Original Research Paper

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Effect of three Collagen Cross Linkers on the Shear Bond Strength of Self : Etch Adhesive System to Dentin at Varying Time Intervals.

Abstract:

Background: When resin composite is bonded to dentin, the bond strength reduces with increase in depth of dentin. This is due to the increase in number of dentinal tubules, their diameter and less amount of intertubular dentin compared to "superficial dentin.21"

Objective: This study proposes to improve bond strength of dentin to composite by biomodifying the dentin substrate prior to application of composite with three different crosslinking agents i;e Glutaraldehyde, Genipin and Riboflavin at varying time intervals.

Method: 42 extracted maxillary incisor teeth were selected. Teeth were sectioned to mesial and distal halves. (n=84). Specimens were divided into 4 groups. Group I was used as control group and Glutaraldehyde, Genipin and Riboflavin crosslinking agents were applied to other groups (II, III, IV) respectively before applying composite resin. These groups were further subdivided into A and B groups based on time of applying crosslinking agent. Shear bond strength was calculated for all specimens using universal testing machine. Results were analysed using Student t test for intra-group and inter-group comparison.

Results: Greater bond strengths were recorded in all the groups compared to the control group. Application of Glutaraldehyde and Genipin as crosslinking agents reported higher shear bond strength compared to application of Riboflavin. The mean shear bond strength values further increased with increased application time of crosslinking agent.

Conclusion: Dentin surface pre-treatment with crosslinking agents at varying time intervals substantially improved the bond strength.

Keywords: Dentin; Crosslinking ability; Glutaraldehyde; Genipin; Riboflavin; Self – etch adhesive; Shear bond strength, Case control study.

Introduction:

Dentin is an arduous mineralized tissue categorized in a three dimensional frame comprises of various tubules. It comprises of 70% minerals, 20% organic matter & 10% fluids. The organic matter mainly made up of Type I collagen (90%), while others are non-collagenous proteins such as phosphor proteins and proteoglycans. [1, 2, 3, 4]"

Hybrid layer, which plays a key role in stronger bond formation between resin and dentin is actually more amenable for failure because of concentration of more stresses. The main problem in bonding arises when it comes to dentin as it has a complex structure compared to enamel.[5]

The reasons for the failure of bonding at the resin – tooth interface are accumulation of water& water affinent monomers in self-etch adhesives & simplified adhesives respectively.[6, 7] Other causes are improper accumulation of resin monomers in hybrid layer, collagenous enzymes that chemically degrade the collagen fibrils which are present at interface, greater reactivity of bonded interface and inadequate polymerization.[6, 8, 9] Uncovered collagen

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fibrils that are susceptible to denaturation and degradation after function occurs due to variations in the depth of demineralized collagen and resin infiltration. These fibrils are more liable to formation of porosities, which have more affinity for water, which will lead to hydrolysis of collagen by Matrix metalloproteinases [MMPs] and Cysteine cathepsins), [6, 10, 11]"

The longevity of adhesive restoration depends on the constant bonding at the resin tooth interface. Constant research has been done to overcome the disadvantages associated with reduction in bond strength progressively. Strateges that became popular are the Introduction of novel adhesive systems and modification of the dentin substrate by application of biomimetic materials.[1, 4, 5] Majority of evolutions in adhesive dentistry emphasized on alterations in bonding agents& their application. But the importance of collagen structure and its stability to bond strength still remains as an unexplored area in the dental field. The longevity of bond between tooth structure and resin is based on the structure & properties of collagen, which can be enhanced by the application of collagen cross linking agents before application of resin.Development of crosslinks within and between the collagen molecules is the rationale behind this.[1, 4, 12, 13, 14]

"Glutaraldehyde (GD)," a chemical crosslinker, is commonly known for fixing human and animal tissues.5The physical properties of various tissues which contain collagen as an integral part are increased by application of Glutaraldehyde. [1, 5, 15] It increases cross linking through reacting with amino groups of "collagen fibrils.[16]"It has similar mechanism of action on dental tissues also. Pretreatment with "Glutaraldehyde" before application of composite resin significantly enhanced bonding at tooth & resin interface on both sound & carious "dentin.[16, 17, 18]"

"Genipin (GE)," is an agent extracted from gardenia fruit "(Gardenia Jasminoides Ellis).[1,19,20,21]""Genipin" has been used for Diabetes mellitus and Jaundice patients in ancient Chinese times.[20,21]" Physical properties of several materials which contain collagen as an integral part has been improved by application of Genipin. Another advantage of Genipin is it is less lethal than Glutaraldehyde[1, 5, 21, 22]"

Riboflavin (vitamin B2) is used for photo- oxidative collagen cross-linking method due to its ability to produce oxygen radicals.Because it does not have any harmful action, it is considered for the treatment of ocular condition like "Keratoconus,"where the physical properties of collagen plays a significant role.[23, 24]. However its applications in dentistry are not yet fully understood. The effect of collagen cross- linking agents on increasing the ultimate tensile strength and stiffness of dentin[1,5,6,11,16,17,19,23] has already been well documented,but limited data was available on their effect on the shear bond strength between resin and dentin.[12]

This in vitro study evaluates the effect of three different collagen cross linking agents i.e.; Glutaraldehyde, Genipin, Riboflavin on the shear bond strength of composite resin bonded to dentin using a self-etch adhesive at varying time intervals.

Materials and Methods:

Extracted human maxillary central incisors needed for the study were collected from the Department of Oral Surgery and stored in 10% buffered formalin solution.

Sectioning of teeth - Each tooth was sectioned longitudinally, parallel to the long axis of the tooth, into a mesial and a distal half by means of a low-speed diamond disc under copious water supply. (Fig 1) Using a diamond disc, the dentin in the proximal wall each of half of the crown incisal to the cemento-enamel junction was removed from the outer surface of the prepared proximal portion of the crown to the pulp chamber, until the remaining dentin thickness was 1-1.5mm (approximately). The specimens were irrigated to remove debris. All the 42 teeth were sectioned into mesial and distal halves (n=84).

Figure 1: Sectioning of each tooth longitudinally using slow speed diamond disc.

Preparation of solutions :

1. 5% Glutaraldehyde (TCI Chemicals) □□□ commercially available.
2. 0.625% Genipin (TCI Chemicals) - 625mg of powder will be dissolved in 1000 ml of distilled water to make 0.625% Genipin solution.
3. 0.1% Riboflavin solution (TCI Chemicals) - 10 mg of powder will be dissolved in 100ml of distilled water to make 0.1% Riboflavin solution.

Division of experimental group :

Prepared teeth were divided into four groups. Group I was taken as control group (n=12) and Group II, III, IV were divided based on the crosslinking agent used for pretreatment i;e Glutraldehyde, Genipin and Riboflavin respectively (n=24).These three groups were further divided into two sub groups each (n=12) based on the pre-treatment time – Subgroup A (5 minutes and subgroup B (10 min).

Bonding protocol :

All the specimens were then mounted in acrylic resin for shear bond strength analysis. The self-etch adhesive Xeno III (Dentsply) was used according to the manufacturer's instructions. An equal amount of liquid A and B (1:1) were mixed for five seconds and applied on the proximal dentin surface. The adhesive was left in place undisturbed for twenty seconds and then gently air dried and light cured for forty seconds. Composite build-up with Ceram X Nano Ceramic Restorative

(DENTSPLY) was done by placing two increments of 2mm thick composite resin, with each increment being light cured for forty seconds. (Fig 2)

Figure 2: Application of self-etch adhesive and composite to each half of the sectioned tooth in increments.

Evaluation of shear bond strength :

The specimens were stored in distilled water at 370c for 24hours. Shear bond strength was determined using a universal testing machine at a crosshead speed of 10mm/min (Fig 3).

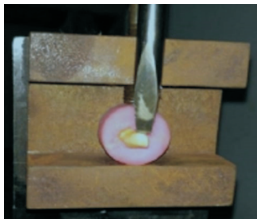

Figure 3: Shear bond strength of the mounted specimens was calculated using universal testing machine.

Shearing load values were recorded through a computer connected to Instron testing machine. The values obtained were in "N" and bond strength were calculated in Mpa using the formula:

Bond strength(Mpa)=Load (N)/Surface area (mm2).

Statistical analysis :

The mean shear bond strength and standard deviation were computed and results were analyzed by ANOVA and student t-test. A p-value <0.05 will be considered as statistically significant.

Results:

The results showed that there was statistically significant difference in mean shear bond strength values between Group I (Control group) and Group II, III and IV where dentin surface was pretreated with crosslinking agents for 5 min and 10 min before application of composite resin. (Table 1 and 2)Similarly the mean shear bond strength was higher in all the subgroups (IIB, IIIB and IVB) where there was increased application time for crosslinking agent (Table 3).

Table I: Mean shear bond strength (MPa) values of control group and Group IIA, IIIA,IVA (Groups with application time of 5 min)

	N	Mean	Std. Deviation	ANOVA	
				F	p-value
Group I	12	4.534	0.550	260.04	<0.001*
Group IIA	12	10.210	0.360		
Group IIIA	12	10.713	0.749		
Group IVA	12	6.398	0.888		

*P value < 0.05 shows significant difference

Table II: Mean shear bond strength (MPa) values of control group and Group IIB, IIIB, IVB (Groups with application time of 10 min)

	N	Mean	Std. Deviation	ANOVA	
				F	p-value
Group I	12	4.534	0.550	239.87	<0.001*
Group IIB	12	10.653	0.343		
Group IIIB	12	11.163	0.782		
Group IVB	12	6.40	0.97		

*P value < 0.05 shows significant difference

Table III: Mean shear bond strength (MPa) within groups

			Mean	SD	Mean difference (95% CI)	T	Df	p-value
Group II	A	12	10.21	0.34	0.44 l (0.43, 0.46)	79.87	11	<0.001*
	B	12	10.65	0.36				
Group III	A	12	10.71	0.75	-0.45 (-0.47, -0.43)	-46.81	11	<0.001*
	B	12	11.16	0.78				
Group IV	A	12	6.398	0.88	0.0008 (-0.93, 0.94)	0.0002	11	0.99
	B	12	6.40	0.97				

*P value<0.05 shows significant difference

Discussion:

Bonding agents that gained popularity in recent times depends on the total-etch technique and the self-etch technique. Simplified self-etch adhesives overcomes the disadvantages that are associated with etch and rinse adhesives such as numerous steps in their usage and greater feasibility for post treatment sensitivity. But despite of its disadvantages, etch and rinse technique is even now treated as the efficient procedure for attaining reliable bond between dentin and resin.[12, 26, 27]

When considered as a substrate for bonding the complex composition and hydrophilic nature of dentin pose a challenge. Diffusion of molecules into deeper layers and change in the water content at surface over a period of time also alters bond strength at resin dentin interface. The physical properties of collagen like rigidity, tensile strength depends largely on the covalent inter-and intra-molecular cross-links formed between its molecules. Certain chemicals which are either available in nature or manufactured artificially are shown to increase these crosslinks in "biological tissues.[1, 12, 28, 29]"

Crosslinking mechanism has tremendous usage in the artificial polymer chemistry and in the living sciences.[11, 24] Its application in dentistry is also well documented. The physical properties of underlying dentin and hybrid layer are remarkably increased due to crosslinking of collagen molecules by use of various chemicals. These chemicals also contribute to reliable long lasting bond by cross-linking collagen in irreversible form and decreasing dentin "matrix biodegradation.[1, 4, 5, 11, 12, 16, 17, 19]"

Glutaraldehyde is the only non-natural cross-linker used in this study. It forms cross-links between an aldehyde and an £-amino group of lysine and hydroxylysine of collagen. When it is applied to living tissues, there is remarkable increase in physical properties.[1,30,31] The problem with glutaraldehyde is its harmful action on biological tissues. To minimize this, a lower concentration is required but this result in "ineffective cross-linking. [19]"

Genipin, a natural crosslinking agent is also used in the present study. It is a Iridoid glycoside with C11H14O5 molecular structure .The reaction mechanisms with living tissues are not well understood. It acts on the unbound amino groups of lysine, hydroxy lysine, or arginine that are present in a collagen molecule or among other collagen molecules to form intra-molecular or intermolecular crosslinks. In fact Genipin polymerizes first and forms intermicrofibrillar crosslinks between fibrils of collagen (oligomericcrosslinks) 1." Many studies have shown that Genipinis safe compared to "Glutaraldehyde.[20] Although the evidence regarding the crosslinking effect of Genipin on collagen is established, its effect on bond strength between resin and dentin is still a matter of research.

Riboflavin is another natural crosslinking agent used in the study. Studies suggest that photooxidation might be the underlying mechanism which helps Riboflavin in collagen crosslinking.[16, 33, 34] When Riboflavin was subjected to ultraviolet light it releases oxide ions, which produce the covalent bonds between the collagen molecules. Also there will be loss of tyrosine and histidine residues in the collagen which indirectly aids in its crosslinking mechanism.But according to Kato et al. these oxide ions are not directly responsible for the bonding to take place.[16,35] Riboflavin because of its yellow tint is preferred over other photosensitizing chromophores such as Porphyrins, Methylene blueetc; in dental field. Cova et al. established that

pho to activated Riboflavin diminishes protein degrading enzymes function and thereby increases the longevity of the adhesion.[16,23]

The results of the present study concluded that application of crosslinking agents prior to application of composite shows considerably greater shear bond strength to dentin. Srinivasulu et al. also established the same that application of crosslinking agents like 6.5 % Proanthocyanidin and 10% Sodium ascorbate to deep dentin significantly improved the shear bond strength of composite to dentin.[12] The rationale behind this is the mechanical properties of collagen are substantially increased by the crosslinking mechanism which makes its fibrils impervious to enzymatic breakdown.[11, 36]."

Group II, where 5% Glutaraldehyde was used as a crosslinking agent showed significantly greater bond strength to dentin when compared to Group I where no crosslinking agent was applied. This in in accordance with the study done by Macedo et al.whereapplication of 5% Glutaraldehyde to caries-affected and sound dentin significantly improved the microtensile bond strength of composite to dentin.17 Group III (Genipin) recorded a higher bond strength compared to Group II(Glutaraldehyde).Similar study was done by Bedran-Russo et al;where they concluded that application of 0.625% Genipin was capable of stabilizing demineralized dentin collagen more effectively when compared to 5% Glutaraldehyde. The reason could be attributed to the specificity of Genipin to form inter micro fibrillar crosslinks via polymerization of GE molecules before "crosslinking.[1]"

Group IV (Riboflavin) showed a higher shear bond strength value than Group I (Control group). These results were in line with the research done by Cova et al. and Chiang et al. who established that Photo activated riboflavin increases dentin bonding by enhancing the dentin stiffness and preserving the enlarging collagen matrix in the hybrid layer. {16, 23}This is because of reactive oxide ions which are released byultraviolet - A radiation exposure that produce the crosslinks between collagen molecules. In mechanism of crosslinking,Riboflavin acts as double edged sword i; e it releases oxide ions that stimulate covalent bonds and also protect from ultraviolet radiation. [23, 24]

Group IV shows significantly lower shear bond strength compared to that of Group II and Group III. The reason could be attributed to the complex mechanism of photo oxidation through which Riboflavin induces crosslinks in collagen, while Glutaraldehyde and Genipin forms crosslinks by simple chemical and ionic interactions between molecules of collagen. Further Riboflavin was not compared with any other crosslinking agents in terms of shear bond strength values in the literature, therefore further investigation is necessary.

In this study crosslinking agents were applied in different time intervals, in contrast to other studies, where prolonged application of crosslinking agent was done[1, 17]" When the application time for crosslinking agent was increased group II and group III showed remarkably greater bond strength in its both subgroups whereas group IV i; egroup treated with Riboflavin reported almost similar bond strength values in its two subgroups. The reason could be application of crosslinking agents for a longer period increases the number of crosslinks (inter, intramolecular& intermicrofibrillar) that are formed between molecules of collagen in both IIB & IIIB subgroups.[1, 14, 23] but not in IV B subgroup as it involves complicated photo oxidationin its crosslinking mechanism, which takes longer time. Further investigation is necessary in this aspect.

Conclusions:

1. Application of crosslinking agent to dentin prior to application of composite results in an increase in shear bond strength between dentin and composite.
2. Shear bond strength increased with increase application of time of crosslinking agent.

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