



## Research Article

### EFFECT OF COLLAGEN STABILIZING AGENTS ON THE SHEAR BOND STRENGTH TO DENTIN: AN *IN VITRO* STUDY

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#### ABSTRACT

**Aim:** This *in vitro* study was designed to evaluate the effect of collagen stabilizing agents on the shear bond strength to dentin. **Materials and Methods:** Thirty premolars were decoronated 2 mm above cemento-enamel junction and restored with composite resin to a height of 2 mm and diameter of 3.5 mm using a cylindrical teflon mould. Teeth were then randomly divided to four groups: Group I (Control): Etching + Rinsing + bonding agent + composite (n = 10); Group II (Riboflavin): Etching + Rinsing + Pre-treatment with Riboflavin + Curing + bonding agent + composite (n=10); Group III (White tea): Etching + Rinsing + Pre-treatment with White tea + bonding agent + composite (n = 10). Samples were then subjected to thermo cycling for 500 cycles at 5° C and 55° C with the dwell time of 30 sec and transfer time of 5 to 10 sec. Then the samples were subjected to shear bond strength evaluation on universal testing machine. Shear load was applied at a cross head speed of 0.5 mm/ minute until failure occurred. The load to failure was recorded individually and statistical analysis was done. **Results:** Group II (Riboflavin) produced the highest mean shear bond strength when compared to white tea. **Conclusion:** According to this study, it can be concluded that pre-treatment by application of Riboflavin improved the shear bond strength to dentin when compared to control and white tea.

**Keywords:** Collagen cross linking agents, Riboflavin, White tea,

#### INTRODUCTION

Adequate bond strength is necessary to withstand the contraction forces during polymerization shrinkage of composite resin and to promote better retention and marginal seal during function<sup>1</sup>. Superior mechanical properties of collagen are desirable for resin restorations. This collagen in the dentine surface gets exposed by acid etching. Subsequent application of bonding agent into the demineralised collagen rich dentin leads to the formation of hybrid layer. Exogenous collagen cross-linking was reported in dental literature as a mechanism to improve the properties of dentine collagen. Improvement of collagen mechanical properties can be achieved by using different collagen cross-linking agents. The dentin surface pre-treatment by these agents may help to increase bond strength values prior to bonding procedures. Several synthetic agents like (glutaraldehyde, carbodiimides, formaldehyde, epoxy compounds and others) and as well as natural agents (proanthocyanidin, genipin, chitosan, propolis, green tea) exhibit exogeneous collagen crosslinking<sup>2</sup>.

Riboflavin helps in the cross linking of dentin collagen by its ability to produce free radicals such as O<sub>2</sub> and O<sub>2</sub><sup>-</sup>. when photo-activated with spectral range from Ultraviolet (UV) to visible light. It forms covalent cross links between adjacent collagen molecules<sup>3</sup>.

White tea is derived from a plant (*Camellia sinensis*). White tea contains catechins like epigallocatechin gallate (EGCG), epicatechin gallate, epigallocatechin, epicatechin. It inhibits MMP release and improves collagen matrix mechanical properties by resisting proteolytic degradation<sup>4</sup>.

Hence, the aim of this study was to evaluate the effect of collagen stabilizing agents like Riboflavin and White Tea on the shear bond strength to dentin. The null hypothesis for this study is that there will be no difference in the shear bond strength of resin composite with or without application of collagen stabilizing agents.

#### MATERIALS AND METHODS

##### Preparation of riboflavin

The 1 % (1 g/100 ml) riboflavin solutions were prepared from riboflavin-5 phosphate (Zenith Nutricorp, USA) dissolved in distilled water. The prepared riboflavin solutions were kept in light-proof test tubes to avoid any light activation of riboflavin before use and applied to dentin specimens at room temperature.

##### Preparation of white tea

10 % white tea extract (United Nilgiri Tea Estates Co. Ltd, Korakundah, India) solution were prepared by weighing 50 g of the tea powder and dissolving it in 500 ml of distilled water and filtered.

##### Shear bond strength evaluation

Thirty non-carious, freshly extracted premolars without cracks or previous restorations, extracted for orthodontic purpose were used in this study. All teeth samples were sterilized by immersing in 10 % formalin solution. Teeth were stored in distilled water until it is used. All teeth samples were decoronated with flexible

diamond disc at 2 mm above the cemento enamel junction from the proximal surface of teeth under copious water cooling. In all the tooth samples dentin was etched for 15 sec with 37 % phosphoric acid (3MESPE). Then the samples were washed and blot dried with absorbent paper pads. Samples were divided into 3 groups.

Group I - Etching-Rinsing-Bonding agent-Composite

Group II - Etching-Rinsing-Pre-treatment with Riboflavin-Light curing-Bonding agent-Composite

Group III - Etching-Rinsing-Pre-treatment with White tea – Bonding agent-Composite

Group II - samples after acid etching were pre-treated with riboflavin for 2 min, cured with visible blue light and Group III samples after acid etching were pre-treated with white tea for 10 min.

In all the Groups, bonding agent (Adper Single Bond- 2, 3M ESPE) was applied and light cured for 30 sec and composite (Filtek Z350, 3M ESPE) restoration was done (height -2 mm and diameter-3.5 mm) using cylindrical Teflon mould and was cured for 30 sec. Light intensity was checked with radiometer before curing. Then the tooth samples were stored in distilled water at 37°C. Samples were then subjected to thermo cycling for 500 cycles at 5°C and 55°C with the dwell time of 30 sec and transfer time of 5 to 10 sec. Wax was coated on the root of all the samples and was embedded in acrylic resin on a split mould. The wax was then removed from the resin block and was filled with light body elastomeric impression material to simulate the periodontal ligament. Then the samples were subjected to shear bond strength evaluation on Universal Testing Machine (Instron). Shear load was applied in a direction of 45° angulations to the bonded interface at a cross head speed of 0.5 mm/minute until failure occurred. The load to failure was recorded individually and the values were tabulated. Statistical analysis was done using SPSS software (version17).

## RESULTS

According to the results obtained, the highest mean shear bond strength value was recorded for group II (Riboflavin) 42.91 Mpa whereas the lowest value was recorded for group III (White tea) 19.93 Mpa (Figure 1).

Multiple comparisons of mean shear bond strength between group I, group II and group III showed no significant difference between group I and group II ( $p = 0.028$ ) (Table 1); group I and III ( $p = 0.110$ ) (Table 2); Whereas there was statistically significant difference between group II and group III ( $p = 0.001$ ) (Table 3). The results proved that pre-treating with Riboflavin increased the bond strength compared to white tea and control group.

## DISCUSSION

G V Macedo *et al* (2009) hypothesized that cross linking in dentin collagen improves bond strength<sup>5</sup>. Dentin/resin interface properties can be better improved by stabilizing the collagen of the hybrid layer by application of external collagen stabilizing agents. The induction of exogenous collagen cross links has been proposed for maintenance, restoration and improvement of tissue function by the production of the mechanically and enzymatically resistant collagen scaffold<sup>6</sup>. The intermolecular cross linking represents the final post translation modification of collagen which forms the basis for the stability and visco-elasticity of collagen fibrils in the hybrid layer.

In the current study, the highest mean shear bond strength value was recorded for group II (Riboflavin) 42.91 Mpa whereas the lowest value was recorded for group III (White tea) 19.93 Mpa and group I (control) was 28.03 Mpa.

Riboflavin was used for dentin surface pre-treatment in this study as it can effectively link dentin collagen and increase bond strength by cross linking dentin collagen due to its ability to produce free radicals when photo activated with a spectral range from UV to visible light<sup>7</sup>. The free radicals such as  $O_2$  and  $O_2^-$  are released when riboflavin is photo activated and light is absorbed, forming covalent cross links between adjacent collagen molecules. Riboflavin is a photo activated free-radical producer with a maximum absorption peak of 270, 366 and 445 nm (Kohlhaas *et al.*, 2006; Spoerletal., 2007) There is an observed reduction of amino acids like histidine and tyrosine during cross linking which leads to the formation of ditryrosine, dimer of tyrosine, that constitutes a possible mechanism in collagen cross linking<sup>8</sup>.

White tea was used in the present study as it is known to have catechins like epigallocatechin gallate (EGCG), epicatechin gallate, epigallocatechin, epicatechin. Epigallocatechin gallate (EGCG) possesses inhibitory effect against matrix metalloproteinases and act as a cross linking agent thus improving the mechanical and enzymatic resistance of collagen matrix<sup>4</sup>. It also exhibits profound inhibitory activity on collagenases that degrade the organic matrix<sup>9</sup>.

Riboflavin pre treatment of dentin was done for 2 min and photo activated by visible light (Shahin *et al.*, 2017, Chiang *et al.*, 2013); white tea for 10 min (F Setal., 2016 Sharafeddin *et al.*, 2015). Following this, Adper Single Bond -2 is applied to infiltrate etched dentin. Though, the advantage of this system leads to highest enamel and dentin bond strengths, the disadvantage is that it is a technique sensitive procedure<sup>10</sup>. The diffusion of resin monomer into the de-mineralized dentin shows a decreasing concentration gradient (Wang and Spencer 2002) leading to exposure of collagen fibers.

In the current study, once the sample preparation was completed thermo cycling was done Thermo cycling regimens may simulate more appropriately the clinical situation<sup>11</sup>. *In vitro* exposure of extracted teeth restorations to cyclic thermal fluctuations has been common in many shear bond strength tests. This is to simulate one of the many challenges that occur in the oral environment. Universal testing machine is widely used to measure the compression, tensile shear, torsion and flexural strength of materials under static loading as the results obtained are reliable. In this study, shear bond strength testing was done using Universal Testing Machine.

According to the results obtained in this study, riboflavin showed statically significant higher shear bond strength when compared to white tea due to the release of free radicals when riboflavin is photo activated and light is absorbed, forming covalent cross links between adjacent collagen molecules. In a plausible explanation there is a reduction in histidine and tyrosine during cross linking leading to the formation of ditryrosine, a dimer of tyrosine which can be a possible mechanism in collagen cross linking mediated by riboflavin. The collagen cross linking could be due to overall decrease in hydroxyl proline release leading to the stiffness of dentin collagen matrix<sup>8</sup>. According to Fawzy *et al* (2012) SEM images of the resin/dentin interface showed a uniform hybrid layer with well-formed long resin tags for 1 % riboflavin<sup>8</sup>. The collagen cross linking effect of photo-activated riboflavin (Arbelaez M C *et al*, Wollensak G *et al*, Cova A *et al*) could explain the overall increase in Shear Bond Strength.

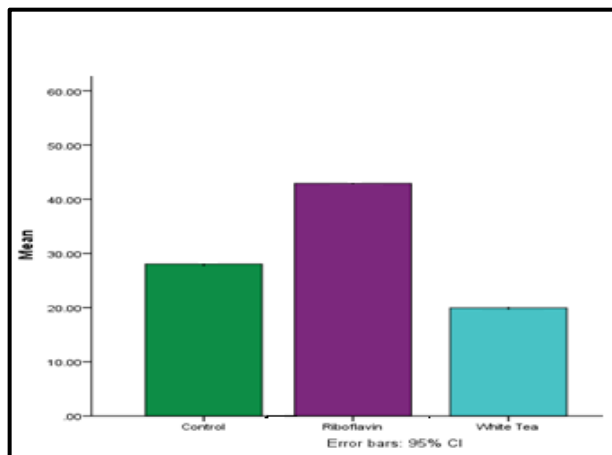


Figure 1 : Mean shear bond strength values between all the groups

Table 1: Mean shear bond strength values between control and riboflavin

Group Statistics					Independent Samples t-test	
Group	N	Mean	SD	SEM	t-Value	P-Value
Control	10	28.0380	11.62514	3.67619	-2.392	.028*
Riboflavin	10	42.9190	15.87412	5.01984		

\*p < 0.05 N- Sample size; SD – Standard Deviation; SEM – Standard Error of Mean

Table 2: Mean shear bond strength values between control and white tea

Group Statistics					Independent Samples t-test	
Group	N	Mean	SD	SEM	t-Value	P-Value
Control	10	28.0380	11.62514	3.67619	1.682	.110
White Tea	10	19.9330	9.85471	3.11633		

\*p < 0.05 N- Sample size; SD – Standard Deviation; SEM – Standard Error of Mean

Table 3: Mean shear bond strength values between riboflavin and white tea

Group Statistics					Independent Samples t-test	
Group	N	Mean	SD	SEM	t-Value	P-Value
Riboflavin	10	42.9190	15.87412	5.01984	3.890	.001*
White Tea	10	19.9330	9.85471	3.11633		

\*p < 0.05 N- Sample size; SD – Standard Deviation; SEM – Standard Error of Mean

White tea showed least shear bond value when compared to other groups. This could be due to the anti-oxidant property of white tea. White tea because of the presence of epigallocatechin gallate which was not rinsed after pre treatment might neutralize the effect of oxygen free radicals retained in the exposed collagen which might interfere with its covalent cross linking.

The Null hypothesis tested in this study was rejected as pre-treatment with collagen stabilizing agents improved the shear bond strength

It is difficult to entirely correlate laboratory findings with the clinical behaviour of any restoration. The best evidence would be achieved with further *in-vitro* and *in vivo* studies with a larger sample size and long-term clinical performance.

### CONCLUSION

Within the limitations of this *in-vitro* study, it can be concluded that the

1. Pre treatment with riboflavin showed statistically significant higher mean shear bond strength value when compared to the other groups.
2. Shear bond strength values after the application of white tea was found to be least as compared to other groups.

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