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# SEALING ABILITY OF BIODENTINE AND SIMVASTATIN FOR REPAIR OF FURCATION PERFORATION USING DYE EXTRACTION METHOD

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#### ABSTRACT

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Aim: This study aimed to evaluate the sealing ability of Biodentine and Simvastatin for the repair of furcal perforation using dye extraction method. **Materials and Methods:** Thirty extracted permanent human mandibular first molars were selected. Access cavities were prepared in all first molars while perforations were made at the furcation areas of only twenty five molars. Grouping of samples was done as follows, Group 1 (n = 10) in which perforations were repaired with Biodentine<sup>TM</sup>, Group 2 (n = 10) in which perforations were repaired with Simvastatin, Group 3 (n = 5) in which perforations were left unrepaired (positive control) and Group 4 (n = 5) in which no perforations were done (negative control). The sealing ability in four groups was evaluated using dye extraction method. Statistical analysis was done using One-Way ANOVA test to compare the mean values of the different groups then pairwise comparisons were performed using Tukey's posthoc test. **Results:** The highest dye absorbance was recorded for the positive control group (0.003 ± 0.002). The mean value of Biodentine group (0.025 ± 0.009) was significantly lower than Simvastatin (0.069 ± 0.011). **Conclusion:** Sealing ability of Biodentine is significantly better than Simvastatin in furcal perforation repair.

KEYWORDS: Biodentine, dye extraction, perforation, sealing ability, Simvastatin

# INTRODUCTION

Perforation is a pathologic or iatrogenic communication between the root canal space and the attachment apparatus. It may be caused by many reasons as extensive carious lesion, pathological resorption and iatrogenic during endodontic procedures. Furcation perforation is most commonly caused by improper use of burs against the pulp chamber floor during access cavity preparation<sup>[1]</sup>. Perforation repair prognosis depends mainly on time, size, location of perforation as well as the sealing ability of the repairing materials. The ideal perforation repair material should be biocompatible, induce osteogenesis and cementogenesis, have good sealing ability and radiopaque. It should also be completely degraded during the repair process to allow for its replacement by new healthy bone and act as a barrier against which the root canal obturating material can be placed<sup>[2]</sup>.

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Repair of perforation defects was done using several materials as; amalgam, calcium hydroxide, glass ionomer cement, zinc phosphate cement, resin modified glass ionomer cement, indium foil, gutta-percha, tricalcium phosphate, dentin chips, hydroxyapatite, super ethoxy benzoic acid, composite resin, and calcium enriched mixture cement <sup>[3-7]</sup>.

As an alternative material to calcium hydroxide, Mineral trioxide aggregate (MTA) was introduced with many advantages as antimicrobial action, biocompatibility, good sealing ability, low cytotoxicity, and ability to set in the presence of blood and moisture contamination. However, MTA still has many drawbacks as long setting time, and difficult handling characteristics <sup>[8-10]</sup>. So, various calcium silicate-based materials (CSMs) have been developed as alternatives to improve its potential disadvantages <sup>[11-13]</sup>.

Biodentine (BD) is relatively new calcium silicate-based material which was introduced as dentin substitute material; it is mainly composed of tricacium silicate, calcium carbonate, zirconium dioxide and calcium chloride as an accelerator to reduce its setting time <sup>[1]</sup>. It's clinically indicated for permanent dentine replacement, pulp capping, repair of root perforation and resorptive defects, retrograde filling and apexification <sup>[14]</sup>.

None of the currently existing perforation repair materials were able to reestablish the normal architecture in furcal perforations areas. Therefore, there is a necessity for introduction of newer perforation repair materials. Simvastatin is a 3 hydroxy 3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor which is cholesterol lowering drug that has been used to reduce the risk of cardiovascular disease. Many studies have recommended simvastatin as a useful material in vital pulp therapy as it exerts a powerful antiinflammatory effect that prevents the inflammatory process induced by lipopolysaccharide (LPS) <sup>[15,16]</sup>. Moreover, Simvastatin is bioinductive material that improves the osteoblastic function resulting in enhanced bone formation <sup>[17]</sup> which is a property required in perforation repair materials.

Sealing ability of furcation repair materials can be assessed by different leakage models including fluid infiltration, dye penetration, bacterial leakage models, dye extraction, air pressure method, an electrochemical method, radioisotope method, metal solution tracers, reverse diffusion method and three dimensional methods<sup>[18]</sup>. In dye extraction methods, teeth are dissolved in acids that release all the dye from the interface and optical density of the solution is measured by spectrophotometer <sup>[19]</sup>.

There is no available information in endodontic literature about the ability of Simvastatin to seal furcal perforations. Therefore, the purpose of this study was to evaluate the sealing ability of Biodentine and Simvastatin in repair of furcation perforation using dye extraction method.

# MATERIALS AND METHODS

Thirty freshly extracted human permanent mandibular first molars with intact furcations were included in this study; these teeth were extracted due to periodontal disease. The exclusion criteria include cracked teeth and those with the extensive carious decay of the crown. The purpose of the study was explained to the patients and informed consents were obtained to use their extracted teeth in the research according to the guidelines adopted by the Research Ethics Committee at Faculty of Dentistry, Tanta University. Teeth were cleaned by removing the hard deposits using curettes and the soft tissues were dissolved by soaking them in 5.25% sodium hypochlorite (Clorox Co., 10th of Ramadan, Egypt) for 10 min and then stored in daily-changed normal saline solution until they were used.

The mesial and distal roots of each molar were amputated 3 mm apical to the furcation area using a diamond disc (Komet; Brasseler, Lemgo, Germany) held at slow speed contrangle handpiece (NSK, Tokyo, Japan) under water cooling. Standard coronal access cavity was prepared in the occlusal surface of each molar with round bur size 3 (Dentsply Maillefer, Tulsa, Oklahoma, USA) at high speed contrangle handpiece (NSK, Tokyo, Japan) and root canal orifices were located with DG 16 endodontic explorer (API, Germany).

Canal orifices and the apical end of each root were acid etched with 37% phosphoric acid gel (3M ESPE Dental Products, St. Paul, MN, USA) for 30 seconds, washed and dried. Then, Adper Single Bond 2 (3M ESPE Dental Products, St. Paul, MN, USA) was applied in two successive coats and light cured using light curing device (Hilux, Benlioglu Inc, Ankara, Turkey) for 20 seconds. The canal orifices and the apical end of each root were then sealed with Filtek<sup>™</sup> Z350 XT flowable composite (3M ESPE, USA) and cured for 40 seconds according to the manufacturer instructions (Fig.1a). To increase the marginal seal, two successive layers of clear nail varnish were applied on all cavity walls and pulpal floor of each molar <sup>[20,21]</sup>

### **Preparation of furcal perforations**

All the teeth roots were placed into putty form of silicone impression material (Speedex® Coltene®, Switzerland) before polymerization to provide a simulated bony socket around their roots after setting. An artificial perforation was prepared nearly in the center of the floor of the pulp chamber of twenty five teeth (Fig.1b) using 0.5 mm round bur (Dentsply Maillefer, Tulsa, Oklahoma, USA) with the slow speed handpiece under water cooling to standardize the size of perforation<sup>[20]</sup>.

## **Grouping of samples**

The teeth were randomly divided into four groups, Group 1: ten molars in which the perforations were repaired with Biodentine<sup>™</sup> (Septodont, Saint-Maur des Fosses, France). Group 2 consisted of ten molars, in which the perforations were repaired with simvastatin (Sigma-Aldrich, St. Louis, MO, USA). Group 3 represented the positive control which consisted of five molars in which perforations were left unsealed while Group 4 represented the negative control, which consisted of five molars without perforations.

#### **Repair of perforations**

In Group 1, perforation repair was performed with BD material in which a single dose container of liquid was twisted open and five drops were poured into the powder capsule and mixed for 30 seconds in amalgamator (Softly8; de Gotzen, Italy) according to the manufacturer's instructions. The capsule was opened and the material was carried with an amalgam carrier and compacted with a hand plugger (Dentsply Maillefer, Tulsa, Oklahoma, USA) at the site of perforation and covered with moistened cotton pellet.

Simvastatin powder was mixed with distilled water to form a creamy mixture and then delivered to the perforation site using a plastic instrument and compacted with a hand plugger (Fig.1c). After sealing of perforations, the samples were kept in 100% humidity for 24 hours to allow the materials to set.

# Measurement of microleakage using dye extraction method

One drop of 2% methylene blue dye (2 g in 100 ml distilled water) was dispensed into the access cavities of all teeth using eye dropper and kept for the next 24 hours. The teeth were rinsed under tap water for 30 min. to remove any residues of the dye. Then, the varnish was scraped using Bard Parker blade no. 15<sup>[22]</sup>.

Each tooth was stored in hermitically sealed vial containing 2 ml of 65% concentrated nitric acid for three days; the supernatant solution from each tooth was then centrifugated for 5 min. at 14000 rpm to release the dye. After centrifugation, the resulting supernatant was analyzed using UV spectrophotometer (UniCo 7200, Shanghi, China) at 550 nm to determine optical density of dye

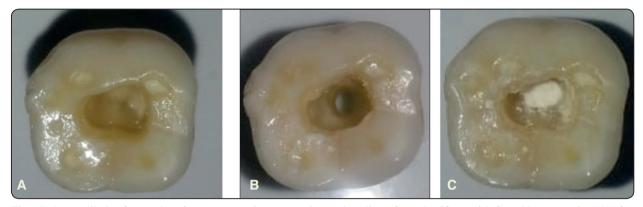


Fig. (1) A mandibular first molar after access cavity preparation and sealing of canal orifices with flowable composite (a), after furcal perforation(b), after perforation repair using simvastatin(c)

absorbance values <sup>[13]</sup>, using concentrated nitric acid as a blank <sup>[23]</sup>.

# Statistical analysis

One-Way ANOVA test was used to compare the mean optical density of dye absorbance values of the different groups at significance level of  $P \le$ 0.05. Then multiple pairwise comparisons between groups were performed using Tukey's post-hoc test using SPSS software version 24 (SPSS inc, Chicago, USA).

# RESULTS

The mean optical density of dye absorbance values and their respective standard deviations (SD) in all groups were shown in Table 1. The highest dye absorbance was recorded for the positive control group while the lowest value for the negative control group as shown in Table 1 and Fig.2.

One-Way ANOVA test revealed a statistically significant difference among the tested groups. Multiple pairwise comparisons showed that Group 1 using BD was statistically significant than Group 2 (Simvastatin group) and both control groups. In addition, Group 2 was statistically significant than negative control group whereas there was no statistically significant difference between Group 2 and positive control group (Table 2).

TABLE	(1)	Mean	optical	density	and	standard
	de	viations	s of dye	absorband	ce val	ues

Groups	no. of samples	Mean±SD	P-value
Group 1	10	$0.025^{b} \pm 0.009$	<0.0001
Group 2	10	$0.069^{a} \pm 0.011$	
Group 3 (positive control)	5	$0.081^{a} \pm 0.013$	
Group 4 (negative control)	5	$0.003^{\circ} \pm 0.002$	

Mean values with the same superscript letters are not statistically significant at  $P \le 0.05$ 

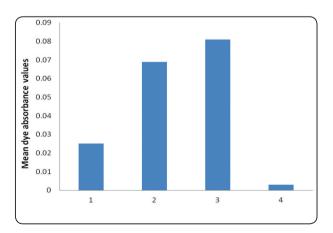


Fig. (2) Bar chart showing mean optical density of dye absorbance values in all groups

Intergroup comparisons	P-value
Group 1 versus Group 2	<0.0001
Group 1 versus Group 3	<0.0001
Group 1 versus Group 4	0.0125
Group 2 versus Group 3	0.2500
Group 2 versus Group 4	<0.0001
Group 3 versus Group 4	<0.0001

# DISCUSSION

Furcal perforations, most commonly occur during access cavity preparation, can cause secondary inflammation of the periodontal attachment which lead to loss of tooth if not treated [24]. In the management of furcal perforations, it is important to arrest the inflammatory process and loss of tissue attachment at the site of the perforation <sup>[25]</sup>. Simvastatin enhances bone morphogenic protein-2 which leads to increase in bone density and stimulates periodontal cells differentiation [26]. It has also anti-resorption action by blocking mevalonate pathway through inhibition of enzyme HMG-CoA reductase. Additionally, it increases levels of alkaline phosphatase, osteopontin and osteocalcin which are osteoblast differentiation markers [27]. So, it was tested in this study as it has desirable properties required for ideal perforation repair material.

Sealing ability of simvastatin in furcal perforation repair was tested in this study in comparison to BD as it was not previously investigated regarding this point in addition to the fact that the degree of tissue response to repaired perforations depends on several factors among them the sealing ability of repair materials<sup>[4]</sup>. Dye extraction method provides more reliable results than dye penetration method since it measures all the dye taken up in the root quantitatively <sup>[28]</sup>. Additionally, it is better than fluid filtration as it is not diminished by time <sup>[29]</sup>. Therefore, in the present study, microleakage was checked using dye extraction method.

The microleakage results are adversely affected by the compatibility of the dye materials and tested materials. The dyes used for assessing microleakage with dye extraction method in other studies are either methylene blue <sup>[30,31]</sup> or basic fuchsin <sup>[32,33]</sup>. Thus, methylene blue dye has been determined spectrophotometrically in this study.

The results of the present study showed that the lowest dye absorbance with negative control group (0.003) which was close to nitric acid (blank) with a value of zero. The positive control group had the highest dye absorbance (0.081) value of all tested groups which showed the accuracy of the technique <sup>[31]</sup>.

In Group 1 using BD; the mean optical density of dye absorbance value was significantly lower than Group 2 (Simvastatin group) and positive control group. This could be attributed to the development of a hydroxyapatite-like layer between dentine and CSMs through the hydration reaction of CSMs suggesting formation of a chemical bond [34]. In addition to its micro-mechanical anchorage to tooth structure [35], its compressive strength, hardness and modulus of elasticity which is close to that reported for human dentine. Moreover, its chemical composition does not contain aluminates that lead to weakening and fragility of the set material [36]. Moreover BD is stable, less soluble, non-resorbable, hydrophilic and produces a tight seal [37]. These results were supported by Sinkar et al [38] and Katge et al <sup>[1]</sup> who reported good sealing ability of BD in furcal perforations and Mir et al [39] who found that the sealing ability of BD is significantly better than MTA and bioactive bone cement.

On the other hand, Group 2 using simvastatin was not significantly different than positive control group which indicate poor sealing ability of this material. This may be due absence of any physical or chemical bond to tooth structure.

## CONCLUSIONS

The sealing ability of Simvastatin is lower comparable to that of Biodentine in repair of furcation perforation.

Further studies should be conducted to test mixture of simvastatin with other biomaterials capable of bonding to tooth structure without affecting other properties of both materials

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