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Original Research Article

Confocal laser scanning microscopic evaluation of depth of penetration and Sealer/Dentin interface between endodontic sealers - An in-vitro study

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ABSTRACT

Objective: An endodontic sealer contributes in obtaining an appropriate seal for the root canal system by establishing an association between the root canal walls and coronal restoration thereby promoting healing. This present research intended to compare the penetrating capacity and to determine sealer/dentin interface among various endodontic sealers.

Materials and Methods: Horizontally sectioned tooth samples were obtained at 3 and 5mm and randomly divided into three groups: Group I - Chemically cured (AH Plus) sealer; Group II - Dual cured (EndoREZ) sealer; Group III – BioCeramic Nano sealer (iRoot SP). All three groups were coated with their respective sealers and obturated using cold lateral compaction technique. The samples were observed using Confocal Microscope for penetrating capacity evaluation and reviewing of sealer/dentin junction at 3 and 5mm depth. The results were statistically analyzed using one-way ANOVA and Tukey's Post-Hoc method.

Results: On depth of penetration evaluation, one-way ANOVA analysis showed Group II exhibited maximum depth of penetration in contrast to Group III and Group I. Group I showed the least average depth of penetration among the other two groups at both 3 and 5mm. On sealer/dentin interface evaluation at 3mm, Group II showed increased interface in comparison with to Group III and Group I. No statistical significance evident among the groups at 5mm.

Conclusion: EndoREZ showed maximum depth of penetration in comparison with iRoot SP and AH Plus sealer at both 3 and 5mm. EndoREZ also showed significant sealer/dentin interface at the level of 3mm when compared to other sealers used.

1. Introduction

The challenge of an root canal procedure depends on the expulsion of the diseased pulp tissue, microbes, their resultant products and noxious debris from the root canal system.[1] Shaping and cleaning procedure is considered the most important step in management of diseased pulp canal which is followed by a three-dimensional obturation.

Obturation eliminates the channels of spillage from the mouth and the periapical tissues into the pulp cavity with a creation of a hermetic seal.[2] The primary purpose of an obturation sealer should be aimed at providing a three-dimensional root canal seal combined with a satisfactory adaptation to the root dentine.[3] Root canal sealers amalgamates the coronal material and the canal wall by occupying the leftover spaces amongst, hence with providing an impermeable seal.[4] The antibacterial effect of sealer is enhanced by the extended penetration of the sealer as it encloses the living bacteria inside the tubules and uncouples them from obtainable nutrient sources for their nourishment.

Endodontic sealers in common, are categorized into distinctive classes according to their principal component, such as zinc oxide eugenol containing, Resins containing predominantly epoxy based and methacrylate based sealers and silicone containing sealers. Sealers containing epoxy

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resin are aquaphobic in contrast to methacrylate resin-based sealers which are aquaphilic, specially when used along with EDTA during smear layer removal from the dentine.[5]

Resin based sealers substitutes traditional sealers containing zinc oxide eugenol, due to its monoblock effect in the root canal system.[6] iRoot SP, a novel bioceramic nano sealer is a calcium silicate-based obturation sealer which demands no additional curing. It has outstanding mechanical and biotic properties along with substantial handling properties. The above mentioned sealer is aquaphilic, insolvable, radiopaque, no aluminium containing sealer with a high pH and requires wetness to strengthen.[7]

The predominant cause for a root canal obturation failure according to enormous studies is the existence of spaces and voids at the sealer/dentin junction. The appearance of such spaces may grant the ingress of fluids in the filled root canal, thereby interrupting the healing process.[8] These spaces may also contribute to the recolonization of microorganisms, leading to endodontic failure demanding retreatment.[9]

The analysis of the sealer/dentin annexation and its deep penetrating ability into dentinal tubules allows in determining the suitable obturating materials and filling techniques which helps in achieving a successful endodontic therapy. The penetrating ability of sealers and the sealer/dentin interface is studied using inummerable microscopic techniques such as stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). On contrasting with standard SEM method, confocal microscopy provides comprehensive information about the existence and dispersion of endodontic sealers between the dentinal tubules in the complete circumference of the radicular canal wall with a comparable low magnification of 100x utilizing Rhodamine-marked sealers emitting fluorescence.[10]

Although finding the adapting ability and penetration capacity of radicular filling materials with this technology appears to be ideal, clinical achievements with these sealers and different obturation approaches have been pending. Thus, the current research aimed at comparing the depth of penetration and to determine sealer/dentin interface of various endodontic sealers using Confocal Laser Scanning Microscope.

2. Materials and Methods

2.1. Preparation of sample

An ethical acceptance was procured before beginning the current research (Ref no: CSP/13/OCT/31/205). Mandibular dentition with solitary roots, thirty in number were kept in 0.1% sodium azide containing normal saline and used for this study. The crown portions of the teeth were sliced using an isomet saw of 0.3mm thickness and the root length was systematized at 14mm. The roots were then arbitrarily divided into 3 groups (Table 1).

2.2. Root canal instrumentation and subsequent obturation

To ensure the presence of a solitary canal, facial and proximal aspect digital radiographs were acquired. The working length was determined post checking of canal patency using a size 10K file which was inaugurated in every canal until the instrument was visualized through the apical foramen. Then, the teeth were subsequently prepared upto F4 size ProTaper Universal System (Dentsply Maillefer, Ballaigues, Switzerland) at the working length. 2ml of 1% sodium hypochlorite was utilized for irrigation procedure after every file instrumentation. To eliminate the smear layer, all canals were flushed using 3 ml of 17% EDTA for a period of 2 minutes followed by 2ml of 1% sodium hypochlorite over 1 minute and aseptic paper points (Dentsply Maillefer) were used for following drying of the canal. The blending of obturation sealers were done adhering to the directions given by their respective manufacturers. To favour assessment under the confocal microscope, every sealer was mixed with Rhodamine B (Sigma-Aldrich, St. Louis, MO) to estimated concentration of 0.1%. The placement of the sealer was performed using master cone gutta percha point with a size 50 (Dentsply Maillefer). The subsequent obturation was done using a cold lateral condensation technique utilizing a size B endodontic finger spreader (Dentsply Maillefer) inserted short of 2-3mm from the determined working length. Additional 2 percent gutta-percha 20 size cones were used until the root canal was filled along its length. The surplus gutta percha was sheared off at the orifice level making a use of a heated plugger.

2.3. Sectioning and image analysis

At the extent of 3 and 5mm from the radicular apex, the teeth were fragmented horizontally. An inverted Leica TCS-SPE confocal microscope (Leica, Mannheim, Germany) was used for investigating the slices of dentin specimens. The absorption and emission wavelengths for rhodamine B dye used were 540nm and 590nm respectively. The images were taken down in the fluorescent mode under confocal laser scanning microscopic (CLSM). Images were documented at an optical zoom of 10x with a 0.4 numeric aperture, and 20x optical zoom with a 0.7 numeric aperture. 10x images were documented at a size of 1550x1550μm^2 with 512x512 pixels resolution. 20x images were recorded at a size of 775x775$\mu m^2$with 1024x1024 pixels resolution. At 20x optical zoom, the area of extended penetration was observed and noted. The extent of sealer penetration into dentinal tubules and sealer/dentin interface was depicted by fluorescence from the microscope. The resin-dentin juncture up to the utmost depth was tracked down as the distance measured for determining the depth of penetration. An electronic measuring ruler, feature available in the image recorder of Confocal microscope was used to record the distance measured. The extent of the sealer penetration was calculated until the outer limit of the visible field in the microscope having the canal wall as the starting point. The space between the filling material and dentin were also measured.

The SPSS 16.0 version software was used to interpret the collected data. The mean and standard deviation were utilized to represent the data descriptive statistics. The one-way ANOVA along with Tukey's Post-Hoc test was applied for performing multivariate analysis. In the above statistical tool, the probability value 0.05 is considered as significant level.

3. Results

In Table 2 , on evaluation of the depth of penetration, one-way ANOVA analysis showed at both 3mm and 5mm, Group II-Dual cured (EndoREZ) exhibited maximum depth of penetration (Figures 3 and 4) when compared with Group III-Bio Ceramic Nanosealer (iRoot SP) (Figures 5 and 6) and Group I-Chemically cured (AH Plus) (Figures 1 and 2). Group I-Chemically cured (AH Plus) showed the least average depth of penetration among the other two groups at both 3 and 5mm.

In Table 3, on sealer/dentin interface evaluation, at 3mm Group II-Dual cured (EndoREZ) (Figure 3) showed increased interface when compared with to Group III-Bio Ceramic Nanosealer (iRoot SP) (Figure 5) and Group I-Chemically cured (AH Plus) (Figure 1). No statistical difference was evident among the groups at 5mm (Figures 2, 4 and 6).

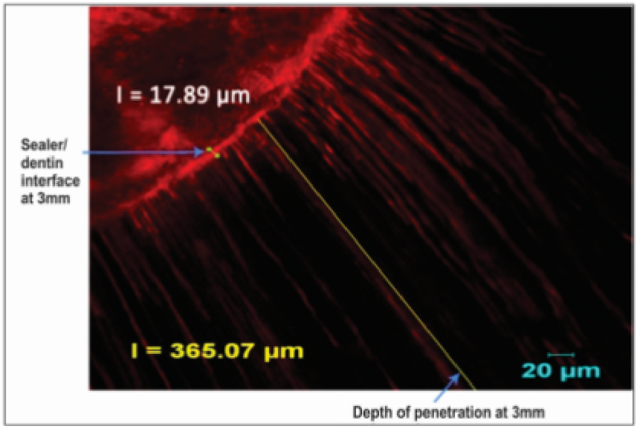

Fig. 1: Depth of penetration and sealer/dentin interface of AH plus at 3mm.

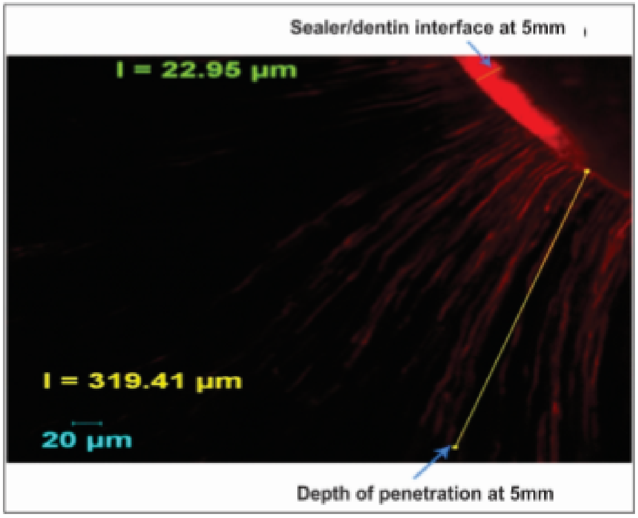

Fig. 2: Depth of penetration and sealer/dentin interface of AH plus at 5mm.

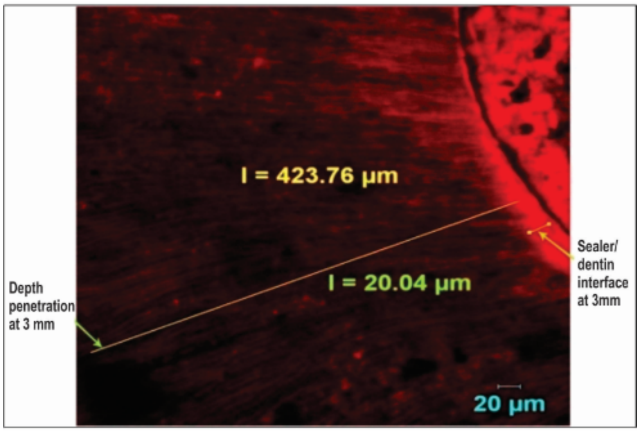

Fig. 3: Depth of penetration and sealer/dentin interface of EndoREZ at 3mm.

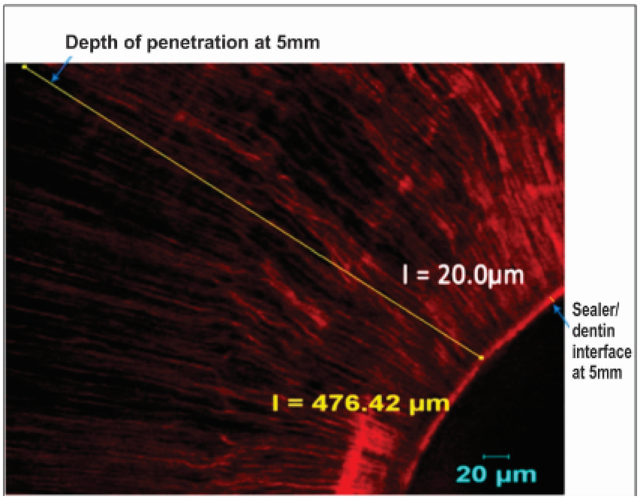

Fig. 4: Depth of penetration and sealer/dentin interface of EndoREZ at 5mm.

Table 1: Composition of endodontic sealers

Experimental groups	Sealers used	Composition	Product details
Group – I	**Chemically cured (AH Plus)** Dentsply Maillifer	Bisphenol-A epoxy resin Dibenzyldiamine, Bisphenol-F epoxy resin Aminoadamantane Tricyclodecanediamine, Zirconium oxide, Calcium tungstate, Silica, Zirconium oxide, Iron oxide, Silicone oil	Ref no 1310000937 & exp date- 2015-07
Group –II	**Dual cured (EndoREZ)** Ultradent, South Jordan, UT	Zinc oxide barium sulfate, pigments, Urethane Dimethacry late resin matrix	Ref. no 5900 & exp. date-2015-06.
Group-III	**Bioceramic Nanosealer (iRoot SP)** Innovative Bio-creamix Inc, Vancouver, Canada ;	Zirconium Oxide. Calcium Silicates, Calcium Phosphate, Calcium Hydroxide, filler and thickening agents.	Ref. no- 5017560U0 & exp. date - 2015-03.

Table 2: Depth of penetration of the sealers

Multiple comparison using One-way ANOVA					
	Groups	**Mean**	**S.D**	**F-Value**	**P-Value**
	AH Plus	373.61	56.99		
3mm	EndoREZ	431.09	15.01	5.408	0.011 **
	iRoot SP	409.28	34.60		
	AH Plus	375.68	48.32		
5mm	EndoREZ	435.27	24.75	6.253	0.006 **
	iRoot SP	420.65	40.99		

Highly Significant at **P ≤ 0.01 level

Table 3: Sealer / dentin interface

Multiple comparison using One-way ANOVA					
	Groups	**Mean**	**S.D**	**F-Value**	**P-Value**
	AH Plus	21.70	2.22		
3mm	EndoREZ	18.60	2.87	4.861	0.016**
	iRoot SP	18.47	2.74		
	AH Plus	21.92	1.99		
5mm	EndoREZ	21.18	1.38	0.768	0.474 #
	iRoot SP	21.13	1.36		

*Highly Significant at P ≤ 0.01 level,
No Significant at P ≤ 0.05 level

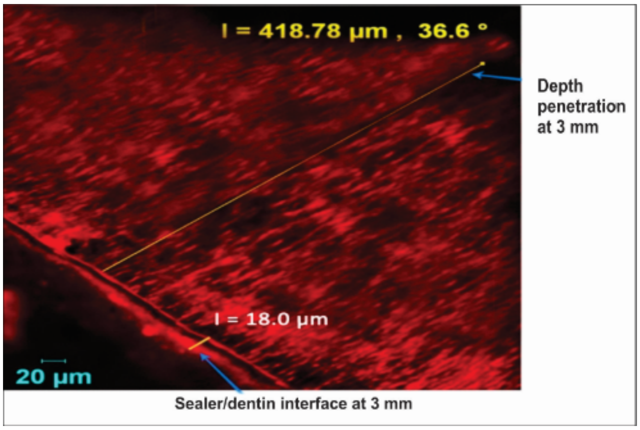

Fig. 5: Depth of penetration and sealer/dentin interface of iRoot SP at 3mm.

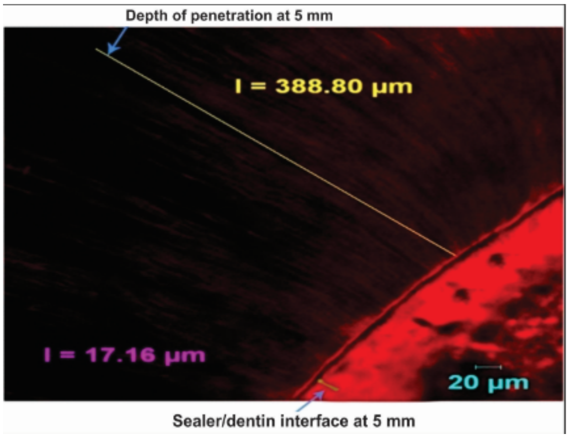

Fig. 6: Depth of penetration and sealer/dentin interface of iRoot SP at 5mm.

4. Discussion

The ideal requirement of an endodontic sealer is to provide an excellent seal when set, should adhere adequately to canal wall and the filling material. Endodontic sealers with dense film will have difficulty in extending into the dentinal tubules and thus pool at the resin-dentin interface[4], often to form a lining of resin at the resin-dentin junction. Therefore, the ultimate outcome of a endodontic filling is to have a dense quantity of gutta percha coated with a minimum volume, thin consistency endodontic sealer, that can penetrate deep into root canal irregularities and dentinal tubules.[11,12] Other advantages of an ideal sealer includes, their probable antimicrobial effects by containment of viable bacteria within the tubules and eliminating them for obtaining nutrient sources.[3]

Epoxy resin-based sealers binds to dentin and are considered to be the "gold standard" sealer. It is based upon by polymerization reaction of epoxy resin amines and is available as a two-component paste root filling sealer.[13] It is biocompatible, well tolerated by underlying tissue, dimensionally stable and is capable of producing a good seal.

In the quest to improve the properties of the gold standard resin-based sealer, a new methacrylate-based resin sealer (EndoREZ) has been developed. EndoREZ is a aquaphilic, two-component, dual cured sealer containing a matrix of urethane dimethacrylate along with zinc oxide, barium sulphate, resins, and pigments. Their merits include enhanced binding to the root dentin and creation of monoblock.[14] The properties of above-mentioned resin sealer were found to be far superior on contrasting with the chemically cured AH Plus sealer.

Recent advancements in nanotechnology introduced a novel non-resin based bioceramic nano sealer, the iRoot SP. It is a hydrophilic calcium silicate-based root canal sealer which is said to have a radiopaque and nonsolvable property. It contains calcium, calcium phosphate, calcium hydroxide, and zirconium oxide, without aluminum. No supplementary curing mediator or mixing is required for setting of the sealer. It requires wetting to set and harden.[15]

Confocal laser scanning microscope instead of using the entire specimen provides better focus with the well-defined optical section. They provide required contrast, clarified image and is detection sensitive.[16] They produce improved light microscopic images of viable cells and tissues.

Maximum depth of penetration was evident in Dual cured EndoREZ sealer followed by Bioceramic Nanosealer iRoot SP and the least depth of penetration was evident in chemically cured AH Plus sealer which was statistically significant at both 3 and 5mm. The sealer dentin interface was statistically significant at 3mm and no significance was evident at 5mm.

The good penetration of dual cured (EndoREZ) sealer can be explained on the basis that it has hydrophilic methacrylate resin monomer additives and low viscosity also it shows good canal adaptation, adequate flow and film thickness. Tay et al 1998 reported that, EndoREZ sealer penetrated to a depth of 800-1200 μm, suggesting that the extent of dentinal infiltration of resin-based sealers may rely on the sealer being drawn into the tubules.[3]

When compared to zinc oxide-based sealers and chemically cured (AH Plus) sealer, dual cured methacrylate sealer creates long resin tags thereby enhancing the bonding between gutta percha and root canal dentin which prevents microleakage and also penetrate deep into dentinal tubules due to their hydrophilic properties.[17]

The second highest depth of penetration in the present study was evident in Bioceramic Nanosealer (iRoot SP) which could be due to their nanosize, excellent level of viscosity which enhances flow of the sealer and increases the depth of penetration into dentinal tubules (Seyda et al 2010).[11] Due to their hydrophilicity and low contact angle, they produce a superior hermetic seal. They exhibit potent antimicrobial action, very biocompatible and insoluble in water preventing its dissolution.

On evaluation of sealer/dentin interface at level of 5 mm, current study showed no significance between the groups, which could be ascribed to the fact of that root apical third possess decreased dentinal tubules in number and if present, they possess small diameter and are mostly closely located. Furthermore, the apical portion of roots shows a pronounced variation in the morphology. The other possible reason may be due to the stress created during obturation using resin-based sealers due polymerisation shrinkage as reported by Hammad et al 2009.[18]

5. Conclusion

EndoREZ showed maximum depth of penetration in comparison with iRoot SP and AH Plus sealer at both 3 and 5mm. EndoREZ also showed significant sealer/dentin interface at the level of 3mm when compared to other sealers used.

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7. Conflicts of Interest

There are no conflicts of interest.

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