enamel bonding, dentine bonding is considered to be more difficult to attain clinically because of many reasons. The exact nature of this bond is unknown; whether it is primarily chemical, micromechanical or a combination of both is a matter of debate. It is well known that dentin has relatively high organic and water content (inorganic -70%, organic - 18% and water 12%) which inevitably complicates bonding procedures[8]. In addition the morphology and compositional nature of dentine is highly variable [5].Apart from these factors the presence of smear layer on dentinal surface and the pulpal response towards these materials further complicates dentine bonding procedures[1]. Finally the polymerization contraction shrinkage of composite resin materials results in formation of gaps at the resin - dentine interface. This in turn causes ultimate bond failure leading to loss of restoration or marginal leakage with recurrent cervico- gingival marginal caries. Only recently have dentine adhesive systems produced laboratory results that approach those of enamel bonding and achieve a predictable level of clinical success.

Dentine adhesion is considered to work on the following strategies-

1. Bonding via resin tag formation in the tubules of conditioned dentine.
2. Formation of precipitate on pre - treated dentine surface followed by chemical or mechanical bonding.
3. Chemical union to either organic or inorganic component of dentine.

Newer adhesive systems rely on a fourth concept for bonding put forward by Nakabayashi etaI in 1982 - "The concept of hybridization of dental hard tissues". Hybridization of dentine is a process that creates a molecular -level mixture of adhesive polymers and dental hard tissues.

Intact mineralised dentine does not permit monomer resin diffusion in clinically relevant time periods. Therefore the dentine has to be suitably conditioned to create channels between collagen fibrils to allow monomers which have good affinity for demineralised dentine

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to diffuse into the substrate. This demineralization permits diffusion of monomers into these hard tissues (enamel and dentine) to form what is called the "Hybrid Layer". Briefly the substitution of resin for mineral in the subsurface of mineralized tissues is the essence of the creation of a hybrid layer[10].Fifth generation bonding agents combined the priming and resin application steps to achieve bonding with one component resin formula. Similar to 4tn generation bonding agents, the 5tn generation bonding agents rely on hybridization to achieve adhesion. These materials generally rely on residual moisture in dentine and hydrophilic water chasing composition to effect resin penetration. The bonding agent contains BISGMA., BPDM (Biphenyl dimethacrylate) and HEMA in an acetone solvent carrier. Some other formulations may contain PENTA (Phosphate adhesion promoter) TEGDMA and an elastomeric UDMA resin in acetone. The composition of Optibond solo is HEMA, GPDM and BISGMA in an ethanol/ water system. It also contains fumed silica and barium fillers to a level found one half of that found in multi component system. Mean shear bond strength achieved exceeded 20 MPA. One bottle systems simplify the clinical procedure by reducing the bonding steps and thus the working time. [1]

Current theories on dentine bonding suggest that 2 fundamental processes are involved in bonding an adhesive to dentine-

1. First the mineral phase must be extracted from the dentine substrate without damaging the collagen matrix; and
2. The micro spaces left back by the mineral must be filled with an adhesive resin that penetrates the exposed collagen fibril network into the underlying partially demineralised dentine.[11]

Concerns have been raised that dentine bonding agents do not fully diffuse through collagen network that remains after acid conditioning. Hydrolysis of these bands of exposed collagen not protected either by resin or by dentine would occur with long term exposure to water. This leads to deterioration of adhesion between resin and dentine resulting in decreased bond strength. Therefore attempts were made to remove the collagen completely and bond the resin directly onto partially demineralised dentine. A two minute exposure of 5% Sodium hypochlorite solution was found to satisfactorily wash of the collagen fibres.[7]

Twenty teeth were divided into four groups of ten teeth each

Group 1-Exposed dentinal surface was acid etched with 37% phosphoric acid gel for 15 seconds followed by rinsing for 30 seconds. Drying was done with oil free air in such a manner not to desiccate the dentine leaving back a moist surface. Optibond Solo(Ker manufacturing company) adhesive was applied and cured for 20 seconds followed by application of composite resin which was light cured later for 40 seconds from all sides. Mean shear bond strength attained was about 18.6530 Mpa which is in accordance with the average bond strength required i.e 17 Mpa.

Group II-The specimens were acid conditioned with 37% phosphoric acid gel for 15 seconds followed by rinsing for 30 seconds. The acid conditioned dentinal surface was exposed to 5% NaOCI for 2 minutes. This was followed by a rinsing and optimal drying. Drying was done in such a manner that a moist surface was left back. Single bond adhesive was applied and cured for 20 seconds. Composite resin of the afore mentioned dimensions were applied and polymerized for 40 seconds from all sides. Mean shear bond strength attained for this group was 21.1330Mpa

The study indicates that bond strength values were considerably higher when the collagen layer after acid conditioning was removed with 5% NaOCI.

This deproteinization of dentinal surface done after acid conditioning has found to be a better substrate for bonding in the case of Optibond solo. Studies have been carried out with different percentages of NaOCI like, 1.5% of NaOCI for 2 minutes and 5% NaOCI for two minutes. Both have shown to eliminate the collagen layer left back after acid conditioning. Here in this study the specimens were exposed to 5% NaOCI for two minutes after acid conditioning.

The phosphoric acid/NaOCI treatments produced a "new dentine substrate "as suggested by Inaba et al in that it had porous but mineralized surface[5].According to the present study for Optibond solo the acid etched and deproteinized dentine surface may represent a more suitable bonding substrate. When NaoCI was applied to the etched Dentin surface it would not only remove the exposed collagen fibrils but also solubilise the fibrils down in to the underlined mineralized matrix, leaving nano-sized porosities within the conditioned substrate.[12]

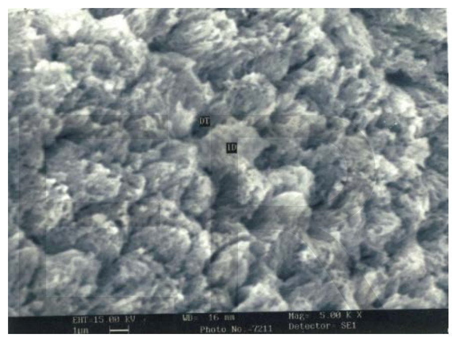

Fig-8-Dentin surface after acid conditioning without NaOCI wash.

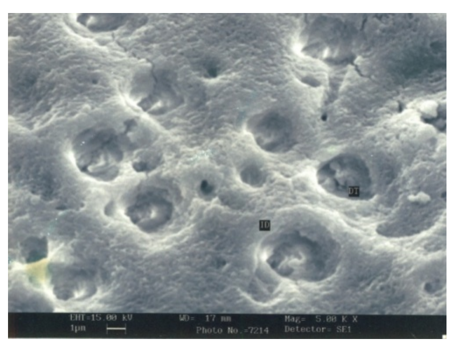

Fig-8- Dentin surface after acid conditioning after NaOCI wash.

The suggested reasons why Optibond solo gives a better bond strength in this new bonding substrate may be due to -

1. The adhesive monomer in Optibond solo is much acidic (pH-1.5-2) and may be able to re-etch the mineral phase of the collagen free dentine surface for a depth of 0.3-0.5 μm which is too shallow to be detected by SEM. This would then create a "nano hybrid layer or

nano RIDL" [5] (resin infiltrated dentine layer) The molecular size of Optibond solo adhesive is much smaller which accounts for its increased penetrability through collagen free mineralized surface. The application of NaOCI after acid etching and before the adhesive resin does not only remove the exposed collagen, produced by acid etching, but also solubilise the fibrils of the underlying mineralized matrix creating submicron porosities within the mineral phase[13]

2. The larger resin tags formed after acid etching and deproteinization contributes for increased total bond strength.
3. The presence of filler particles inside the resin tags of Optibond solo has been recently demonstrated by Perdiago in 1995. Thus the resin tags may permit more filler particles to enter into the tags there by increasing the shear bond strength.

The more retention and bond strength provided by Optibond solo on acid conditioned/ NaOCI dentine substrate can be due to the formation of a new phenomenon known as "Reverse Hybrid Layer". In conventional hybrid layer formation, the mineral phase of dentine is removed by acid conditioning and replaced by resin which is later polymerized.

In "reverse hybrid layer" formation acid conditioning removes the smear layer and exposes the collagen fibres of the dentine matrix. This is followed by application of NaOCI which not only removes the exposed collagen fibres but also solubilises the fibrils down into the underlying mineralized matrix to create submicron porosities within the mineral phase. Cylindrical channels (0.1 μm in diameter) previously occupied by collagen fibres are now available for resin infiltration within the mineralized matrix .More over NaoCI produced an acid -base resistant zone under hybrid layer thereby resisting secondary caries.[5]

Acid etching of mineralized dentine decreases its modulus of elasticity from a relatively stiff 17 GPA to a very low of 5 Mpa due to the removal of apatite crystals. Infiltration of resins into demineralised dentine partially restores the stiffness of matrix to a level of about 2 -6 Gpa. If the modulus of elasticity of acid etched/ deproteinized dentine is higher than 6 Gpa the higher apparent bond strength of Optibond solo may be due to improved mechanical properties of the substrate as well as the increased diameter of resin tags in dentinal tubules.

Another advantage of Optibond solo adhesive is that it contains fumed silica and barium fillers. These fillers provide better bond strength and also resist polymerization shrinkage stress preventing the detachment of the adhesive from the underlying hybrid layer. These observations provide evidence for an "elastic bonding concept" in which a sufficiently thick and relatively elastic or un or semi filled adhesive resin may have absorbed in part the polymerization shrinkage of composite material by elastic elongation preventing the interface from detachment. [14]

This enhanced bond strength may not be present in the case of some other bonding systems as NaoCI is an oxidising agent and interfere in the free radical polymerisation of resin cement at the resin dentin interface{13}

Research studies conducted regarding this new concept of bonding by C. Prati et al on the effect of removal of surface collagen fibrils on resin - dentine bonding showed that high bond strength can be obtained via "reverse hybrid layer" phenomenon caused by acid etching followed by NaOCI treatment when fifth generation bonding agents were used. Paulette Spencer et al examined the nature of unprotected protein i.e collagen left back after acid conditioning at the dentine adhesive interface. They found that this unprotected protein left back after acid conditioning served as the major inadequacy in long term bond stability.

The present study agrees with Gwinnet's conclusion that the collagen layer does not significantly contribute to the interfacial strength of resin to dentine.

VI SUMMARY

20 recently extracted premolar teeth were randomly divided into 2 groups of ten teeth each. The occlusal surfaces of the teeth in the four groups were ground to expose dentine and reduced to obtain a flat surface of dentine perpendicular to the long axis of the teeth. A suitable mould of specific dimension was kept ready to mount all forty specimens to enable carry out shear bond strength studies. The teeth were sectioned just below the cervical line to facilitate embedding of these specimens in autopolymerising resin moulds with only the occlusal surface exposed.

The resin was mixed to a fairly thick consistency poured into the rectangular mould and the prepared specimens with flat surfaces were embedded in the acrylic material and allowed to set. The master rectangular metal mould was designed to fabricate blocks that would fit the chuck of Hounsefield tensometer machine. The procedure of preparation of specimens was separate for all twenty specimens. These specimens were kept in normal saline solution at 37°C.

The specimens were grouped as follows -

Group I. Optibond Solo bonding agent was applied on the dentinal surface without 5% NaOCI wash after acid conditioning.

Group II. 5% NaOCI wash for two minutes was carried out after acid conditioning of dentin. Optibond Solo bonding agent was applied.

Sodium hypochlorite treatment of the acid conditioned dentine substrate was done to remove the collagen fibrils left back suspended after acid conditioning. In both the groups the dentinal surface were cleaned with pumice slurry and gently dried with oil free air. Acid conditioning was carried out with 37% phosphoric acid gel for 15 seconds followed by rinsing and drying to remove excess water. Optibond solo adhesive was used for bonding the specimens in group I and group II.. The specimens in group II were treated similarly as group I except for a two minute exposure with 5% NaOCI after acid conditioning. This was followed by rinsing and drying to remove excess moisture. Composite resin was applied over the adhesive for both groups using a Teflon mould with specific dimension to obtain a cylinder of restorative material. All twenty specimens were transferred to the Houns field tensometer for shear bond strength studies. Two specimens each for the two groups were prepared separately for SEM study. Bonding with and without 5% NaOCI treatment was done in above mentioned manner for all groups. Composite resin application was over the entire dentinal surface alone. The specimens were sectioned vertically to expose the resin/ dentine interface followed by standard SEM specimen preparation. All specimens were evaluated under SEM and photographs taken accordingly.

VII CONCLUSION

The present study compares shear bond strength of composite resin on exposed dentinal surface using a fifth generation bonding agent with and without hybrid layer. The exposed collagen fibres after acid conditioning which constitutes the basis for hybrid layer formation was washed off with 5% NaOCI for 2 minutes.

1. Shear bond strength values were shown to be lowest when Optibond Solo adhesive(Group 1) was used without removal of collagen fibers.
2. Group II, where Optibond Solo were used after removal of collagen fibers showed better bond strength values when compared to group I where Optibond Solo was used without removing collagen fibers after acid conditioning
3. SEM evaluation of the two groups were carried out. The results of SEM examination confirmed effective removal of collagen fibers left back after acid conditioning when exposed to 5% NaOCI for 2 minutes.

The present study concludes that acid conditioning 5% NaOCI treatment creates a dentine substrate which provides more shear bond strength for OptibondSolo adhesive .The study emphasizes on the importance of removing the collagen fibrils left back after acid conditioning completely. This may prevent the long term bond degradation of composite resins and subsequent micro leakage and discoloration

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