Clinical evaluation of adhesive restorations with and without Proanthocyanidins in Non Carious Cervical Lesions– 1 Year Follow up

Lekha Santhosh^{1,*}, B.S. Wasima², Srinivas Panchajanya³, Srirekha Aswathnarayan⁴, Jaya Kumar T⁵

^{1.5}Professor, ²PG Student, ³Reader, ⁴Professor & HOD, Dept. of Conservative Dentistry & Endodontics, The Oxford Dental College & Hospital, Bengaluru, Karnataka

*Corresponding Author:

Email: lekh_santhosh@yahoo.com

Abstract

Introduction & Objective: The deterioration of the hybrid layer in an adhesive restoration takes place over time resulting in the failure of restoration. The objective of this study was to compare the clinical efficacy of dentin adhesive applied over proanthocyanidin(PA) treated dentin for composite restorations in Non Carious Cervical Lesions (NCCLs) using the modified US public health service (USPHS) criteria.

Materials and Method: Seventy NCCLs in selected subjects were assigned to two groups. In experimental group, the PA solution was applied during the bonding procedure and restored with nanofilled composite. The second group served as a control, where resin restoration was placed without modifying the dentin. Two examiners recorded clinical performance after 1week, 6 months and 12 months using modified USPHS criteria. Observation was recorded and statistical analysis was done.

Results: PA group showed better result for various clinical criteria in comparison to the control group at 6 months and 12 months but there was no statistical significance. When evaluated within the group at different time interval the control group showed statistically significant difference for the retention (p=0.009) and marginal adaptation (p=0.002). There was a loss of retention at 6 months and no further loss at the end of one year.

Interpretation and Conclusion: PA conditioning during the adhesive procedure for composite restoration in NCCLs did not improve the various clinical parameter in comparison to control group when evaluated for a period of 1year.

Keywords: Proanthocyanidins, Non-Carious Cervical Lesions, Collagen.

Introduction

An adhesive is used in restorative dentistry to promote a strong bond between the restorative material and the dentin substrate. However, restorations, most often fail due to marginal failure at the gingival floor, suggesting that the dentin adhesive interface is the weakest link.⁽¹⁾

During bonding to dentin, the adhesive monomers thoroughly infiltrate and encapsulate the exposed collagen fibrils, creating the hybrid layer. However, deterioration of this hybrid layer takes place due to various physical and chemical factors. It has been found bacterial enzymes, host-derived that matrix metalloproteinases (MMPs) play a role in degradation of the exposed collagen.⁽²⁾ An application of an acid during the etching procedure can further lead to activation and increased expression of MMPs in the substrate, thus increasing the risk of an enzymatic breakdown of the newly formed hybrid layer.^(3,4)

Collagen in biological tissue is strengthened by the formation of native cross-linkers that provide the fibrillar resistance against enzymatic degradation as well as increase tensile properties.⁽⁵⁾

Proanthocyanidins(PA) is a natural collagen crosslinker, a MMPs inhibitor,⁽⁶⁾ potent antioxidant and belongs to a subgroup of the flavonoid compounds with low toxicity.⁽⁷⁾ In vitro studies have shown that using PA as a bio modifying agent, improved the stability of dentine matrix, when used during bonding procedure.^(8,9) A recent study has shown that application of PA was capable of improving dentin collagen's biological stability within clinical time period of 10s.⁽¹⁰⁾ Hence PA can be tried in a clinical setup too. There are no clinical studies on long-term stability of resin dentine interface with PA being used for modifying the demineralized collagen during the bonding procedure.

Null hypothesis was that there is no improvement in the clinical durability of the dentin adhesives by bio modification of collagen with PA. Hence, this study was designed to assess the clinical durability of dentin adhesives by modification of collagen with 95% PA during the bonding procedure for the placement of composite resin restorations in Non Carious Cervical Lesions (NCCLs) for a period of 1 year, using modified US public health service (USPHS) criteria.

Materials and Method

Ethical clearance from the Institutional Ethical Committee was obtained and informed patient's consent was taken. Healthy patients between 30-50 years of age with one or more NCCLs ('U' shaped lesions) present bilaterally with adjacent and opposing teeth were selected. Patients undergoing orthodontic treatment and bruxers were excluded. Fractured, restored or carious teeth also were not included. Seventy NCCLs were thus selected and using split mouth design, were allotted to either experimental or control group. The lesions were categorized in terms of sensitivity, cervico incisal height, lesion depth and presence of wear facets were evaluated. Before the adhesive procedure, the teeth were cleaned with the pumice water slurry.

For each patient freshly prepared experimental solution was obtained by dissolving Grape Seed Extract-95% PA powder (Perennial Life sciences Privates Limited, Delhi) in distilled water to obtain 3.75wt% solution.

Experimental group: The sites were acid etched with 37% phosphoric acid etchant gel (Scotch bond Etchant, 3M ESPE) following the manufacturers' instruction. Freshly prepared PA solution was applied for 10s using a disposable applicator followed by application of an ethanol based adhesive (Adper single bond 2,3M ESPE) and light cured for 20s using a standardized LEDtion^R (light curing unit, Ivoclar vivadent). Restoration was build up with a nanofilled composite (Filtek supreme XT) in two or three increments beginning from occlusal wall to the gingival margin, and light cured each increment for 20s.

Final contouring and polishing of the restorations were performed at the same appointment, using an extra fine grit needle shaped diamond point followed by polishing (Shofu kit and polishing pastes Deor core).

The control group was restored similar to protocol described above without the application of PA.

Two calibrated examiners who were fully blind to the treatment evaluated the restorations. The evaluations were performed at three different periods -1 week, 6months and 12 months using modified USPHS criteria.⁽¹¹⁾ The study data was analysed using SPSS [Statistical Package for Social Sciences IBM, Corp]. Chi square test was used to compare the proportional distribution of the study parameters amongst the two groups & at different time intervals.

Results

Thirteen male and three female patients (average age of 43 years) having a total of 70NCCLs were selected for the study. All the 15 patients (100%) attended the 12-month recall. The two examiners evaluations were analysed with Cohen's Kappa Coefficient. The kappa coefficient (k) was greater than $0.85(k.\ge0.85)$. 'p' value for the examiners inter evaluation reliability was less than 0.001 and hence highly significant.

There was no statistically significant difference between control and experimental group at 6months and one-year period with respect to all criteria. The lesion characteristics namely- sensitivity, cervico incisal height, lesion depth and presence of wear facets did not show any correlation.

Discussion

Dentine is an organized biological tissue having elastic properties due to its higher organic content when compared to enamel. The organic matrix of dentin is composed of type I collagen which have intrinsic cross links that increase the structural stability. The pro MMPs that are entrapped or bound in dentin during its formation can be catalyzed to active enzymes after being released from dentin by lowering of the pH to 4.5 or below. Mazzoni et al have shown that it is the host derived MMPs which is responsible for degradation of collagen.⁽¹²⁾ The collagen cross-linking agents enhance mechanical properties of dentin matrix, reduce biodegradation rates of collagen, increase the properties of the dentin resin bonds, and extend the life of an adhesive restoration.⁽¹³⁾

Various agents, natural and synthetic have been introduced in dentistry to inhibit the protease enzymes as well as to increase the strength of the collagen fibrils. They are carbodiimide, chlorhexidine digluconate (CHX), glutaraldehyde (GD), PA, genipine (GE) and hesperidin. CHX inhibits MMPs and cysteine cathepsins but has no cross-linking ability. GD has collagen cross-linking effect, with inhibitory effect on collagenases but has high cytotoxicity.⁽¹⁴⁾ GE and PA are natural cross-linking agents, are biocompatible, nontoxic and show vast biological activities and have been successfully used in the pre-treatment of biological tissues to improve their mechanical properties and degradation resistance.⁽¹⁵⁾

PAs are a class of bioflavonoids that are naturally occurring plant metabolites available in pine bark, elm tree and grape seed. PA or condensed tannin is composed of condensed flavon-3-ol subunits, catechin, epicatechin, and epicatechin-3-O-gallate and linked mainly through C4-C8.⁽¹⁶⁾ PA has high ability to bind to proline-rich proteins, like collagen and facilitate the enzyme proline hydroxylase activity that is essential for collagen biosynthesis.^(17,18) Liu et al. found that the poorly infiltrated demineralized dentin at the bottom of the hybrid layer can be mechanically strengthened by PA bio modification and it contributed to the stabilization of the bonding interface.⁽¹⁹⁾ PA, improved the ultimate tensile strengths and stiffness of demineralized dentine collagen.(20,21)

Several studies have included PA in adhesive resins and have shown beneficial outcomes however, the addition of PA into dental adhesive or in self-etch adhesives may disturb the resin polymerization and degree of conversion, subsequently affecting the mechanical properties of the resin. PA readily scavenges free radicals⁽²²⁾ and may hamper the polymerization of adhesives resins. Therefore, in this clinical trial PA was not incorporated in the adhesive resin. The alternative use of PA, as a primer agent is an approach that is more favourable and hence was followed in the study.

The demineralized collagen, when pre-treated with PA for 15-30s, could withstand 24h of collagenase digestion without any perceivable change in collagen's fibrillar structure and characteristic banding pattern. Liu et al found that treatment of 2wt% or more concentrated PA for 30s was enough to render the demineralized dentin collagen protected from degradation. Their

unpublished data revealed that PA was strikingly effective in improving the acid etched dentin's stability against collagenase degradation following only 10s of treatment.⁽¹⁰⁾ PA solution was prepared by adding grape seed extract (containing over 90% PA) to deionized water to a concentration of 3.75 wt.%. With the said concentration, they found that PA treatment as short as 10s could enhance collagen's resistance toward enzymatic challenge.⁽¹⁰⁾ This timing is clinically feasible. Hence, this clinical study was done with similar concentration, time and mode of application of PA.

In the present study, PA group showed a better result for various clinical criteria in comparison to the control group at 6 months and 12 months but there was no statistical significance. Thus, null hypothesis was accepted.

Failure could be at the adhesive layer of the hybrid layer in both the groups, which may indicate that in the experimental group the cross-linked dentin matrix mechanically strengthened only the bottom of the hybrid layer in which demineralized dentin was inadequately infiltrated by resin. PA cannot prevent the hydrolytic break down of polymers that constitute these bonds between dentin and resin, this could explain why the restorations of PA treated cavities did not show a better clinical result compared to the control group.

Many factors are involved for failure of cervical adhesive restorations, which are inadequate moisture control and cuspal movement during occlusion. Clinical studies also associate tooth flexure with low retention in cervical restorations, showing that the paraxial loading moment developed into the tooth has negative effects at the cervical restorations interface.⁽²³⁾ The repetitive cyclic of para functional loadings may induce a failure in the cervical region of the restoration which undergoes strain softening, generating micro-cracks at the restoration margin.⁽²⁴⁾ Hence, the result has to be judged considering the above mentioned failures in NCCLs.

Further studies have to be conducted with longerterm follow up for a period of two or three years for validating the result. PA Application time greater than 10s should be carried out as in few in-vitro studies have shown success.

Conclusion

Within the limitations of the study, it can be concluded that PA conditioning during the adhesive procedure for composite restoration in NCCLs did not show difference in the various clinical parameter in comparison to control group when evaluated for a period of twelve months.

References

1. De Munck J, Van Landuyt K, Peumans M, Poitrven A, Lambrechets P, Braem M et al. A critical review of the

durability of adhesive to tooth tissue: methods and results. J Dent Res 2005;84:118-32.

- 2. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host derived enzymes during aging. J Dent Res 2004;83:216-21.
- 3. Sano H. Micro tensile testing, Nano leakage, and biodegradation of resin dentin bonds. J Dent Res 2006;85:11-4.
- Lehmann N, Debret R, Romeas A, Magloire H, Degrange M, Bleicher F, et al. Self-etching increase matrix metalloproteinase expression in the dentin pulp complex. J Dent Res 2009;88:77-82.
- Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of cross linkers to dentin collagen enhances the ultimate tensile strength. J Biomed Mater Res B, Appl Biomater 2006;80(1):268-72.
- Ferreira D, Slade D. Oligomeric Proanthocyanidins: naturally occurring o-heterocyclic. Nat Pro Rep 2002;19:517-41.
- 7. Fine AM. Oligomeric Proanthocyanidin complexes: history, structure, and phytopharmaceutical application. Alter Med Rev 2000;5:144-51.
- Castellan CS, Bedran-Russo AK, Karol S, Pereira PN. Long-term stability of dentin matrix following treatment with various natural collagen cross-linkers. J Mecha Behav Biomed Mater 2011;4(7):1343-50
- Castellan CS, Pereira PN, Grande RH, Bedran-Russo AK. Mechanical characterization of proanthocyanidin-dentin matrix interaction. Dent Mater 2010;26(10):968-73.
- 10. Liu Y, Chen M, Yao X, Xu C, Zhang Y, Wang Y. Enhancement in dentin collagen's biological stability after proanthocyanidins treatment in clinically relevant time periods. Dent Mater 2013;29:485-92.
- 11. Cvar J, Ryge G. Criteria for the clinical evaluation of dental restorative materials. US DHEW Document, USPHS, San Francisco. 1971;1-42.
- Mazzoni A, Tjaderhane L, Checchi V et al. Role of MMPs in caries progression and bond stability. J Dent Res 2015;94:(2):241-51.
- 13. Bedran Russo AK, Yoo KJ, Ema KC, Pashley DH. Mechanical properties of tannic acid treated dentin matrix. J Dent Res 2009;88(9):807-11.
- Umashankar PR, Mohanan PV, Kumari TV. Glutaraldehyde treatment elicits toxic response compared to decellularization in bovine pericardium. Toxicol Int 2012;19(1):51-58.
- Nagpal R, Singh P, Singh S, Tyagi SP. Proanthocyanidin: a natural dentin bio modifier in adhesive dentistry. J Res Dent 2016;4:1-5.
- Han B, Jaurequi J, Tang BW, Nimni ME. Proanthocyanidin: A natural crosslinking reagent for stabilizing collagen matrices. J Biomed Mater Res Part A 2003;65:118-24.
- 17. Hagerman AE, Butler LG. The specificity of proanthocyanidin- protein interactions. J Biol Chem 1981;256:4494-7.
- Ku CS, Sathishkumar M, Mun SP. Binding affinity of proanthocyanidin from waste Pinusradiata bark onto proline-rich bovine achilles tendon collagen type I. Chemosphere 2007;67:1618-27.
- Liu R, Fang M, Xiao Y, Li F, Yu L, Zhao S, Shen L, Chen J. The effect of transient proanthocyanidins preconditioning on the cross-linking and mechanical properties of demineralized dentin. J Mater Sci Mater Med 2011;22(11):2403-11.
- 20. Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of cross linkers to dentin

collagen enhances the ultimate tensile strength. J Biomed Mater Res B Appl Biomater 2006;80(1):268-72.

- Bedran-Russo AK, Pashley DH, Agee K, Drummond JL, Miescke KJ. Changes in stiffness of demineralized dentin following application of collagen cross linkers. J Biomed Mater Res B Appl Biomater 2008;86(2):330-34.
- Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol 1990;186:343-55.
- Heymann HO, Sturdevant JR, Bayne S, Wilder AD, Sluder TB, Brunson WD. Examining tooth flexure effects on cervical restorations: a two-year clinical study. Am J Dent Assoc 1991;122:41–7.
- Ichim I, Li Q, Loughran J, Swain MV, Kieser J. Restoration of non-carious cervical lesions. Part I. modelling of restorative fracture. Dent Mater 2007;23:1553–61.