A comparative *in vitro* study of sealing ability of bioceramic and resin based sealer using two protocols of irrigation

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Сравнително ин витро изследване на способността на херметизиране на кореновите канали с биокерамика и епоксиден силър при прилагане на два ирригационни протокола

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Summary

Aim: *To compare the sealing ability of the bioceramic sealer Total Fill BC and the resin based sealer AH Plus using two protocols of irrigation – classic and modified with chitosan-citrate 0.6% as a final irrigating solution.*

Materials and Methods: *Forty one-rooted human teeth (n = 40) were used for the purpose of this in vitro study. All teeth were prepared using crown-down technique with rotary nickel-titanium instruments ProTaper Universal (Dentsply, Sirona, USA). Two irrigating protocols are used for the purpose of this in vitro study: first (classical) protocol - 10 ml 2.0% NaOCl, 10 ml 17% EDTA and 10 ml saline, and second (modified) protocol - 10 ml 2.0% NaOCl, 3 ml 0.6% chitosan-citrate and 10 ml saline. All teeth are randomly allocated into four groups: group I (n = 10) – the first (classical) irrigating protocol was applied and the root canals were obturated with AH Plus (Dentsply Sirona, USA); group II (n = 10) – the second (modified) irrigating protocol was applied and the root canals were obturated with AH Plus (Dentsply Sirona, USA); group III (n = 10) – the first (classical) irrigating protocol was applied and the root canals were obturated with Total Fill BC (FKG, Germany) and group IV (n = 10) – the second (modified) irrigating protocol was applied and the root canals were obturated with Total Fill BC (FKG, Germany); The teeth were immersed in dye (1 % methylene blue) for 72 hours. All teeth were sectioned longitudinally and the penetration depth of the dye (in millimeters) was observed under light microscope (magnification x 40). Measurement of the dye penetration was realized using the software program Image-Pro Plus 6.0 (Media Cybernetics, USA).*

Results: *There was a statistically significant difference between groups with different protocol of irrigation and between groups with different protocol of obturation.*

Conclusion: *It was found that the application of chitosan-citrate 0,6% as final irrigating solution contributes high sealing ability both of the premixed bioceramic sealer Total Fill BC and the epoxy based sealer AH Plus.*

Keywords: *bioceramic sealer, resin based sealer, apical microleakage, chitosan-citrate.*

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Резюме

Цел*: Да се сравни херметизиращата способност на биокерамичния силър TotalFill BC с епоксидния силът AH Plus като се използват два протокола на иригация – класически и модифициран, включващ хитозан-цитрат 0,6% като финален иригант.*

Материали и методи*: Четиредест еднокоренови човешки зъби (n = 40) са използвани за целта на настоящето изследване. Всички коренови канали са препарирани чрез crown-down техника с никел-титанови инструменти ProTaper Universal (Dentsply, Sirona, USA). Два протокола на иригация са използвани за целите на изследването – първи (класически) – 10 ml 2,0% NaoCl, 10 ml 17% EDTA и 10 ml физиологичен разтвор и втори (модифициран) - 10 ml 2,0% NaoCl, 3ml хитозан-цитрат 0,6% и 10 ml физиологичен разтвор. Всички зъби са разпределени на случаен принцип в четири групи: I група (n = 10): приложен е първи (класически) протокол на иригация и кореновите канали са обтурирани с AH Plus (Dentsply Sirona, USA; II група (n = 10): приложен е втори (модифициран) протокол на иригация и кореновите канали са обтурирани с AH Plus (Dentsply Sirona, USA); III група (n = 10): приложен е първи (класически) протокол на иригация и кореновите канали са обтурирани с Total Fill BC (FKG, Germany) и IV група (n = 10): приложен е втори (модифициран) протокол на иригация и кореновите канали са обтурирани с Total Fill BC (FKG, Germany). Зъбите са потопени в багрило (1% метиленово синьо) за 72 ч. След това всички зъби са рязрязани надлъжно като апикалното просмукване на багрилото е оценено чрез светлинен микроскоп (увеличение x 40). Изчислението на пенетрацията е чрез софтуерната програма Image-Pro Plus 6.0 (Media Cybernetics, USA).*

Резултати*: Установена е статистически значима разлика между групите с различен протокол на иригация и между групите с различен метод на обтурация.*

Заключение*: Установено е, че приложението на разтвор на хитозан-цитрат 0,6% като финален иригационен разтвор, подпомага запечатваща способност както на биокерамичния силър Total Fill BC, така и на епоксидния силър AH Plus.*

Ключови думи*: биокерамичен силър, епоксиден силър, апикално микропросмукване, хитозан-цитрат.*

Introduction

A main purpose of endodontic treatment is to prevent microbial contamination of the root canal system and/or to remove as much as possible microbes to ensure clinical and radiographic success. Numerous endodontic filling materials have been developed for achieving good sealing ability and adhesion to the root canal dentin. An ideal root canal sealer should adhere firmly to both dentin and core filling materials [1]. The strong bond of the endodontic sealer to the dentin is extremely important for preserving the integrity of the obturation material, which is directly related to the prevention of microleakage [2]. Various adhesive obturation systems have been introduced in endodontics in an attempt to obtain a "monoblock" in which the core material, sealing agent and root canal dentin form a single cohesive unit within the root canal [3].

Previous research demonstrated ability of chitosan to enhance the mechanical properties of dentin collagen and to reinforce collagen constructs [4]. Chitosan is a natural linear polysaccharide obtained by the deacetylation of chitin, which is found in crab and shrimp shells. Biocompatibility, biodegradability, bioadhesion and lack of toxicity of chitosan are of a great importance in dental medicine [5,6].

AH Plus (*Dentsply Sirona,* USA) is an epoxy-based sealer, which used with gutta-percha is suitable for lateral and vertical condensation techniques [7].

With both antimicrobial and sealing properties, premixed bioceramic materials are one of the few materials available in endodontics that contribute to endodontic treatment success [8].

Total Fill BC (*FKG*, Germany) is a bioceramic sealer recognized for its biological properties such as biocompatibility, bioactivity and antibacterial activity, as well as for its excellent physico-chemical properties [9, 10, 11].

The study hypothesis was that the use of chitosan-citrate solution 0.6% as a final irrigant could contribute to a better adhesion of the bioceramic sealer to the root canal dentine.

Aim

To compare the sealing ability of the bioceramic sealer Total Fill BC and the resin based sealer AH Plus using two protocols of irrigation – classic and modified with chitosan-citrate 0.6% as a final irrigating solution.

Materials and Methods

Sample selection and preparation

Forty one-rooted human teeth (n = 40) were used for the purpose of this in vitro study. All teeth were mechanically cleaned of any dental deposits and/or calculus by curettes and stored in saline at 4ºC temperature. All teeth were selected with straight root canals which were type I based on Vertucci's classification [12]. Before root canal preparation the crown was cut in the zone of the cement-enamel junction (CEJ) and the root length was standardized about 12 mm (+/- 1 mm). The working length (WL) is standardized with K-file #10 per 1 mm from the apex of the tooth as the instrument is inserted into the root canal until its tip is slightly protruded from the apex of the tooth and then was reduced with 1 mm. The tips of the roots are sealed with sticky wax for maximum simulation of the clinical situation [13]. All teeth were prepared using crown-down technique with hand steel instruments K-files #10 and #15 (*Dentsply, Sirona*, USA) and with rotary nickel-titanium instruments ProTaper Universal (*Dentsply, Sirona*, USA). The final preparation of the root canals is with file F4 (ProTaper Universal, *Dentsply, Sirona*, USA).

Two irrigating protocols are used for the purpose of this in vitro study.

- first (classical) protocol - 10 ml 2.0% NaOCl, 10 ml 17% EDTA and 10 ml saline
- second (modified) protocol - 10 ml 2.0% NaOCl, 3 ml 0.6% chitosan-citrate and 10 ml saline

Preparation of solution chitosan-citrate 0.6%

A method for preparation of solution chitosan-citrate 0.6% has been developed. Low molecular weight chitosan (with high bioadhesive capacity) was used as 0.6 mg of chitosan was dissolved in 100 mL 1% citric acid. The selected concentrations of both reagents fulfil the requirements for dissolving chitosan at pH 4.5.

All teeth are randomly allocated into four groups:

Group I (n = 10) – the first (classical) irrigating protocol was applied and the root canals were obturated with AH Plus (*Dentsply Sirona,*USA);

Group II (n = 10) – the second (modified) irrigating protocol was applied and the root canals were obturated with AH Plus (*Dentsply Sirona,* USA);

Group III (n = 10) – the first (classical) irrigating protocol was applied and the root canals were obturated with Total Fill BC (*FKG*, Germany);

Group IV (n = 10) – the second (modified) irrigating protocol was applied and the root canals were obturated with Total Fill BC (FKG, Germany);

All root canals were obturated with single cone technique using standartized gutta-percha cones (F4) and with the specified for the group sealer. After the root canal obturation all teeth were coronary obturated with the composite material Filtek Z250 (*3M ESPE*, USA). Finally all samples were imbedded in wet sponge which simulated the periapex and forced the hardening process.

Sample preparation for light microscopy observation

The entire root surface was painted with two coats of varnish to within 2 mm of the apex (figure 1). The teeth were immersed in dye (1 %

methylene blue) for 72 hours. All samples were then rinsed with distilled water and the varnish was removed. All teeth were sectioned longitudinally and the penetration depth of the dye (in millimeters) was observed under a light microscope (magnification x 40). Measurement of the dye penetration was realized using the software program Image-Pro Plus 6.0 (*Media Cybernetics*, USA) (figure 1). The comparative statistical analyses were performed with Bootstrap for Independent Samples t-test, SPSS 19 (*IBM*, USA).

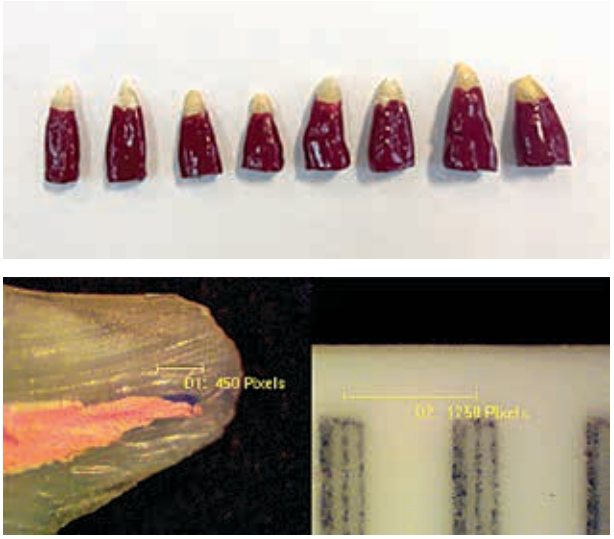

Fig. 1. *Assessment of the dye penetration between the root canal obturation and the dentin wall using the software program Image-Pro Plus 6.0*

Results

In group I the microleakage mean values of AH Plus and the classic protocol of irrigation were 1,80 +/- 0,12. In group II (modified protocol of irrigation and obturation with AH Plus) mean values were 0,84 +/- 0,14. In group III (classic irrigating protocol and obturation with Total Fill BC) mean values were 0,97 +/- 0,29 and in the last group IV (modified protocol of irrigation and obturation with Total Fill BC – 0,25 +/- 0,11 (figure 2).

There was a statistically significant difference between the groups with different protocol of irrigation (between group I and II – p = 0,046, between group III and IV – p < 0,001). It has been also determinate a statistical difference between the groups with different protocol of obturation and classic protocol of irrigation (between group I and III – p < 0,001). Between the groups with different protocol of obturation and same protocol of irrigation – modified it has not been determinate a significant change (between group II and IV – p = 0,478) (table 1).

Tab. 1*. Comparison of mean value of vertical apical microleakage between all groups*

I group	III group	I group	II group
II group	IV group	III group	IV group
p	p	p	p
0,046	<0,001	<0,001	0,478

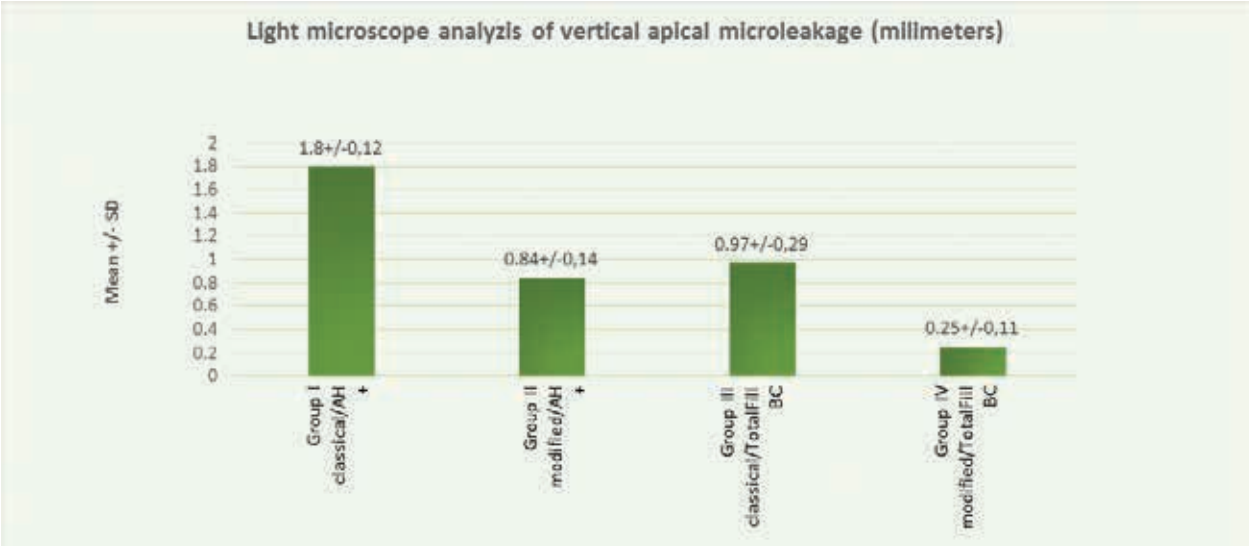

Fig. 2. *Light microscope analysis of vertical apical microleakage in millimeters (SD – Standard Deviation)*

Next light microscopic images represent the apical vertical penetration of the dye methylene blue 1% in all four groups (figures 3 – 6).

Discussion

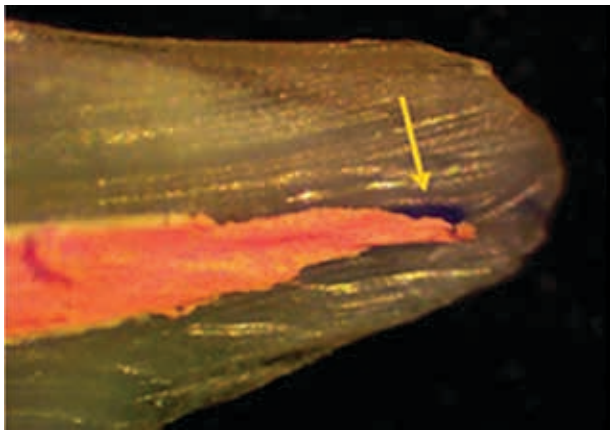

***Fig. 3**. Light microscopic image of a longitudinal sectioned root canal, irrigated with the classic irrigation protocol, and obturated with AH Plus (magnification x 40)*

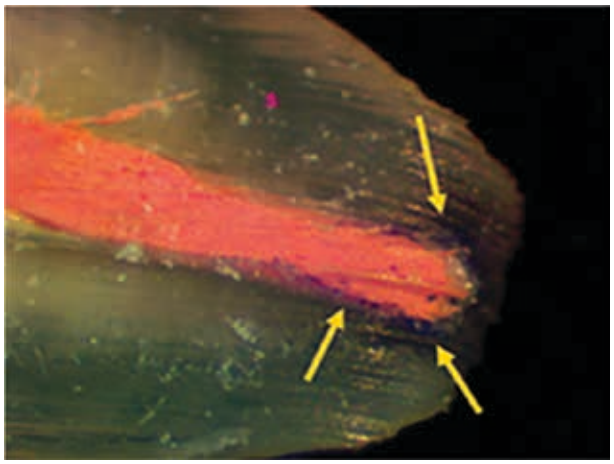

***Fig. 4.** Light microscopic image of a longitudinal sectioned root canal, irrigated with the modified irrigation protocol, and obturated with AH Plus (magnification x 40)*

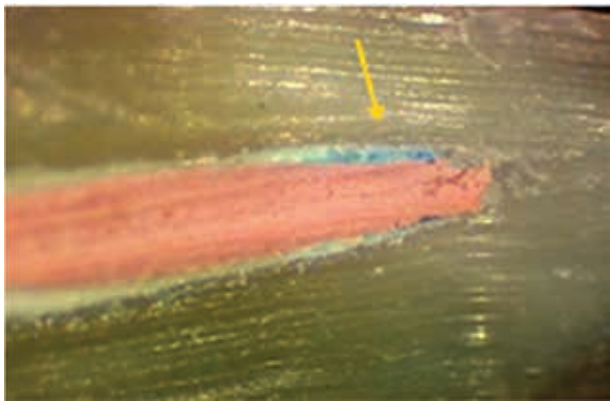

***Fig. 5.** Light microscopic image of a longitudinal sectioned root canal, irrigated with the classic irrigation protocol, and obturated with Total Fill BC (magnification x 40)*

***Fig. 6.** Light microscopic image of a longitudinal sectioned root canal, irrigated with the modified irrigation protocol, and obturated with Total Fill BC (magnification x 40)*

The aim of creating an endodontic monoblock after removal of the dental pulp is not a new concept in endodontics. After pulpectomy the root canal must be filled and a high priority is the obturation material to achieve a good adhesion to the dentine walls [14, 15]. The microleakage - coronary, lateral and / or apical, is a major clinical problem that subsequently leads to failure of endodontic treatment. Endodontically treated teeth have significantly reduced mechanical strength due to creating an endodontic access, the instrumental shaping of the root canals, as well as due to desiccation of dentine tissues [16, 17]. Various factors, such as root canal anatomy, the apical diameter, the chemical and mechanical preparation, the final irrigation and the degree of root canal contamination can be predispositions for microleakage into the root canals [14, 15].

In this study the sealing possibility of two root canal sealers was analyzed – an epoxy based and a bioceramic sealer, using two irrigating protocols - classic and modified (with chitosan-citrate solution 0,6%). The chitosan-based irrigants showed a satisfactory ability to remove the smear layer and open the dentin tubules for subsequent sealing of the root canal system [4]. Due to its high chelating ability for various metal ions in acidic conditions, it has been applied widely in the removal or recovery of metal ions in different industrial areas. The structure of

chitosan is similar to that of extracellular matrix proteins, such as proteoglycans and glycosaminoglycan. Some studies showed that chitosan and their derivatives interacted with and neutralized matrix metalloproteinases (MMPs), which improved the resistance of dentin to degradation [5, 6].

In previous study as a consequence of SEM investigation with different concentrations of chitosan-citrate (from 0.1 – 0.6%), the chitosan-based irrigant solution (at a concentration of 0.6%) showed a satisfactory ability to remove the smear layer and open the dentin tubules for subsequent sealing of the root canal system [4].

The bioceramic sealers are hydrophilic, they do not shrink and are insoluble in tissue fluids. Adhesion between bioceramic sealers and root canal dentine is influenced by the moisture present in the tooth. As the components of the bioceramic material enable the formation of calcium hydroxide and hydroxyapatite, they ensure an excellent bond to both the dentin and the filling material. Meanwhile they allow the practitioner to perform the microbial control without removing dentin unnecessarily and leaving a stronger root for restorative reconstruction [8]. One reason for the hardening of premixed bioceramic sealers could be the lack of moisture in the root canal system, especially at already endodontically treated teeth [18]. Epoxy resin based sealer have good adhesive properties to the root canal dentin, have excellent biocompatibility and minimum risk of a postoperative inflammatory reaction. AH Plus has many advantages over other sealers, but the ability to ensure a hermetic root canal obturation is debatable in the literature [19, 20]. This sealer showed better long-term sealing ability compared to conventional sealers due to its reported expansion over time. It is biocompatible and more radiopaque, has a shorter setting time (approximately 8 h), lower solubility, and a better flow characteristics compared with AH26 [7].

The data from the light microscopic analysis distributed in the four groups in this *in vitro* study are as follows – in group I the microleakage mean values of AH Plus and the classic protocol of irrigation were 1,80 +/- 0,12, in group II (modified protocol of irrigation and obturation with AH Plus) mean values were 0,84 +/- 0,14, in group III (classic irrigating protocol and obturation with Total Fill BC) mean values were 0,97 +/- 0,29 and in the last group IV (modified protocol of irrigation and obturation with Total Fill BC) – 0,25 +/- 0,11 (figure 2). Statistically, there was a significant change between the groups with different protocol of irrigation (between group I and II – $p = 0,046$, between group III and IV – $p < 0,001$). It has been also determinate a statistical difference between the groups with different protocol of obturation and classic protocol of irrigation (between group I and III – $p < 0,001$). Between the groups with different protocol of obturation and same protocol of irrigation – a significant change has not been determined (between group II and IV – $p = 0,478$) (table 1).

In the study, we confirm the good sealing ability of the bioceramic sealer Total Fill BC (mean 0,97 +/- 0,29) (figure 5), while at the same time confirming the hypothesis of better adhesion of the bioceramic sealer to the root canal dentine using solution chitosan-citrate 0,6% as final irrigating solution (mean 0,25 +/- 0,11) (figure 6). We confirm the high conditioning ability of the chitosan citrate solution 0,6% as a chelator of the root canal dentin. The results were in agreement with another study in which solution of chitosan-citrate 0,2% was used, with high efficiency in eliminating the smear layer that should enable a better three-dimensional root canal sealing [21].

Conclusion

In the limitations of this *in vitro* study, it was found that the application of chitosan-citrate 0,6% as final irrigating solution contributes to the high sealing ability of both the premixed bioceramic sealer Total Fill BC and the epoxy based sealer AH Plus.

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