



Original Article

บทวิทษฬฬกร

Assessment of leakage of roots filled with Epiphany[®] and Resilon[®] after final irrigation with 2% chlorhexidine

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Abstract

Objective The purpose of this *in vitro* study was to compare the leakage of roots filled with Epiphany[®] and Resilon[®] after final irrigation with 2% chlorhexidine.

Materials and methods Seventy single-rooted teeth were prepared using crown down technique and divided to three experimental groups of twenty samples each and positive and negative control groups of five samples each. In each experimental group, root canal was irrigated with 2.5% sodium hypochlorite and 17% EDTA then final irrigated with sterile water (group 1), 2% chlorhexidine (group 2) or 2.5% sodium hypochlorite followed by 2% chlorhexidine (group 3). The experimental groups were filled with laterally compacted Resilon[®] cones and Epiphany[®] sealer. The leakage of glucose was evaluated by measuring its concentration once a week for a total period of 28 days using a glucose penetration model. Data of glucose concentration that leaked through the filled root canals were not normally distributed. Therefore, they were statistically analyzed by the nonparametric test (Friedmann test and Kruskal-Wallis test). The level of significance was set at $p = .05$.

Results The increasing glucose concentration values among each time intervals in the same experimental group were statistically significant different. However, no statistically significant differences were found among the three experimental groups at each time interval.

Conclusion This study showed that 2% chlorhexidine when used after 17% EDTA and 2.5% sodium hypochlorite did not adversely affect the leakage of root canal system filled with Epiphany[®] and Resilon[®].

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Key words: chlorhexidine; Epiphany[®]; leakage; Resilon[®]

Introduction

The ideal root filling material should entomb residual bacterial after instrumentation, seal the root canal space, prevent re-infection from coronal leakage, and stop apical penetration of tissue fluids from reaching surviving bacteria in the root canal system.¹ Gutta-percha has universally been accepted as the gold standard for root canal filling materials. It appears to be the least toxic and tissue-irritating root canal filling material available. However, gutta-percha does not adhere to the dentinal walls and consequently, a sealing agent is required.

Recently, an innovative adhesion material, Resilon[®] has become available for filling the root canal space. Resilon[®] is a thermoplastic synthetic polymer-based root canal filling material, which contains bioactive glass and radiopaque fillers. Resilon[®] has same handling properties as gutta-percha. It can be softened with heat and dissolved with solvents such as chloroform for retreatment purpose. Similar to gutta-percha, Resilon[®] are available as master cones in all ISO (International Organization for Standardization) sizes and accessory cones in different sizes. In addition, there are Resilon[®] pellets, which can be used for the backfill in the warm thermoplasticized technique. Epiphany[®] sealer manufactured specifically for using with Resilon[®] is a dual curable dental resin composite sealer. This sealer forms a bond to the core filling material and the cleaned dentinal wall, hence creating a "monoblock".^{2,3}

An endodontic irrigant should ideally exhibit powerful antimicrobial activity, dissolve organic tissue remnants, disinfect the root canal space, flush out debris from the instrumented root canals, provide lubrication, and have no cytotoxic effects on the periradicular tissues.⁴ Unfortunately, no irrigating solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer.

In an effort to remove the smear layer completely, many authors suggest the use of several solutions. 15-17% neutral EDTA solution is effective in de-mineralizing the dentine, and can be used to remove the smear layer. However, it does not dissolve organic matter. On the other hand, sodium hypochlorite solution acts on pulp tissue remnants and has antimicrobial properties. Combined use of sodium hypochlorite and chlorhexidine within the root canal could gain an additive antimicrobial action and a tissue dissolution property. In addition, the combined use of sodium hypochlorite and chlorhexidine are less toxic than sodium hypochlorite alone. However, the combined use of sodium hypochlorite and chlorhexidine within the root canal arise the residual organic matter on the root canal wall which may alter the sealing ability of the root canal sealer.⁵

To date, no studies have demonstrated the effect of final irrigation of chlorhexidine on leakage between Epiphany[®] root canal sealer and radicular dentin. The purpose of this *in vitro* study was to compare the leakage of roots filled with Epiphany[®] and Resilon[®] after final irrigation with sterile water, 2% chlorhexidine or 2.5% sodium hypochlorite followed by 2% chlorhexidine.

Materials and methods

Selection and preparation of teeth

A total of 70 extracted human teeth with single and straight root were collected and stored in 0.2% thymol solution. The teeth were soaked in distilled water for 24 hours before use to eliminate traces of thymol. Each tooth was decoronated to give approximately 15 mm of root length from the coronal surface to the apex of the root with a low speed saw (IsoMet[®], Buehler). An operating microscope (Carl Zeiss Surgical, Inc., Thornwood, NY) was used to inspect the roots for cracks under 25 x magnifications.

Instrumentation and obturation of root canals

The working length was determined visually by subtracting 1 mm from the length of a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) at the apical foramen. The middle and coronal thirds were prepared using ISO size 1, 2, 3, and 4 Gate Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland). All teeth were instrumented with a crown-down technique, using a set of ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) as recommended by manufacturer. The apical portion of the canal was instrumented to size 40 master file with K-Flex-o-files (Dentsply Maillefer, Ballaigues, Switzerland). The purpose of this preparation regimen was to create a uniform size of canal and to overcome the variation in natural morphology. Each canal was irrigated with 3 ml of 2.5% sodium hypochlorite solution using a 27-gauge needle after each instrument and ensured patency by extrusion of the file beyond the apical foramen. The needle was inserted as deep as possible into the canal without binding. All canals were irrigated with 5 ml of 17% EDTA solution for 1 minute to remove the smear layer. All root canals were enlarged by only one operator to minimize operator variation.

Twenty teeth were assigned to three experimental groups. Five teeth each were assigned to the control groups.

After root canal preparation was completed, the root canals were final flushed with sterile water (group 1), 2% chlorhexidine (group 2) or 2.5% sodium hypochlorite followed by 2% chlorhexidine (group 3). Each canal was dried with paper points.

Root canal obturation

Group 1, 2, 3: root canals were filled with Epiphany® and Resilon® (Pentron Clinical Technologies)

A self-etching primer (Epiphany® primer; Pentron Clinical Technologies) was placed into the canal with paper point. One drop of the primer was used for each root. Excess primer was removed using paper point, leaving the internal surfaces moist with primer. The remaining solvent evaporated with a gentle air spray for 5 seconds. Epiphany® root canal sealer was dispensed onto a mixing pad and placed with a master cone. The canal was then filled with Resilon® cone using the lateral condensation technique, which using a spreader size D11Ts (Dentsply Maillefer) and size 15 accessory Resilon® cones. The tip of each accessory cone was lightly coated with sealer. When the Resilon® filling was completed, endodontic plugger was used for vertical compaction and the coronal surface was light cured for 40 seconds to polymerize the sealer. The deeper resin sealer then polymerizes by chemical curing during the following 30 to 60 minutes.

Positive control group: Root canals were filled using laterally compacted Resilon® cones without sealer.

Negative control group: Root canals were sealed using laterally compacted gutta-percha and Epiphany® sealer and completely covered with silicone.

After the obturation, all root canal specimens were examined with a microscope at 25x magnification to ensure that there were no cracks or craze lines in the roots. Moreover, in all groups postoperative radiographs were taken to ensure that all root canal specimens were properly obturated without voids. Root canal specimens inadequately filled or cracked were excluded and replaced by new samples. Then, all root canal specimens coated with silicone, except for the 4 mm of apical part in order to allow glucose penetration via apical region. All root canal specimens were stored at 37°C and 95% humidity for one week to allow the material to set completely.

Glucose penetration model–preparation and measurement

Each root was connected to a 17-cm-long glass tube and sealed with silicone. The assembly was then placed in a sterile centrifuge tube with a rubber cap. Two milliliters of 0.1% benzoic acid solution (pH 7.0) were dispensed into the sterile centrifuge tube. The 4 mm apical portion of root canal specimen was immersed in the solution. Benzoic acid was used to inhibit the growth of microorganisms that might influence the glucose readings. The tracer used in the present study was 1 mol L^{-1} glucose solution (pH 7.0).

All specimens were subjected to reduce pressure before the glucose solution was injected into the glass tube. The glass tube was connected to vacuum pump (Bio-Rad Laboratories, 3300 Regatta Boulevard, Richmond, CA 94804) by using Three-way, Double oblique, Pressure, Solid glass plug, Stopcock size 2 mm (Fortune Scientific Co., LTD). The air was removed from the root canal specimen and glass tube until the pressure in the system was stable at 20 inch Hg measured by a manometer that connected to the vacuum pump. After maintaining the pressure of 20 inch Hg for 5 minutes, the glucose solution was released into the glass tube by opening the three-way stopcock, without allowing air to enter the glass tube.

About 5 ml of the glucose solution, containing 0.1% benzoic acid was released into the glass tube until the solution was 14 cm above the root canal specimens. This level of glucose solution created a hydrostatic pressure of 1.5 KPa or 15 cm H_2O .⁶

All specimens were stored in the incubator at 37°C and 95% humidity through observation period. A solution of 50 μl was drawn from the centrifuge tube using a micropipette at 1, 7, 14, 21 and 28 days. The same amount of 0.1% benzoic acid was added to

the centrifuge tube reservoir to maintain a constant volume of 2 ml. The sample was then analyzed with a Glucose liquicolor in a spectrophotometer at a wavelength of 500 nm. Concentration of glucose in the centrifuge tube was presented in mmol L^{-1} .

Data of glucose concentration (mM) that leaked through the filled root canals were not normally distributed. Therefore, they were statistically analyzed by the nonparametric test (Friedmann test and Kruskal-Wallis test). The level of significance was set at $p = .05$.

The Ethical community of Dental faculty of Chulalongkorn University approved this research study.

Results

In positive control group, glucose leakage was detected from the first day which increased over times. In negative control group, glucose leakage could not be detected in all apical reservoirs throughout the experiment (Table 1).

Descriptive statistical data of leakage amount of glucose concentration measured in all experimental groups at each time interval were shown in Table 1. Each experimental group showed the increasing glucose leakage concentration from the beginning to the end of experimental period (Table 1). The glucose concentration values increased significantly at times in the same experimental group when compared using Friedmann test ($p < .05$). However, no statistically significant differences were found among the three experimental groups at each time interval (1, 7, 14, 21 and 28 days) when compared using Kruskal-Wallis test ($p > .05$).

The rates of glucose leakage (mM/week) among each experimental group were shown in Figure 1. In group 1 and 2, the rate of leakage was gradually

Table 1 Glucose leakage concentration (mM) in three experimental groups and control groups at each time interval after obturation.

Groups		Glucose concentration (mM)				
		Day 1	Day 7	Day 14	Day 21	Day 28
Group 1 (sterile water)	Mean ± SD	0.11 ± 0.35	3.01 ± 7.32	4.96 ± 9.05	7.78 ± 13.65	10.74 ± 18.28
	Median	0	0.04	0.57	1.07	1.32
	Min-Max	0-1.51	0-31.62	0-28.16	0-43.42	0-61.82
Group 2 (2% chlorhexidine)	Mean ± SD	0.33 ± 0.99	0.62 ± 1.45	1.47 ± 3.90	2.18 ± 6.31	3.07 ± 9.09
	Median	0	0	0.01	0.10	0.20
	Min-Max	0-3.56	0-4.23	0-17.02	0-28.32	0-41.03
Group 3 (2.5% sodium hypochlorite, 2% chlorhexidine)	Mean ± SD	0.76 ± 2.22	2.03 ± 4.44	5.39 ± 10.31	7.47 ± 12.97	9.90 ± 16.51
	Median	0	0	0.69	1.48	2.04
	Min-Max	0-8.96	0-14.58	0-36.90	0-44.16	0-54.64
Positive control group	Mean ± SD	15.56 ± 27.23	48.54 ± 5.51	255.30 ± 148.25	269.41 ± 151.62	279.64 ± 158.75
	Median	0.38	45.32	208.52	215.94	221.72
	Min-Max	0-63.06	43.59-56.21	98.78-446.14	112.81-470.07	122.71-494
Negative control group	Mean ± SD	0	0	0	0	0
	Median	0	0	0	0	0
	Min-Max	0	0	0	0	0

increased except in the third week, the rate of leakage in group 1 was slightly decreased. In group 3, rate of glucose leakage in the second week of experiment was more increasing as compared with other experimental groups, and then it became stable until the end of the experiment. However, the rates of glucose leakage concentration were not statistically significantly different between experimental groups at each time point.

Discussion

The glucose penetration model is a new possibility to evaluate the sealing ability of root canal filling. It has been introduced by Xu *et al.*⁶ as a further development of the model of the fluid transportation concept that might be more sensitive than the measurement with an air bubble.

The model used in this study was modified from that of Xu *et al.*⁶ The modification was firstly the use of vacuum to reduce air bubbles.⁷⁻⁹ Air bubbles could be entrapped in the glucose system and gap between the root canal filling materials and the canal wall. Tracer penetration was affected negatively by air entrapped in the gap between the root canal filling materials and the canal wall, resulting in the failure to demonstrate the full extent of the void. Although the use of pressure may have no clinical relevance, it has the practical advantage of accelerating leakage detection. Secondly instead of using epoxy resin or sticky wax, the contact between tooth and glass tube was sealed with silicone. This was proved to provide better seal in moist environment (95% humidity). Finally specimens were placed in closed system at 37°C 95% humidity. This

model eliminates the effect of glucose evaporation on glucose concentration measurements within 28 days.

In positive control group, glucose leakage concentration was gradually increasing in the first week, and then became stable at the second week through the end of the experiment. In contrary, glucose leakage could not be detected in the negative control group. Glucose leakage could be detected in all experimental groups. It should be noted that the concentration value of glucose leakage was lower than previous studies. This maybe due to the longer distance of the root canal fillings. In this study the root canals were filled up to 15 mm as compare with the 4 mm from apex in other studies.^{6,10} In addition, the lesser number of dentinal tubules in the apical part might lead to compromised bonding apically.¹¹

In this study the root canals were irrigated with 2.5% sodium hypochlorite and 17% EDTA before final flush with sterile water (group 1), 2% chlorhexidine (group 2) or 2.5% sodium hypochlorite followed by

2% chlorhexidine (group 3). There were no significant differences of glucose leakage concentration measured at each time point between all experimental groups. However, the glucose leakage concentration in each experimental group was found significantly increasing from the beginning to the end of experimental period.

In the experimental group 2 and 3, there were combinations of irrigants which resulted in color change and precipitation. Combination of 2% chlorhexidine and 17% EDTA (group 2) demonstrated the formation of a white viscous solution, whereas combination of 2.5% sodium hypochlorite and 2% chlorhexidine (group 3) demonstrated the formation of the dark-brown precipitation.

Although we found no significant difference between group 2 and group 3, group 3 showed more glucose leakage concentration at the end of experimental period when compared with group 2. The dark-brown precipitation occurred when sodium hypochlorite and chlorhexidine were combined. This

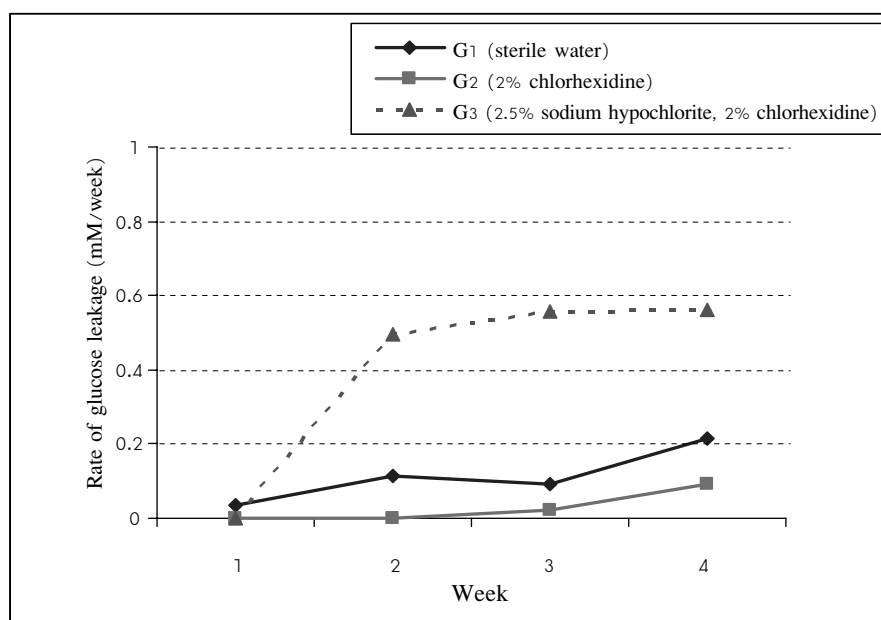


Fig. 1 Rates of glucose leakage (mM/week) in three experimental groups at 1, 2, 3 and 4 weeks.

might interfere with the sealing of root canal filling by forming gaps between Epiphany® sealer and dentin. In cross-sections of filled roots, gaps were observed between the dentin and Epiphany® layer.¹²

Recent studies have reported on the occurrence of color change and precipitation when sodium hypochlorite and chlorhexidine are combined.^{13,14} Furthermore, concern has been raised that the color change might have some clinical relevance¹⁴ because of staining, and that the precipitation might interfere with the sealing of root canal filling. Basrani *et al.*¹⁵ demonstrated that the precipitate of sodium hypochlorite mixed with chlorhexidine contains a significant amount of para-chloroaniline (PCA), a hydrolysis product of chlorhexidine. The amount of PCA directly increased when the concentration of sodium hypochlorite increased. Bui *et al.*¹⁶ compared the effect of irrigating root canals with a combination of sodium hypochlorite and chlorhexidine on root dentin and dentinal tubules. Their findings showed that there were significantly fewer patent tubules when compared with the negative control group which irrigated with sterile water. Because of the sodium hypochlorite/chlorhexidine precipitate tends to occlude the dentinal tubules. In addition, the presence of this precipitate on the root surface might affect the seal of an obturated root canal, especially with resin sealers which required a hybrid layer.

In group 2, there was less glucose leakage concentration at the end of experimental period when compared with other experimental groups. The white viscous solution occurred when EDTA and chlorhexidine were combined. To date, no studies have demonstrated chemical properties of the white viscous solution or its effect on root dentin. Marending *et al.*¹⁷ proposed an irrigation regimen in which sodium hypochlorite was used throughout instrumentation followed by EDTA, and chlorhexidine was used as a final irrigant.

In group 1, the final irrigant was sterile water as recommended by manufacturer of Epiphany® sealer. Although, there was no precipitate and color change but the result showed glucose leakage concentration and the rate of the leakage was gradually increasing in the same pattern as in group 2. The cause of glucose leakage might be explained firstly from the volumetric shrinkage that occurs concurrently with polymerization of the resin. If the resin-tooth bond is too weak, polymerization forces will debond the resin from the tooth resulting in microleakage.¹⁸ Secondly during experiment, the root specimens were immersed in the apical reservoir, therefore dissolution of sealer might occur and permit gaps formation between root canal dentin and root filling materials. This may result in increase in leakage over time. Versiani *et al.*¹⁹ found that solubility of Epiphany® sealer did not conform to ANSI/ADA standardization (3.14%). Water sorption and solubility play an important role in microleakage in long term observation.

Under the condition of this study, combination of 17% EDTA and 2% chlorhexidine showed the least glucose leakage concentration eventhough the formation of white viscous solution occurred. Further studies on the chemical properties of the white viscous solution in combined irrigation with 17% EDTA and 2% chlorhexidine and its effect on root dentin need to be investigated.

Conclusion

There were no statistically significant difference of glucose leakage in root canals filled with Epiphany® and Resilon® after final irrigation with sterile water, 2% chlorhexidine, or 2.5% sodium hypochlorite followed by 2% chlorhexidine after irrigated with 17% EDTA. Thus, this study showed that 2% chlorhexidine when used after 17% EDTA and 2.5% sodium hypochlorite did not adversely affect the leakage of root canal system filled with Epiphany® and Resilon®.

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บทคัดย่อ

วัตถุประสงค์ เพื่อศึกษาเปรียบเทียบการร่วซึมของรากฟันที่อุดด้วยอิพิฟานี® และเรซิลอน® หลังจากล้างครั้งสุดท้ายด้วยคลอร์เฮกซิดีนร้อยละ 2

วัสดุและวิธีการ นำฟันรากเดี่ยวจำนวน 70 ซี่ มาขยายคลองรากฟันด้วยวิธีควรวาดวนน์ และล้างคลองรากด้วยโซเดียมไฮโปคลอไรด์ร้อยละ 2.5 อีดีทีเอร้อยละ 17 โดยแบ่งเป็นกลุ่มทดลอง 3 กลุ่ม ๆ ละ 20 ซี่ และกลุ่มควบคุมบวกและลบกลุ่มละ 5 ซี่ ในกลุ่มทดลองจะแบ่งตามชนิดของน้ำยาล้างคลองรากฟันที่ใช้ล้างครั้งสุดท้าย คือ น้ำที่ปราศจากเชื้อ (กลุ่มที่ 1) คลอร์เฮกซิดีนร้อยละ 2 (กลุ่มที่ 2) และโซเดียมไฮโปคลอไรด์ร้อยละ 2.5 ตามด้วยคลอร์เฮกซิดีนร้อยละ 2 (กลุ่มที่ 3) โดยกลุ่มทดลองจะอุดคลองรากฟันด้วยเทคนิคแลเทอร์ลคอมแพคชันด้วยอิพิฟานี® และเรซิลอน® โดยใช้แบบจำลองการซึมผ่านของกลูโคสในการประเมินการร่วซึมของความเข้มข้นกลูโคสทุกสัปดาห์จนครบ 28 วัน ซึ่งข้อมูลมีการแจกแจงไม่ปกติ จึงนำข้อมูลที่ได้อาวิเคราะห์โดยใช้การทดสอบพรีดแมนและครัสคัล-วอลลิส ที่ระดับนัยสำคัญทางสถิติเท่ากับ .05

ผลการศึกษา ในกลุ่มทดลองกลุ่มเดียวกันพบว่า ความเข้มข้นของกลูโคสเพิ่มขึ้นในทุกช่วงเวลาแตกต่างกันอย่างมีนัยสำคัญทางสถิติอย่างไรก็ตามไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติในช่วงเวลาเดียวกันระหว่างกลุ่มทดลอง

สรุป การศึกษานี้แสดงว่าการล้างครั้งสุดท้ายด้วยคลอร์เฮกซิดีนร้อยละ 2 หลังจากล้างด้วยโซเดียมไฮโปคลอไรด์ร้อยละ 2.5 และอีดีทีเอร้อยละ 17 ไม่ได้มีผลต่อการร่วซึมของรากฟันที่อุดด้วยอิพิฟานี® และเรซิลอน®

(จ ทันต จุฬฯ 2551;31:135-44)

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