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A Histopathological Evaluation of Periradicular Tissues after Root Canal Filling with Gutta-Percha/AH_{26} or Resilon/Epiphany in Dogs' Teeth

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Abstract: Root canal obturation is a critical factor in success or failure of a root canal treatment. The material that is used for root canal obturation must be able to provide a perfect seal. The aim of this study was to evaluate *in vivo* the response of the periradicular tissues after endodontic treatment and root filling with gutta-percha/AH_{26} or resilon/epiphany in dogs' teeth without coronal seal. About 40 root canals with vital pulps in 2 dogs were instrumented and obturated in a single session and randomly assigned to two groups based on kind of root filling; gutta-percha/AH_{26} or resilon/epiphany. After 3 and 6 months, the animals were euthanized and the jaws were removed and submitted for histologic processing. Longitudinal sections were obtained and stained with hematoxylin and eosin and examined under light microscopy. Inflammatory status was determined by pathologist and analyzed using Wilcoxon and Mann-Whitney tests. No significant difference found between the two groups in 3 and 6 months ($p>0.05$). Also, no significant difference was observed in the intensity of inflammation in group gutta-percha/AH_{26} between 3 and 6 months ($p>0.05$). But significant difference was observed in the intensity of inflammation in group resilon/epiphany between 3 and 6 months ($p<0.05$). Under circumstances of this study, there was no apparent advantage of using resilon/epiphany over gutta-percha/AH_{26} and in controlled conditions both of these materials provide comparable seal. This study also showed the importance of the coronal restoration in preventing unfavorable periradicular tissue reaction.

Key words: AH_{26}, gutta-percha, resilon, root canal filling materials, tissue reaction, Iran

INTRODUCTION

The role of bacteria and their byproducts in the development of pulp and periapical lesions has been well established (Leonardo *et al*., 2007). The aim of endodontic treatment is to prevent or eliminate microbial challenge from the root canal system. Disinfecting of the root canal space is achieved through mechanical and chemical means (Bergenholtz, 1974) followed by placing a root canal filling. Additionally, the quality of the coronal seal in root-filled teeth has been considered to be an important etiologic factor in treatment success (Ray and Trope, 1995).

It appears that gutta-percha and sealer is the weak point in endodontics (Torabinejad *et al*., 1990; Khayat *et al*., 1993; Shipper and Trope, 2004; Shipper *et al*., 2004). Filling of the root canal with this even by the most technically proficient operator will not result in a seal that is dependable. Many different materials have been proposed as root canal fillings but none have replaced gutta-percha that is universally accepted as the gold standard filling material. Because gutta-percha presents no adhesion to the tooth structure, ideally it should be replaced by a better sealant material. Resilon, a synthetic thermoplastic resin material has been developed that performs like gutta-percha has the same handling properties and for re-treatment purposes could be softened with heat or dissolved with solvents like chloroform. According to the manufacturer, the resilon cones contain a blend of dimethacrylates and exhibit adhesion both to root dentin and to methacrylate-based sealers such as epiphany which is a dual curable dental resin composite sealer. This sealer when used with the resilon forms a filling resistant to bacterial penetration and considered as Resilon Monoblock System (RMS) (Shipper *et al*., 2004). In *in vitro* investigations, the superiority of this system to reduce or prevent coronal microleakage is shown (Shipper *et al*., 2004, 2005; Tunga and Bodrumlu, 2006; Stratton *et al*., 2006). Although, this claim has been challenged by other investigations, gutta-percha was not shown to be superior material (Tay *et al*., 2005; Shemesh *et al*., 2006;

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Baumgartner *et al*., 2007; Biggs *et al*., 2006). The cytotoxicity and bio-compatibility of resilon/epiphany system is monitored in several researches that showed satisfactory results (Grecca *et al*., 2011; Garcia *et al*., 2010). Due to optimum cytotoxicity, biocompatibility and microleakage results, it is necessary to assess resilon/epiphany in *in vivo* condition where the host defense response also plays a role and where a histological evaluation can determine the presence/absence of apical periodontitis, the disease of interest in endodontics. A few studies are performed in *in vivo* models that suggested the less apical periodontitis after using resilon/epiphany in comparing gutta-percha/sealer (Leonardo *et al*., 2007; Shipper *et al*., 2005; Brasil *et al*., 2010). Clinical outcome assessments have also been shown to be favorable with the use of resilon/epiphany (Debelian, 2006; Conner *et al*., 2006). The purpose of this study was to evaluate *in vivo* the periradicular tissue response after root canal filling with the resilon/epiphany system compared with gutta-percha/AH_{26} in dogs' teeth without coronal restoration.

MATERIALS AND METHODS

In this interventional-experimental study, there were 24 sound pre-molar teeth of two male mixed Iranian dogs weighing 10-20 kg and aging 1 year (12 pre-molars in each dog). The anesthetic induction was achieved by intravenous administration of Asperonazin then intravenous administration of 11 mg kg^{-1} ketamin (Park-Davis, France) followed by oral intubation 1-2% Halotan. The dogs additionally received a local anesthetic with 2% Lidocaine+1/80000 epinephrine (Daroupakhsh-Iran) as infiltration to provide regional nerve block anesthesia and bleeding control.

After oral examination and found all the teeth sound with no caries, there were taken periapical radiography to make sure if there is any periodontal disease and the apices are closed. All the process was done in complete aseptic condition. The teeth were isolated with rubber dam and disinfected with 10% povidine iodine solution. Mesiodistally, access cavity were prepared and canals were instrumented with profile rotary instruments (Dentsply, Tulsa Dental, Tulsaok) and a k-file # 40 (Kerr, Romulus, MI) at working length. Irrigation was done with 15 mL of 1% NaOCl with 27 gauge needles during instrumentation final irrigation with 10 mL of 17% EDTA (TitriplexIII, Merck Chemical, Germany) was performed. The canals were dried with sterile paper points (Dentsply, Dentry GmbH, Konstanz, Germany).

About 48 canals in 24 pre-molars were divided to two test and two control groups as follow; left mandibular and maxillary pre-molars in two dogs were chosen to fill with gutta-percha/AH_{26} (Dentsply, Dentry GmbH, Konstanz, Germany).

Right mandibular and maxillary premolars were filled with resilon/epiphany system (Pentron Clinical Technologies, LC, USA) All canals in two test groups (10 teeth in each group) were obturated with lateral condensation using a # 40 master cone, a finger spreader # 30 and accessory cones. In resilon/epiphany group before filling, the self-etched epiphany primer (Pentron Clinical Technologies, Walling fold, CT) was introduced to canals by a sterile paper point and excess was removed by another paper point. Epiphany sealer was entered to canals with lentulu spiral (Dentsply Maillefer, Johnson city, TN). After complete filling with resilon cones and epiphany sealer, the LED light (LED blue phase, Vivadent) was used 40 sec at orifices of canals for setting the epiphany. The canals in another test group were filled with gutta-percha/AH_{26}. The canals in positive control group (2 teeth) were filled with single cone of gutta-percha/AH_{26} or resilon/epiphany. The canals in negative control group (2 teeth) were obturated as same as two test groups. After obturation of all canals (test and control), a sterile cotton pellet was placed in the access cavity and the crown was filled with glass ionomer (Fuji IX, GC Co., Tokyo, Japan). After 2 days, the access cavities in two test groups and positive control group were opened to oral cavity for exposuring oral enzymes and microorganisms. One dog after 3 months period and another dog after 6 months were euthanized by vital perfusion after anesthesia with 2 mg kg^{-1} ketamin and 0.15 mg kg^{-1} Rumpan IV. The left and right common carotid arteries were then exposed and the jaws perfused with 10% formaldehyde. Jaw blocks were resected and fixed in 10% formaldehyde, decalcified in formic acid and embedded in paraffin. Serial longitudinal sections of 5 micron were cut and hematoxilin and eosin stained. Histopathological evaluation was performed blindly by one oral pathologist according to the following predetermined scale (Table 1).

Table 1: The scale using by pathologist to evaluation of specimens

Score	0	1	2	3
Intensity of the inflammatory infiltration	Absent	<30	30-60	>60
The amount of macrophage and plasma cell	Absent	<10	10-25	>25
The amount of fibroblast	>30	10-30	5-9	1-4
Tissue condition	Mature fibrosis+ collagen fibers	Immature fibrosis+ little collagen fibers	Granulation tissue	Necrosis centers
PDL condition	Regenerated	Regenerated	Degenerated	Degenerated
Cementum condition	Deposition	Deposition	Resorption	Abscess
Bone condition	Deposition	Deposition	Resorption	Abscess

The data was analyzed by Wilcoxon and Mann-Whitney tests

RESULTS AND DISCUSSION

Both two dogs tolerated the operative procedures well throughout the observation period and there were found no inflammation and sinus tract related to treated teeth. Negative control specimens had zero score at the end of both 3 and 6 months. One positive control specimen had 2 score at the end of 3 months and the other one had 3 score of it. Both positive control specimens had 3 score at the end of 6 months. Findings of this study are shown in Table 2 (Fig. 1-8). Wilcoxon test showed no significant difference between gutta-percha/AH_{26} and resilon/epiphany at 3 ($p = 0.24$) and 6 months ($p = 0.56$). Mann-Whitney test showed no significant difference between 3 and 6 months specimens in gutta-percha/AH_{26} group ($p = 0.84$). But this test showed the intensity of inflammatory reaction was increased significantly in resilon/epiphany group by time lapse ($p = 0.02$) (Fig. 9 and 10). One of the most important reasons for endodontic failure is coronal microbial leakage. Torabinejad *et al*. (1990) found that 50% of the teeth without coronal seal were contaminated along the whole length of the root filling after 19 and 42 days depending on the organism. Additional *in vitro* studies have confirmed the high leakage rate of gutta-percha and sealer root fillings within 30 days using either lateral or vertical techniques (Khayat *et al*., 1993; Shipper and Trope, 2004). Therefore, coronal seal is critical for periapical health after root canal treatment. But coronal restoration may be postponded, ignored or failed for some reasons thus is proposed to use root canal filling materials that are preferable in coronal seal.

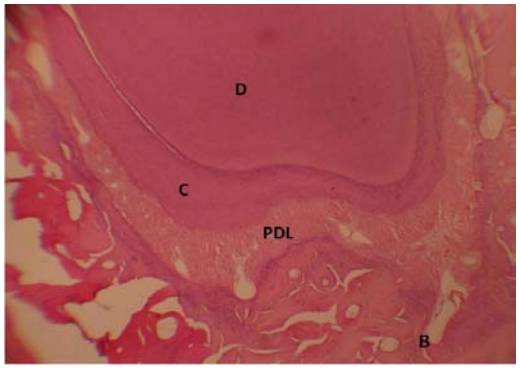

Fig. 1: Histopathological feature of specimen in negative control group. Score 0 of inflammation. H and E staining ×40 magnification

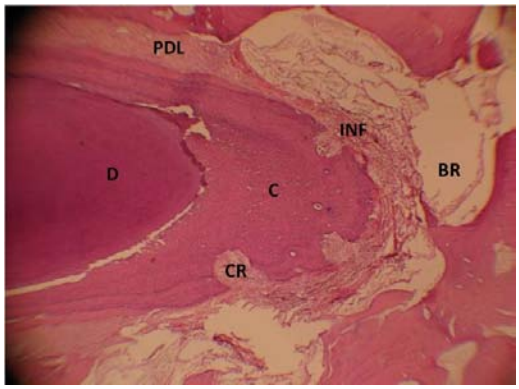

Fig. 2: Histopathological feature of specimen in positive control group. Score 3 of inflammation. H and E staining ×40 magnification

Table 2: The frequency and frequency percent of specimens of different groups

Groups	Time	-	0	1	2	3
Gutta-percha /AH_{26}	3 months	Frequency	-	6	4	0
		Percent	-	60	40	0
	6 months	Frequency	-	3	4	3
		Percent	-	30	40	30
	Overall	Frequency	-	9	8	3
		Percent	-	45	40	15
Resilon/epiphany	3 months	Frequency	1	7	2	0
		Percent	10	70	20	0
	6 months	Frequency	-	3	6	1
		Percent	-	30	60	10
	Overall	Frequency	1	10	8	1
		Percent	5	50	40	5
Positive control	3 month	Frequency	-	-	1	1
		Percent	-	-	50	50
	6 month	Frequency	-	-	-	2
		Percent	-	-	-	100
	Overall	Frequency	-	-	1	3
		Percent	-	-	25	75
Negative control	3 months	Frequency	2	-	-	-
		Percent	100	-	-	-
	6 months	Frequency	2	-	-	-
		Percent	100	-	-	-
	Overall	Frequency	4	-	-	-
		Percent	100	-	-	-

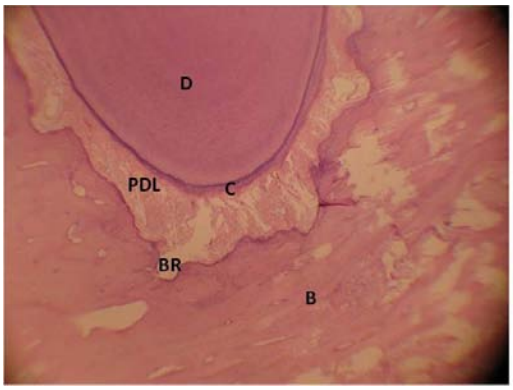

Fig. 3: Histopathological feature of specimen in gutta-percha/AH_{26} group. Score 1 of inflammation. H and E staining ×40 magnification

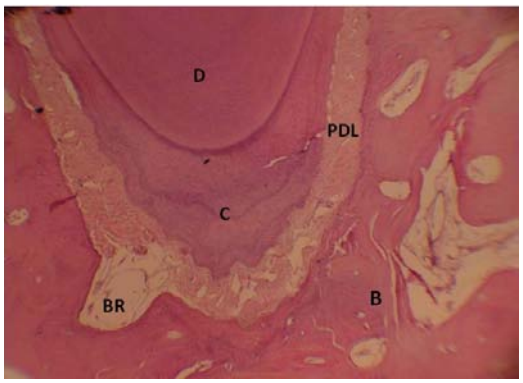

Fig. 6: Histopathological feature of specimen in resilon/epiphany group. Score 2 of inflammation. H and E staining ×40 magnification

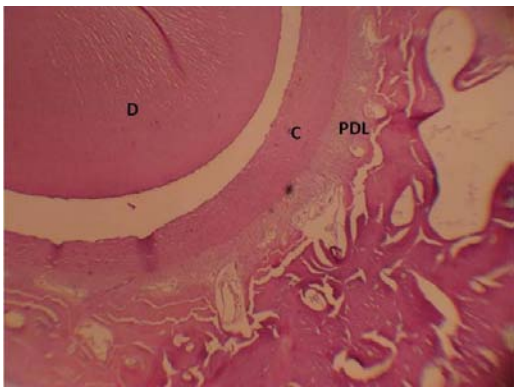

Fig. 4: Histopathological feature of specimen in resilon/epiphany group. Score 1 of inflammation. H and E staining ×40 magnification

Fig. 7: Histopathological feature of specimen in gutta-percha/AH_{26} group. Score 3 of inflammation. H and E staining ×40 magnification

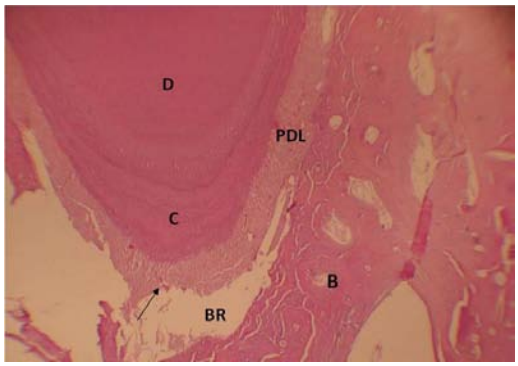

Fig. 5: Histopathological feature of specimen in gutta percha/AH_{26} group. Score 2 of inflammation. H and E staining ×40 magnification

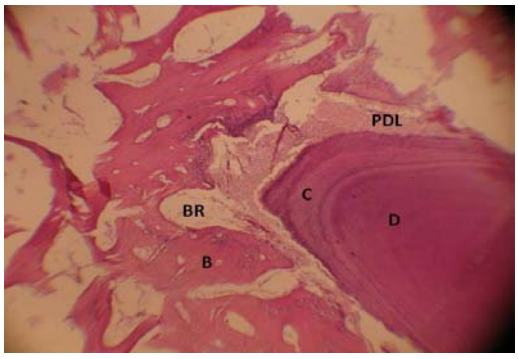

Fig. 8: Histopathological feature of specimen in resilon/epiphany group. Score 3 of inflammation. H and E staining ×40 magnification

The results of this study also emphasized on importance of placing coronal restoration because all specimens (except for 1 specimen in resilan/epiphany group) showed inflammation at the end of 3 months.

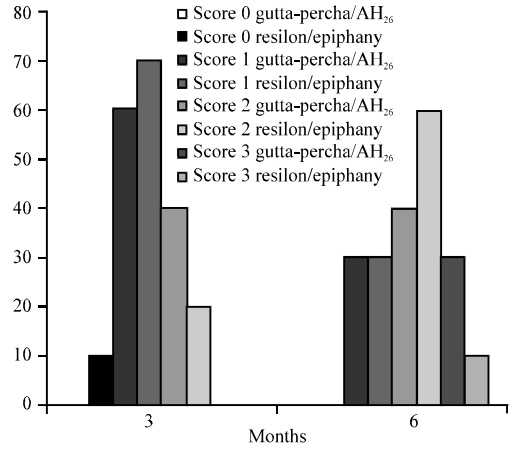

Fig. 9: The frequency percent of specimens of two test groups at 3 and 6 months

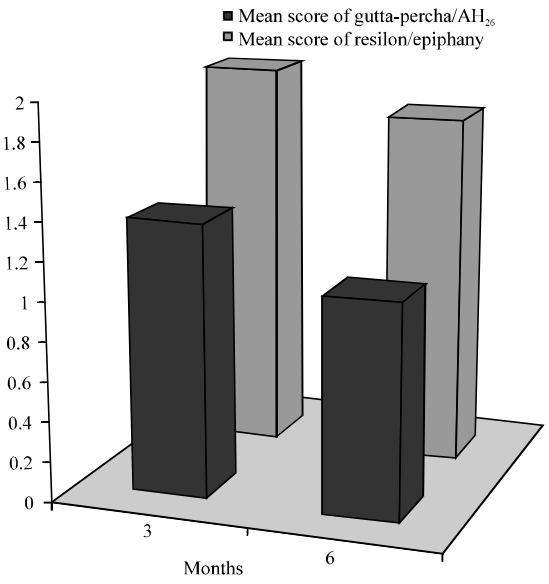

Fig. 10: The mean score of inflammation of two test groups at 3 and 6 months

It is accepted gutta-percha combined with traditional root canal sealers do not prevent the coronal to apical migration of bacteria and/or their byproducts when challenged (Shipper *et al*., 2004, 2005; Stratton *et al*., 2006; Shemesh *et al*., 2006; Baumgartner *et al*., 2007), thus the researchers have attempted to replace this by the new thermoplastic materials such as resilon/epiphany system. In fact, this *in vivo* study is a continuation of other *in vitro* studies showed that there is no significant difference between resilon/epiphany and gutta-percha/AH_{26} but the *in vitro* studies are unable to determine the effects of saliva enzymes and oral micro-organisms on root filling structure also immune system response to stimulators. Under circumstances of this study, there was no apparent advantage of using resilon/epiphany over gutta-percha/AH_{26} and in controlled condition both of these materials provide comparable seal. This finding is in contrast to Leonardo *et al*. (2007) that found the less inflammatory reaction in resilon/epiphany than gutta-percha/sealapex group. The difference may be related to the type of used sealer; seal apex that is a most soluble and least antibacterial sealer of $Ca(OH_2)$ based sealers (Johnson and Gutmann, 2006). This sealer needs to 3 weeks to complete setting that not considered in Leonardo et al study. But in this study, all specimens were coronal sealed by 48 h is needed for complete setting of AH_{26} may be another reason for difference between two study. The results of this study are also in contrast to Shipper *et al*. (2004) that found the less periapical inflammation after using resilon/epiphany than gutta-percha/AH_{26} after coronal microbial inoculation. This difference may be related to exposure of specimens in the study to oral environment and saliva that was not done in Shipper *et al*. (2005) study. Water penetration can cause hydrolysis and softening of resin elements that have negative effects on the bonding at interface of resilon cone/epiphany sealer and epiphany sealer/canal walls that consequently caused leakage. Softening is accompanied by liquid absorption by resin resulted in swelling and altering resin mechanical characteristics. Hydrolysis can cause debound between collagen fibers and resin polymers. This process is accelerated by the bacterial enzymes (Peumans *et al*., 2005; Hashimoto *et al*., 2000). By more resin damaging the leakage also increases. Resilon destruction by the enzymes such as Lipase and Esterase is approved.

By considering the before mentioned reasons, it could be concluded that continued saliva leakage and proteolytic enzymes and bacterial products are caused in failure of bonding in resilon/epiphany system by time laps resulted in more leakage and inflammation at the end of 6 months. The finding of this study was in agreement to Brasil *et al*. (2010) that found biocompatibility of resilon/epiphany equivalent to gutta-percha/AH_{26}.

CONCLUSION

Under circumstances of this study, there was no apparent advantage of using resilon/epiphany over gutta-percha/AH_{26} and in controlled conditions both of these materials provide comparable seal. This study also showed the importance of the coronal restoration in preventing unfavorable periradicular tissue reaction.

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NOMANCLATURE

D = Dentine
C = Cementum
B = Bone
PDL = Periodontal Ligament
CR = Cementum Resorption
BR = Bone Resorption
INF = Inflammatory cells
GT = Granulation Tissue

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