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Evaluation of apical seal and tubular penetration of a novel bioactive glass sealer, bioceramic sealer and resin–based sealer: an In-Vitro study

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Abstract

INTRODUCTION. The majority of endodontic failures are caused primarily by inadequate sealing of the root canal. Seepage of fluids is likely to occur if apical seal is not properly established.
AIM. This in-vitro study was to evaluate the apical seal and tubular penetration of a novel bioactive glass sealer: NISHIKA CANAL SEALER BG, bioceramic sealer: CERASEAL and epoxy resin–based sealer: AH PLUS.
MATERIALS AND METHODS. 49 extracted human single rooted mandibular 1st premolar teeth with fully formed apices were taken and decoronated at the Cemento Enamel Junction for standardized working length of 14 mm. All samples were instrumented upto size F3 of ProTaper Gold files. After complete irrigation protocols with Sodium Hypochlorite, Saline, Ethylene diamine tetra acetic acid and Chlorhexidine, samples were divided into 3 groups according to the sealers used. Obturation was done using single cone technique. All the specimens were put in 1% methylene blue dye for 72 hours after keeping them in incubator for 48 hours. Teeth were split into two halves, one visualised for dye penetration and other for tubular penetration and scoring was done.
RESULTS. Kruskal Wallis test revealed that there were significant differences in microleakage and tubular penetration between all the groups ($p = 0.01$). Nishika Canal Sealer BG had better apical sealing ability and tubular penetration followed by CeraSeal and AH Plus.
CONCLUSIONS. Within the limitations of the study, it was concluded that, Nishika Canal Sealer BG has the maximum apical sealing ability and tubular penetration when compared to CeraSeal and AH Plus

Keywords: stereomicroscope, sealer, scanning electron microscope, microleakge, bioceramic, bioactive glass

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Оценка апикального герметизма и тубулярной пенетрации нового биоактивного стекло-герметика, биокерамического герметика и герметика на основе смолы: экспериментальное In-Vitro исследование

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Резюме

ВВЕДЕНИЕ. Большинство неудач эндодонтического лечения обусловлено недостаточной герметизацией корневого канала. При отсутствии надежного апикального уплотнения возможно просачивание жидкостей, что может привести к неудаче лечения.
ЦЕЛЬ. Настоящее in vitro исследование направлено на оценку апикального герметизма и тубулярной пенетрации нового биоактивного стекло-герметика NISHIKA CANAL SEALER BG, биокерамического герметика CERASEAL и герметика на основе эпоксидной смолы AH PLUS.

МАТЕРИАЛЫ И МЕТОДЫ. Для исследования было отобрано 49 удаленных однокорневых нижних первых премоляров с полностью сформированными апикальными отверстиями. Все зубы были подвергнуты декапитации на уровне цементно-эмалевого соединения, обеспечивая стандартизированную рабочую длину 14 мм. Подготовка корневых каналов проводилась с применением файлов ProTaper Gold до размера F3. После выполнения стандартного протокола ирригации с использованием гипохлорита натрия, физиологического раствора, этилендиаминтетрауксусной кислоты (ЭДТА) и хлоргексидина, образцы были разделены на три группы в зависимости от применяемого герметика. Обтурация проводилась методом одного конуса.
После завершения обтурации все образцы были помещены в 1% раствор метиленового синего на 72 часа после предварительного выдерживания в инкубаторе в течение 48 часов. Затем зубы были расщеплены на две половины: одна использовалась для оценки проникновения красителя, другая – для анализа тубулярной пенетрации с последующей балльной оценкой.
РЕЗУЛЬТАТЫ. Анализ с применением критерия Краскела-Уоллиса показал наличие статистически значимых различий в показателях микроподтекания и тубулярной пенетрации между всеми группами (p = 0,01). Nishika Canal Sealer BG продемонстрировал наилучшие показатели по апикальному герметизму и тубулярной пенетрации, за ним следовали CeraSeal и AH Plus.
ВЫВОДЫ. С учетом ограничений данного исследования было сделано заключение, что Nishika Canal Sealer BG обладает максимальной апикальной герметичностью и глубиной тубулярной пенетрации по сравнению с CeraSeal и AH Plus.

Ключевые слова: стереомикроскоп, герметик, сканирующий электронный микроскоп, микроподтекание, биокерамика, биоактивное стекло.

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INTRODUCTION

Endodontic treatment is fairly predictable in nature with reported success rates up to 86–98% [1]. The majority of endodontic failures are caused primarily by inadequate sealing of the root canal. Ideally, the root filling material should seal the root canal system and favour tissue repair [2]. Seepage of fluids is likely to occur if apical seal is not properly established [1]. For good sealing, the filling material must be able to adhere to the dentinal wall while preventing invasion of microorganisms [3].

To ensure the long-term effectiveness of root canal treatment, three-dimensional root canal obturation is crucial [4]. When considering a three-dimensional filling of the root canal system, the capacity to seal imperfections and penetrate dentinal tubules is necessary [5]. This capability is influenced by the material's fluidity, that enables the sealers to access the regions that instruments might not have reached [6]. Apart from the apical and coronal leakage, micro gaps between the sealer and dentinal tubules also play an important role in clinical success of the treatment.

Good tubular penetration and adaptation of the sealer ensures adequate stability, reduced microleakage, increased fracture resistance and effectively prevent bacteria from entering into the tubules [7]. It also reduces the micro-gaps between the material and the dentinal walls. Adequate tubular penetration can also three dimensionally fill the root canal system. Creating a fluid tight apical seal prevents any ingress of microorganisms into the root canal system.

Endodontic treatment approaches have evolved in response to technology improvements and this has led to significant rise in success rates of the root canal treatment [8]. Root canal sealer along with gutta-percha, have been demonstrated to be necessary for a successful obturation, as the sealer needs to bond with dentin along the canal wall. However, standard root canal sealer based on Grossman's formula is barely ideal because it is neither adequately adhesive nor binds completely with dentin [8].

The purpose of this in vitro study was to compare the sealing ability and tubular penetration of new bioactive glass sealer NISHIKA CANAL SEALER BG (Nippon Shika Yakuhin), along with commonly used bioceramic sealer – CeraSeal (META BIOMED) and epoxy resin – based sealer AH Plus (DENTSPLY) in oval canals.

MATERIALS AND METHODS

Samples of 49 extracted human single rooted mandibular 1st premolar teeth with fully formed apices which were extracted due to orthodontic reasons were taken for the study after obtaining ethical clearance from the institution. These were analyzed for any anatomical variations and internal resorption by taking RVG. All the samples were inspected under magnification for any cracks along the tooth surface to avoid other routes of leakage. Standardization of root length to 14 mm were done by decoronating them near the cemento-enamel junction (CEJ). Only apical foramen with diameter smaller or equal to size #10 K-files were selected. In order to standardize the samples for preparation, the file was introduced into the canal and pushed beyond the apex until the tip of the file was seen through it.

The working lengths were determined using a #10 K files upto the apical foramen for standardization. Instrumentation of the root canals were instrumented up to size F3 of ProTaper Gold rotary files using Crown down technique.

The canals were irrigated with a disposable syringe and a 30 G side vented needle using 2 ml of 3% NaOCl throughout the instrumentation. A #10 K-file was used to maintain apical patency throughout the preparation. Subsequent to instrumentation with files saline irrigation was done and upon completing instrumentation, the canals were rinsed with 2.5 ml of 17% EDTA solution followed by agitation with EndoActivator (EA) for 30 sec and was flushed with saline. The canals were then irrigated with NaOCl. Lastly irrigation was performed with 2 ml of saline. Master cone was selected for all the samples (size F3 GP) and this was confirmed using RVG. All the canals were then dried with paper points.

Samples were then divided into 3 experimental groups. Group 1: Obturation was done by using a F3 GP cone and Canal Sealer BG with a single cone technique, following the manufacturer's instructions. Group 2: Obturation was done by using a F3 GP cone and AH Plus sealer with a single cone technique. Group 3: Obturation was done by using a F3 GP cone and CeraSeal with a single cone technique, following the manufacturer's instructions. CeraSeal was pumped into the canal and the master cone was fitted till the working length.

The positive control group included two teeth that had a single cone with no sealer. The negative control group included two teeth that were unobturated and coated fully with nail varnish.

The access of all the samples was then sealed with intermediate restorative material. All the samples were then coated with two layers of fingernail varnish leaving only 1 mm of the apical foramen to remain exposed. Negative controls were completely coated with fingernail varnish including the apical foramen portion.

All the samples were then allowed to set in the incubator at 37^0 C for 42 hours and were then immersed in 1% methylene blue dye for 72 hours. After removal from the dye, the roots were rinsed in tap water, and the fingernail varnish was completely removed by scraping with a Bard-Parker number 11 blade in order to facilitate easy splitting of the roots.

The buccal and lingual portion of the roots were grooved longitudinally with a cylindrical diamond point in a high-speed handpiece and with the help of a chisel and mallet they were split into halves. Out of two halves, one half was used for leakage evaluation, and the other half was used for tubular penetration evaluation.

The amount of microleakage on the fractured side of the spilt root was measured from the apex to the highest extent of dye penetration in the coronal direction. Scoring was performed by using a stereomicroscope at 10X magnification to examine the dye's full extend.

For scanning electron microscopy (SEM) evaluation, all the specimens were vacuum dried, sputter coated with gold, and viewed under SEM. The penetration depth of sealer into dentinal tubules were examined at cervical, middle, and apical third of the root and scoring was done according to the depth of penetration of sealers into the tubules by an independent observer. Scoring for microleakage and tubular penetration was given according to Attur et al. [9] (Table 1).

Statistical Analysis

Kruskal Wallis Test followed by Dunn's post hoc test was used to compare the mean penetration depth for micro leakage & sealer penetration into dentinal tubules scores between 3 groups. The level of significance was set at $p < 0.05$.

RESULTS

The mean Dye penetration score for Group 1 was 0.47 ± 0.64, for Group 2 was 1.47 ± 1.06 and for Group 3 was 0.67 ± 0.72. These differences in the mean Dye penetration scores for Micro Leakage between 3 groups was statistically significant at $p = 0.01$ (Table 2).

The mean Penetration depth of sealer for Group 1 was 3.53 ± 0.64, for Group 2 was 2.60 ± 0.91 and for Group 3 was 3.20 ± 0.94. These differences in the mean Penetration depth of sealer into dentinal tubules between 3 groups was statistically significant at $p = 0.02$ (Table 3).

Table 1. Scoring criteria according to Attur et al. [9]

Таблица 1. Критерии оценки согласно Attur и др. [9]

Index	Score	Value
Dye penetration for microleakage in root canal walls	0	No visible dye on the root canal walls
	1	Dye visible on the root canal walls
	2	Penetration of dye up to half of the length longitudinally
	3	Penetration of dye more than half of the root surface longitudinally
Penetration depth of sealer into dentinal tubules	0	No penetration
	1	1–20 μ
	2	21–40 μ
	3	41–60 μ
	4	More than 60 μ

Table 2. Comparison of mean Dye Penetrations scores for Micro leakage between 3 groups using Kruskal Wallis Test

Таблица 2. Сравнение средних показателей проникновения красителя для оценки микроподтекания между тремя группами с использованием критерия Краскела-Уоллиса

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	15	0.47	0.64	0	2	0.01*
Group 2	15	1.47	1.06	0	3	
Group 3	15	0.67	0.72	0	2	

*Statistically Significant
*Статистически значимо

Table 3. Comparison of mean Penetration depth of sealer into dentinal tubules between 3 groups using Kruskal Wallis Test

Таблица 3. Сравнение средней глубины проникновения герметика в дентинные канальцы между тремя группами с использованием критерия Краскела-Уоллиса

Groups	N	Mean	SD	Min	Max	*p*-Value
Group 1	15	3.53	0.64	2	4	0.02*
Group 2	15	2.60	0.91	1	4	
Group 3	15	3.20	0.94	1	4	

*Statistically Significant
*Статистически значимо

DISCUSSION

The root canal filling materials prevent microbes and its exudates from communicating into the periradicular tissues. A significant proportion of failures in endodontic treatment and retreatment is attributed to the difficulty in obliterating accessory canals, apical deltas, anastomoses, fins, and irregularities of the root canal system and failure to get a fluid tight apical seal [9].

Among various types of sealer used today AH plus has gained popularity due to its radiopacity, biocompatibility, ease of use and availability. AH Plus is an epoxy-bis-phenol resin–based sealer that also contains adamantine and bonds to root canal [10]. As a kind of epoxy resin–based sealer, AH Plus is used frequently in clinical work and is usually chosen as the control in studies on the properties of new sealers because of its long track record.

CeraSeal is calcium phosphate based bioceramic sealer dispenced using a pre-mixed syringe. CeraSeal sealers have excellent sealing adaptation and biocompatibility, as well as rapid tissue recovery [11]. Calcium silicate produces Calcium Aluminate Hydrate gel and Calcium Silicate Hydrate gel by absorbing the moisture from surrounding tissues in the root canal and some crystallization of Calcium Hydroxide.

Nishika Canal Sealer is developed from BG-based biomaterials and originally intended for both dental pulp and bone regeneration therapies. This is a two-phased paste; Paste A containing silica dioxide, bismuth subcarbonate, and fatty acids, wherein Paste B contains calcium silicate glass, magnesium oxide and silica dioxide, etc. this two-paste system hardens when exposed to heat or moisture [10].

Bioceramic root canal sealers can promote physical and chemical bonding with dentin by creating hydroxyapatite precipitates at the dentin sealer interface after it sets. Conventional sealers can shrink as they harden and dissolve in tissue fluids, creating a space that allows microbes to escape [12]. It is well known that bioceramic interact with dentin along the root canal to provide biomineralization and forms a hybrid layer along the dentine which is rich in mineral. [13; 14]. A big advantage for bioceramic sealer is the ability to bond in moist dentin. This property was evaluated and bioceramic sealers showed high bond forces in moist dentin, over epoxy resin sealers [15; 16].

The tubular penetration of endodontic sealers majorly depends on their physico-chemical properties, complete smear layer removal and permeability of the tubules, depending on the anatomical root canal zone of the teeth. Number and diameter of dentinal tubules decrease apically in the root canal. To achieve good amount of tubular penetration, the particle size of the material must be smaller than the tubule diameter for it to be well suited for penetration.

This present study evaluated the penetration ability using SEM. Even though micro-CT can produce better 3D imaging, SEM is still preferred for tubular penetration studies. This is because micro-CT observations might be less sensitive compared with the sectioning method in terms of void detection. This is in accordance with a study by Kim et al. [17]. Volumetric 3D analysis is better with micro-CT wherein magnification is better with 2D SEM. SEM was preferred over sealer staining using fluorescent dyes because dyes have shown to be unsuitable in precisely indicating sealer penetration depth according to recent study by Sina Schmidt et al. [18].

Likewise, many techniques are employed to assess root canal sealer capacity for apical sealing. One popular, simple, and quick way to test the sealers microleakage is through the linear measurement of dye penetration. Endodontic microleakage happens at the coronal or apical part of the root canal obturation, each having its own repercussions. Muliyar et al. discussed the role of microleakage in endodontic failures and the importance to address and control it properly to ensure a successful endodontic outcome [4; 19].

In this present study AH Plus Sealer has showed the maximum score for microleakage and the least score for tubular penetration. Even though previous studies have proved that epoxy resin–base AH Plus has superior adaptation and provides tight seal to prevent microleakage, the results of the present study, contradicted this. This might be due to the use of single cone obturation which was used in all three groups. AH Plus combined with warm vertical compaction method of obturation has shown better seal and tubular penetration [16]. Bioceramic based sealers are preferably used along with single cone obturation methods as high temperatures from warm vertical compaction can interfere with the interaction between the sealer and moisture content which help in forming the mineral infiltration zone as the warm vertical compaction desiccates the root canal because of its high temperature.

The newer generation bioceramic sealers have particle size averaging 0.2 μm whereas epoxy resin – based AH Plus has a particle size of about 8 μm. This can be an attributing factor for the sealer penetration into the tubules in this present study. Bioactive glass and bioceramic sealers flow better even in the presence of moisture, but in case of AH Plus sealer it is not the same.

Stereomicroscopic images revealed linear dye penetration of the samples. Digital images were acquired from the stereomicroscope. Samples were scored according to the presence of dye along the walls of the canals. If the dye is seen on the tip of the walls of the canal, then they were scored 1 (Fig. 1). If the dye is seen at half the length of root canal, then it is scored 2. If dye has leaked into more than half the length of the root canal, then it is scored as 3 (Fig. 2).

Under SEM evaluation, Nishika Canal Sealer and CeraSeal showed higher sealer penetration into the dentinal tubules than AH Plus. SEM images revealed tubular penetration of sealers in which the particles were seen to penetrate in to the tubules at different depths. Sealer particles were seen penetrated and scattered into the dentinal tubules at 1000X magnification. Sealer particles were seen into the dentinal tubules at 1000X magnification (Fig. 3).

Fig. 1. Dye seen on the tip and walls of the canal

Рис. 1. Краситель виден на верхушке и стенках канала

Fig. 2. Dye is seen in more than half the length of the root canal

Рис. 2. Краситель виден более чем в половине длины корневого канала

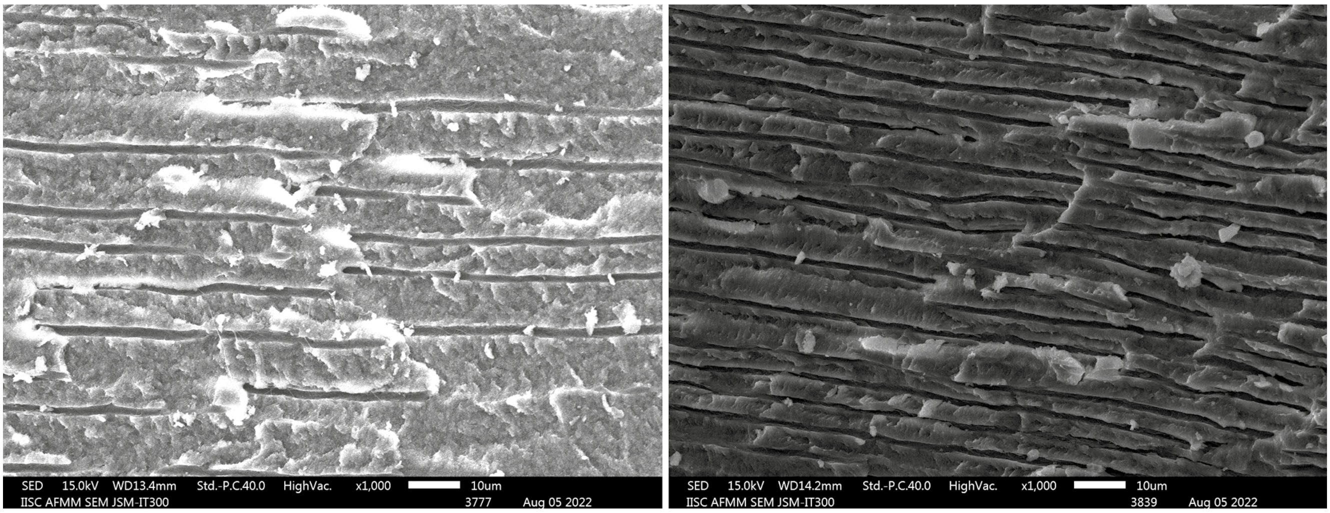

Fig. 3. Sealer particles into the dentinal tubules at 1000X magnification

Рис. 3. Частицы силера в дентинных канальцах при увеличении 1000X

GP-sealer-tubule interface was also analysed by the SEM. Some of the images were discarded due to inability to calculate the penetration depth because of debris over the tubule surface.

SEM images revealed that sealers were well adapted to the dentinal walls in most of the specimens. Agitation of the sealers could have helped the sealers for better penetration and should be tested in future studies. This present study also revealed that, greater the penetration depth, lesser the microleakage and vice versa.

CONCLUSION

Within the limitations and experimental conditions of this study, it can be concluded that, The Nishika Canal Sealer BG had significantly more apical seal compared to CeraSeal and AH Plus, most of the samples showed microleakage irrespective of the sealer used and the bioactive glass sealer had significantly more tubular penetration compared to CeraSeal and AH Plus. Despite the fact that there was significant difference in all three groups, further studies are required to evaluate this in large samples for a long-time success.

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