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A stereomicroscopic analysis of microleakage of epoxy based resin sealer by using three different natural antioxidants after use of sodium hypochlorite - An Invitro study

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Abstract

Introduction : 88% of endodontic failures are attributed to apical microleakage between obturating materials and dentin. Sodium hypochlorite is a widely used root canal disinfectant. It has the ability to dissolve the organic component of the smear layer. However, remnants of this strong oxidizing agent or its oxidative by-products such as hypochlorous acid and hypochlorite ions are reported to inhibit the free radical polymerization of methacrylate resins and adhesion of epoxy resins used in

the canal. So in this study we aim to analyze the effect of three different natural antioxidant treatments on the dentin after sodium hypochlorite irrigation on reducing microleakage

Method: 36 freshly extracted single canal teeth were collected. Teeth were decoronated at cement enamel junction followed by cleaning and shaping. Then canals were irrigated with 5.25% sodium hypochlorite as final irrigant. Then the 36 samples were divided into 4 groups (n=9) based on the antioxidant groups. Then canals were obturated with epoxy based resin sealer and gutta percha. IRM was used for temporary filling and the samples were stored in distilled water for 7 days. Microleakage was evaluated by stereomicroscope using silver nitrate dye.

Result: The results demonstrated that 5.25% NaOCl caused significant increase ($P<0.05$) in the microleakage, but this can be reversed by 6.5% Proanthocyanidin significantly more than 50% Aloe vera and 10% Alpha tocopherol.

Conclusion: 6.5% Proanthocyanidin significantly reduces microleakage more than 50% Aloe vera and 10% Alpha tocopherol.

Keywords: Antioxidants, Proanthocyandin, microleakage, aloe vera, alpha tocopheraol, stereomicroscope.

Introduction

Success of endodontic therapy depends on proper cleaning, shaping, and obturating the radicular space by establishing a fluid impervious seal**.** [1]Complete disinfection of root canal is achieved through chemomechanical preparation using manual, rotary, and/or reciprocation instruments that are associated with endodontic auxiliary chemical agents[2] Various irrigants which are used during cleaning and shaping procedure have the potential to affect bonding of subsequently placed adhesive restorations either by directly affecting the bonding procedure or by affecting the structural and mechanical properties of bonding substrate i.e., pulp chamber dentin**.**[3]

Sodium hypochlorite (NaOCl) is the gold standard irrigant used in endodontic due to its ability to dissolve organic matter and its antimicrobial properties**.**[2] Even though Sodium hypochlorite provides gross debridement, disinfection, lubrication and dissolution of tissues, there is no single irrigant that alone covers all of the ideal requirements from an irrigant.[3] Adverse effect of NaOCl include toxicity, promotion of structural changes in organic dentin components (mainly Type 1 collagen) and its effects on the mechanical properties of dentin, such as reducing flexural strength and the elastic modulus. It even lacks residual antimicrobial activity. [2]

Ari H, Erdemir A -stated that 5% Naocl causes depletion in calcium and phosphorus levels and in mechanical properties of dentin, such as elastic modulus, flexural strength and microhardness, which further contributes to a reduction in the micromechanical interaction between adhesive resins and NaOCl-treated dentin**.** [3] On contact with organic matter Naocl breaks down into chloramines and protein derived radical intermediates. These breakdown products are capable of having an adverse effect on the pyridinoloine crosslinks occurring in type 1 collagen**.** Remnants of Naocl or its oxidative byproducts such as hypochlorous acid and hypochlorite ions inhibit the free radical polymerization of methacrylate resin and adhesion of epoxy resin used in the canal**.** [4]

Various studies have shown that the compromised mechanical properties of Naocl treated dentin could be reversed by application of an different antiox-idant**.**[3]Antioxidants are the substances that have potential to neutralize the free radicals, it can even restore the redox potential of the oxidized dentin

substrate. Sodium ascorbate and proanthocyanidins (PA) are some of the most researched antioxidants in dentistry.PA is more potential in neutralizing free radical as compared to sodium ascorbate. PAs are naturally occurring plant metabolites, which are a part of group of polyphenolic compounds known as flavonoids,

Proanthocyanidin is found in high percentage in grape seed, cranberries, leaves of bilberry and birch, and bark of several trees. PAs from grape seed extract (GSE) are composed primarily of 36% oligomeric and polymeric procyanidins, namely, catechin or epicatechin and <5% of flavan-3-ol monomeric catechins.[4]

Aloe barbadensis miller (Aloe leaves) is another antioxidant used in the study which possesses anti-inflammatory, antimicrobial ,moisturizing ,wound healing and pain relief properties. Anthraquinones the substance present in aloe vera is responsible for its antimicrobial effect [5]

Vitamin E also called as alpha-tocopherol is a natural occurring and a very powerful antioxidant.

Its mechanism of action is quiet similar to that of sodium ascorbate. It has the ability to reverse the compromised bond strength of dentin caused by use of Naocl by scavenging free radicals and molecular oxygen,. The beneficial effect of alpha-tocopherol is attributed to its antioxidant and alcohol solvent effect.[6]

Hence, the aim of this study is to assess the microleakage of epoxy based sealer by using three different natural antioxidants after use of sodium hypochlorite

Material and Method

Thirty-six teeth extracted for periodontal and/or orthodontic reasons with single roots and single canals were collected and stored in distilled water until use. The teeth were decoronated at the cementoenamel junction and standardized to a length of 12 mm with the help of a low-speed diamond disk under water spray. Patency of each root canal was checked using a K-file (#15) (Mani Inc., Tochigi, Japan) and working length was established 1 mm short of the apex. Cleaning and shaping were performed by crown-down technique, using ProTaper shaping and finishing files (Dentsply, Maillefer, Ballaigues, Switzerland), up to size F4. During the preparation of the canal, a total of 5 ml of 5.25% NaOCl (Photon) was used for irrigation between instruments. After instrumentation, a final rinse with 5 ml of 17% ethylenediaminetetraacetic acid (EDTA) (Prevest EDTA Solution) was done to remove the smear layer. Finally, the canals were irrigated with 5 ml of 5.25% NaOCl. The samples were divided into four groups (n = 9) based on the final treatment protocol:

Group 1- Saline

Group 2- 6.5% proanthocyanidin

Group 3 –50% aloe vera

Group 4- 10% alpha tocopherol

Antioxidants used in the study i.e 6.5% proanthocyanidin[7], 50% aloe vera[8] and 10% alpha tocopherol [6]solution where prepared .Then the canals were flushed with their respective solutions, keeping a standardized quantity and contact time of 5 ml and 5 min, respectively. The root canals of the samples in Groups 2, 3 and 4 were additionally rinsed with 5 ml of distilled water and the canals of all the samples were dried with paper points (Meta). The canals were coated with ADSEAL sealer (Meta Biomed America) with the aid of a lentulospiral (Mani Inc., Tochigi, Japan) and obturated using #F4 gutta-percha (Sure Endo). The root samples were coronally sealed with intermediate restorative material (IRM, Dentsply DeTrey, Konstanz, Germany) and were stored in distilled water for 7 days.

After 7 days samples were coated with nail varnish expect at apical 2mm and then immersed in 50% silver

nitrate (Nice Chemicals) for 12 hours and then dried under fluoresecent light for next 8 hours. Samples were then viewed under stereomicroscope under 30X magnification. Grading for penetration depth evaluation was done in accordance with the Kytridou V et al [9]

Result

Comparison of marginal leakage among four study groups

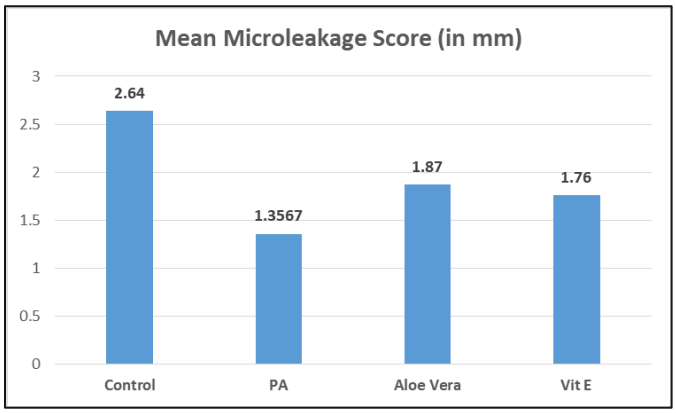
Groups	N	Mean	Std. Deviation	p value
Control	9	2.6400	.23335	0.001*
PA	9	1.3567	.30623	
Aloe Vera	9	1.8700	.43655	
Vit E	9	1.7600	.40417	

One Way ANOVA test; * indicates significant at p≤0.05

Pairwise comparison of leakage score

Pair	Difference	p value
Control vs PA	1.2833	0.001*
Control vs Aloe Vera	0.77	0.001*
Control vs Vit E	0.88	0.001*
PA vs Aloe Vera	-0.5133	0.021*
PA vs Vit E	-0.4033	0.095 (NS)
Aloe Vera vs Vit E	0.11	0.912 (NS)

Post hoc tukey test; * indicates significant at p≤0.05; NS: Non-significant



Minimum microleakage seen in PA group (1.35mm) followed by vitamin E group (1.76mm)

Aloe vera shows 1.87 mm microleakage and the maximum leakage is seen in control group (2.64mm)

Pairwise comparision shows there is significant difference in microleakage between control and PA group(1.2833),control and aloe vera group(0.77) ,control and vitamin E group(0.88) and PA and aloe vera group (-0.5133).

Discussion

The aim of endodontic therapy is to achieve a complete hermetic seal of the root canal and prevention of coronal and apical microleakage [10]

Microleakage, whether coronal or apical, adversely affects the success of root canal therapy**.** Many factors which can lead to microleakage during the course of root canal treatment like: isolation, patient co-operation, canal anatomy, root morphology, operator skill, root canal sealing and the choice of filling material. Cleaning and shaping, and three-dimensional obturation of the root canal system are essential for promoting periapical healing.[11]

For the success of root canal treatment it is essential to prevent coronal and apical microleakage of bacteria and their by-products and this can be achieved by an adequate three-dimensional seal of root canal system. Ingle stated that most failed cases of root canal treatment are due to percolation of fluid from inflamed periapical tissue into improperly obturated canals. Allen and Strindberg emphasized on the importance of complete sealing of the root canal system along with a fluid tight seal for long term success of root canal treatment**.** [12]

To counter this apical leakage problem many advances in root canal sealers have been done one of which is introduction of resin based sealers. Resin-based sealers not only can penetrate the dentinal tubules, but also can adhere to the exposed dentin surface. [12]

In a study by Lee et al he concluded that resin-based sealers have high bond strength compared to other sealers. Resin sealer penetrates deeper into the dentinal tubules due to its flow ability and long-term polymerization time and it even have a very low shrinkage while setting and long-term dimensional stability.[1]

In this study we have used ADSEAL (an epoxy based resin sealer) which is one of the recent type of resin based sealer. Adseal is a type of resin-based sealer that contains bismuth phosphate and zinc-oxide mixed with vinyl polymer available in two paste-containing tubes. [13] It has excellent chemical, physical properties and sealing ability because of which it is considered superior to other types of epoxy based resin sealers. [10] It even shows better penetration into root canal irregularities thereby increasing its micromechanical bond to root dentin, but Naocl which continues to be the main irrigant contribute to a decrease in this micromechanical interaction between dentin and resin.

Studies have shown that pretreatment of root canal with antioxidant before application of resin based sealers is capable of reversing the Naocl induced effects by interacting with byproducts of Naocl and resulting in its neutralization. [4] In this study we have used newer antioxidants such as alpha tcopherol, aloe vera which have never been studied previously and compared it with PA. We have used all the naturally occurring antioxidants as they have been proven to be safe in various clinical applications.

In our study, Aloe vera on comparision with control group shows a significant reduction in microleakage as it contain polyphenolic compounds such as alone A and B The antioxidant effect of Aloe vera mainly due to polysaccharides found in parenchyma tissue of Aloe vera leaves and other substances containing in Aloe vera leaves namely polyphenols, indoles and alkaloids. This material can neutralize the free radicles therefore Aloe vera extract could reverse the compromised bond strength. In a study by Barandozi, he said that antioxidant activity of Aloe vera is not only caused by a single chemical component but the action of several components containing in Aloe vera that work synergistically. [14] Anthraquinone present in aloe vera is also responsible for its antioxidant property. [8]

Alpha tocopherol on comparison with control group shows a significant reduction in microleakage as it is a chain breaking antioxidant that prevents propagation of free radical reaction. Furthermore presence of alcohol in the composition of 10% alpha tocopherol in this study adds to its antioxidant property since 10% alpha tocopherol was not miscible in water. Thus, the reduction in microleakage is not only due to the antioxidant agent of α-tocopherol but also due to the presence of alcohol .It even allows free radical polymerization of adhesive resin. [14]

Proanthocyandin is a naturally occurring antioxidant found in high concentration from natural sources such as grape seed extract, cocobeans, pine bark extract and cranberries. [8] Grape seed extract chosen for this study as it contain 10% higher conc of PA's. [7]Grape seed extract is a natural antioxidant that contains 98% oligomeric proanthocyanidin complexes (OPCs). It has antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and anti-allergic properties. OPC contains multiple electron donor sites (hydroxyl sites) that allow it to bind to free radicals by donating its hydrogen atoms. Gallic acid which is present in the content of proanthocyandin also increases the free radical scavenging activity. [8]

PA has the ability of collagen cross-linking which could be another possible reason behind its improved bond

strength,PA's stabilizes and increases cross linking of type 1 collagen.[4] Kalra et al. in this study observed that PAs enhances biodegradation resistance and they also maintain the interfacial seal between resin sealer and root dentin. Ethanol and acetone are usually used as solvents in the extraction of PAs from GSE. Sarac et al. stated that these solvents could effectively be used to remove water from the dentin surface and aid better penetration of resin into the dentin. This could have also improved the action of PAs on the root dentin [4]

Thus PA significantly reduces microleakage to a much greater extend when compared with aloe vera, alpha tocopherol and control group as it shows much greater degree of oxygen free radical scavenging potential and it is even 50 times more effective antioxidant than alpha tocopherol and aloe vera. [3]

Conclusion

Within the limitation of this invitro study,it can be concluded that-

- Use of NaOCl as a final irrigant significantly increases the microleakage of ADSEAL sealer to root canal dentin.
- Use of PA, Alpha tocopherol and Aloe vera as final irrigating solutions reduces the microleakage of ADSEAL sealer to NaOCl treated dentin.
- Alpha tocopherol is as good as Proanthocyanidin in improving the adhesion of epoxy resin sealer to root dentin.

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