Marginal Adaptation of Calcium Silicate-based Materials used in Furcal Perforation Repair: A Comparative in Vitro Study

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Abstract: The purpose of this research was to study the marginal adaptation of eight calcium silicate-based materials when used to seal large furcal perforations, compared with one resin-modified glass-ionomer cement by scanning electron microscopy (SEM). Conclusion:All tested calcium silicate-based materials showed good marginal adaptation and are suitable for large furcal perforation repair.

Keywords: calcium silicate cements, mineral trioxide aggregate, marginal adaptation, furcal perforation

1. Introduction

Mineral trioxide aggregate (MTA) was developed for endodontic purposes. It was first introduced as a root-end filling material by Torabinejad et al. in 1993. Now it is regarded as gold standard repair material for a wide scope of clinical applications including perforation repair, pulp capping, apexification and the repair of internal and external root resorption, etc. [3].

Numerous investigations have been performed on it since its introduction till now [1], [2], [3]. Results from most of these studies indicated that MTA may be considered to be almost ideal endodontic material due to its unique qualities. Generally, it hardens in humid conditions, seals endodontic space almost hermetically and exhibits minimal microleakage. It is practically almost insoluble in water and shows minimal expansion when setting [1], [4], [5], [6], [7].

MTA displays significant advantages in terms of its biocompatibility. When used as pulp capping, perforation repair and root-end material it shows minimal inflammatory response. [2], [8]. It is osteoconductive and stimulates the adjacent tissues to produce mineralized tissues – dentin, osteodentin, cement and new bone. Thus it provides biological sealing of the apex, pulp chamber or perforations [1], [3], [8]. MTA is the first restorative material which not only provides an effective seal of root perforations [9], [10], [11] but consistently allows for the cementum overgrowth, and may facilitate the regeneration of the periodontal ligament [3], [8].

In a comprehensive literature review Parirokh and Torabinejad discussed the drawbacks of MTA. The main disadvantages of this material are a discoloration potential, presence of toxic elements in the material composition, difficult handling characteristics, long setting time, high material cost, and the difficulty of its removal after curing [1].

MTA is available as two trade products - ProRoot MTA and MTA-Angelus both in gray and white forms (gMTA and wMTA). Making an attempt to overcome MTA deficiencies new MTA-like cements with similar chemical composition and indications have been developed [12], [13], [14]. All MTA and MTA-like cements are calcium silicate-based materials (CSMs).
BioAggregate (Innovative Bioceramix, Vancouver, Canada) is a white cement, whose chemical composition is similar to the white ProRoot MTA, but with some differences. It is composed of bioceramic nano-particles, including primarily calcium silicate, calcium phosphate, amorphous silicate oxide, calcium hydroxide and hydroxyapatite. It containts a significant amount of tantalum oxide for radiopacity instead of bismuth oxide [14], [15]. Bio-Aggregate appears to be a novel biocompatible [16] and nontoxic biomaterial and has the ability to induce mineralization-associated gene expression in osteoblast cells [17]. Bioaggragate and MTA exhibit equal antimicrobial effectiveness against *Enterococcus faecalis* [18].

Aureoseal (G. Ogna e Figli, Muggiò, Italia) is a modified tetracilicate cement based on Portland cement (PC) and radiopaque agents. Regarding its physical properties and chemical compounds it may be identified as gray form [13], [16]. When hardens in acid environment Aureoseal showed lower surface hardness than white MTA [20]. It is biocompatible [13], [19], but it exertes some cytotoxic effect when tested on mouse fibroblast cells [21].

Biodentine (Septodont, Saint-Maur -des-Fossés, France), according to manufacturer`s information, is a new material, that is the first all-in-one bioactive and biocompatible dentin substitute, developed on the base of an unique Active Biosilicate Technology, and designed to treat damaged dentine both for restorative and endodontic indications. It poses outstanding sealing properties and reduces microleakage. Its mechanical properties supersede those of MTA and glass ionomer cements (GIC), making it suitable to repairing endodontic perforations [22].

Resin-modified glass ionomer cements (RMGIC) have superior physical properties compared to conventional glass ionomere cements (GIC): significantly greater bond strength, best marginal adaptation and adhesion, least microleakage [23], [24].There is no difference in caries outcome between RMGIC and resin-based composite [25].When used as subgingival restorations they elicited a better periodontal response than dental amalgam [26]. Apart from their advantages they have some disadvantages. According to Selimović et al they are less biocompatible than conventional GIC [27].

According to manufacturer`s information GC Fuji VIII GP exhibits excellent biocompatibility and physical properties: reliable bond strength, good tensile strength (30 Mpa), high flexural strength (52 Mpa). It reaches 90% of its mechanical properties within just 10 min [28].

The three-dimensional hermetic seal is the main requirement to the materials for perforation repair. It is a complex result of marginal adaptation, adhesion, solubility and volume changes of the applied materials. The gap size between the dentin and material and the fluid leakage represent the quantitative manifestation of the materials` sealing ability [3]. Many studies have investigated the microleakage of the two basic types of MTA - ProRoot MTA and MTA-Angelus, but only a few their marginal adaptation. There are several reports about gap size produced by them as a root-end materials [7], [9], [10], [29] but no any data about there marginal adaptation as a furcal perforation repairing materials. In regard to Aureoseal, BioAggregate and Biodentine, as well as for GC Fuji VIII (RMGIC) we found only few data about their marginal adaptation, gap size and microleakage in the literature. There is no evidence of any comparative data about the gap size of the basic and new CSMs as materials for furcal perforation repair.

The purpose of this study was to compare the marginal adaptation of eight calcium silicate-based cements when used to seal large furcal perforations, compared with one resin-modified glass-ionomer cement by scanning electron microscopy (SEM).

2. Materials and Methods

Preparation of teeth for longitudinal sections

Ninety three freshly extracted human permanent maxillary and mandibular molars were used in this study. The molars were intact or had minimal restorations or caries lesions. Teeth with root fusion were excluded. After extraction the teeth were fixed in 10% buffered formalin for 2 weeks. Then they were cleaned to remove soft tissues and tartar and stored in physiological saline with a few thymol crystals before use.

Molars were decoronated 3 mm above the cemento-enamel junction using a slow-speed diamond saw (PHM, Plovdiv, Bulgaria) under constant water spray. A standardized endodontic access opening was made in each tooth. Root canals were cleaned and shaped up to #40 K-files (Beutelrock, VDW GmbH, München, Germany) using a step-back technique and then filled with Cortisomol (Produits dentaires Pierre Roland, Merignac, France) as a sealer and gutta-percha (Meta, Korea) using a single cone technique. Large perforations were made perpendicular to the center of the pulp chamber floor between mesial and distal roots by using a steel round bur ISO 014 (Komet, Gebr. Brasseler, Germany).

Gap size measurement of MTA products using SEM is a common method to evaluate the marginal adaptation of these materials to dental tissues [6],[9],[11], [29]. Making an attempt to simulate the real clinical conditions of the periodontal tissues adjacent to the perforation site we used a clinically oriented in vitro model. A piece of 4 cm x 2 cm gauze was placed in the furcation area so that its ends stayed free in occlusal direction. A silicone impression material Stomaflex Putty (Spofa Dental, a Kerr company, Jícín, The Czech Republic) was mixed to provide a bony socket simulation. The tooth roots were placed into the unset silicone and then removed when polymerization had finished. Then the “sockets” were filled with Stomaflex Light (Spofa Dental, Kerr company, Jícín, The Czech Republic) and the teeth were again inserted into them. Apical surfaces of the perforations were kept wet by moisturizing the free ends of gauze with physiological saline. Furthermore, this model prevented materials from over-extension in furcation area and allowed us to work without any internal matrix.

The teeth were randomly divided into nine equal groups of 10 teeth each. Three additional teeth with unrepaired perforations served as a positive control group in order to observe the SEM image of the dentine in this area.

Perforation sites were cleaned with sodium hypochlorite 2.6% (Dentsply, St. Quentin en Yvelines, France), dried and sealed with various materials as follows:

- Group 1 - white MTA-Angelus (Angelus, Londrina, Brazil)
- Group 2 - gray MTA-Angelus (Angelus, Londrina, Brazil)
- Group 3 - white ProRoot MTA (Dentsply, Tulsa, Johnson City, TN)
- Group 4 - Aureoseal (G. Ogna e Figli, Muggiò, Italia)
- Group 5 - BioAggregate (IBC Inc., Vancouver, Canada)
- Group 6 - Biodentine (Septodont, Saint-Maur-des-Fossés, France)
- Group 7 - white Portland cement (Titan cement CEM I 52,5N) (Zlatna Panega, Bulgaria)
- Group 8 - gray Portland cement (Titan cement CEM II/B-L 32,5 R) (Zlatna Panega, Bulgaria)
- Group 9 – GC Fuji VIII (RMGIC) (GC Corp., Tokio, Japan)

All calcium silicate cements were mixed according to the manufacturer`s instructions on a glass slab with cement spatula to produce homogeneous paste, inserted into the perforation with Dovgan carrier (SybronEndo Corp. Orange, CA) and compacted to the level of pulp floor with hand pluggers. A wet cotton pellet was placed in contact with the cements for 24 h. The coronal access was sealed with temporary filling material Coltosol F (Coltene, Whaledent, Switzerland).

GC Fuji VIII capsules were prepared in accordance with the manufacturer`s instructions with an amalgamator (Amalga Mix 2, Gnatus, Brazil) and inserted into perforation site using capsule applier (GC America Inc., Chicago IL, USA).

A chemically cured composite resin material Compolux (Septodont, France) was used for coronal sealing of all teeth. All teeth were taken out of the silicone model and stored in a closed container in 100% humidity at 37°C for 24 hours. All procedures were performed by one researcher.

Scanning Electron Microscopy (SEM)

Using a slow-speed diamond sow (PHM, Plovdiv, Bulgaria), the teeth were longitudinally sectioned to reveal the restorative materials. The sectioned specimens were mounted on an aluminum stubs, sputter-coated with a gold layer and examined under Philips SEM 515 (Philips, Eindhoven, the Netherlands) at an accelerating voltage of 25 Kv at different magnifications – from x25 to x6000. The magnification x2000 was chosen to be the most suitable for measuring the gap size in the dentin-material interface.

SEM photomicrographs were made and printed from four corners of each specimen at magnification x2000. The distance between the dentine walls of perforation and materials was measured to the nearest 0.01 µm at other four equidistant points of each micrograph. As a result, 16 measurements were made for each sample, i.e. 160 measurements per group.

All data was processed with a SPSS 15.0 statistics program (SPSS, Inc. Chikago, IL). Descriptive statistics (mean, standard error, standard deviation, confidence interval) was used to evaluate the materials` gap size. The results also were submitted to Mann-Whitney tests for evaluating whether two independent samples are from the same population. The significance of the differences between the nine groups was examined by using t-test. α-level was set at 0.05 and $p<0.05$ was considered as statistically significant.

3. Results

The SEM examination of the samples` longitudinal sections showed different gap size between the materials and dentinal walls in the various groups. There was no sample without gap. Table 1 shows the mean values of the width of the gap, standard errors, standard deviations, confidence intervals, minimum and maximum of the values and statistical differences between groups. Figure 1A is representative picture of a perforation site filled with various cements (x40). There are shown the 4 points where micrographs were made at magnification x2000. Figure 1B reveals one of these micrographs where the measurements are made at another four points.

Figure 2 shows the box-plots of the gap size measurements, which illustrate the median, minimal and maximal gap size of the materials, as well as the variance in each experimental group. Figure 3 shows scanning electron micrographs of the dentinal walls-cements interface of all nine materials.

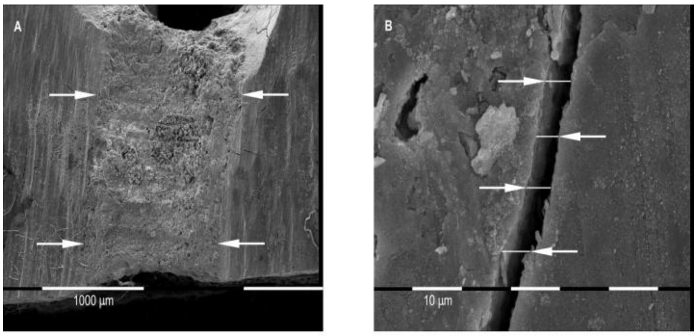

Figure 1. (A) SEM photomicrograph (original magnification x40) of longitudinal section of original sample, filled with wProRoot MTA, with arrows showing four points where another four micrographs were made. (B) SEM photomicrograph (original magnification x2000) of dentin-material interface of original sample, with arrows showing four points where gaps were measured.

Table 1. Gap size produced in each group – mean values, standard errors, standard deviations, confidence intervals, minimum and maximum of the values and significant difference. Same letter reveals no significant difference whereas different letter reveals statistically significant difference at $p<0.05$

Table 1. Gap size

Materials	*Mean*	*Std. Error*	*95 % CI for Mean*	*Std. Dev.*	*Min.*	*Max.*
Angelus white	6.87^{a}	0.58	5.56-8.18	1.83	3.72	10.17
Angelus gray	6.37^{a}	0.68	4.82-7.93	2.17	3.44	9.89
ProRoot white	$5.89^{a,b}$	0.80	4.07-7.71	2.54	2.90	10.02
Aureoseal	$6.25^{a,b}$	0.84	4.34-8.15	2.66	2.99	11.83
Bioaggregate	$5.62^{a,b}$	0.50	4.49-6.75	1.57	2.90	7.64
Biodentine	$7.28^{a,d}$	0.65	5.79-8.76	2.07	3.98	9.93
Portland white	$4.02^{b,c}$	0.68	2.46-5.56	2.16	1.43	7.86
Portland gray	2.53^{c}	0.35	1.74-3.33	1.11	1.54	4.69
Fuji VIII	9.37^{d}	0.78	7.58-11.45	2.48	6.59	14.09

Gray Portland cement had the smallest gap (2.53±1.11 µм), whereas GC Fuji VIII had the largest gap (9.37±2.48 µм). The mean value increased in the following order

gPortland<wPortland<BioAggregate<wProRoot<Aureoseal< gAngelus<wAngelus<Biodentine<Fuji VIII.

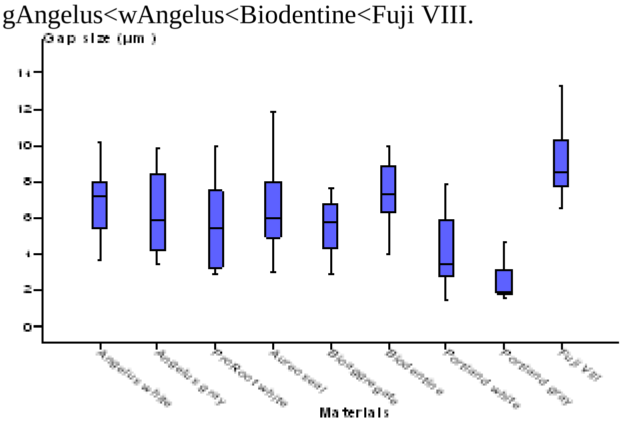

Figure 2: Box-plots of the gap size measures, which illustrate the median, minimal and maximal gap size of the materials, as well as the variance in each experimental group.

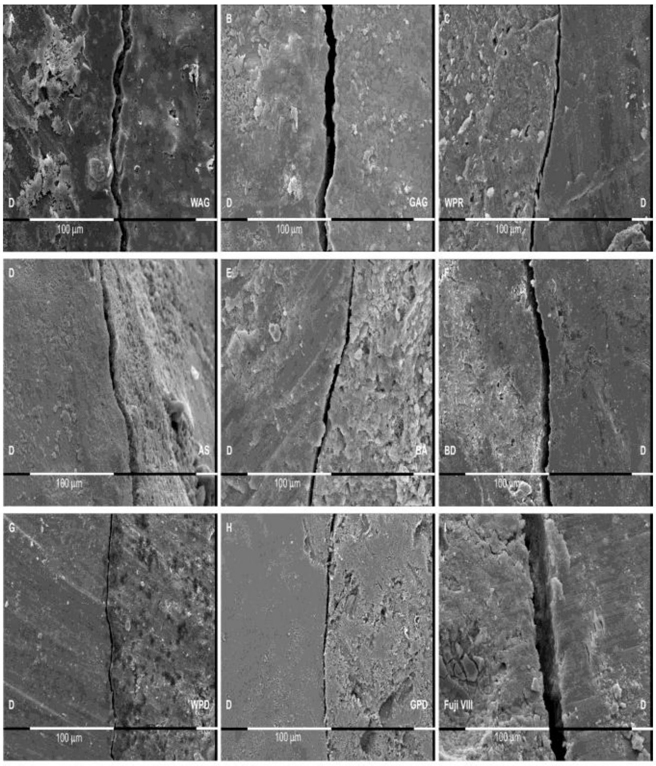

Figure 3. SEM photomicrographs of dentin-material interface of original samples (original magnification x500), filled with: (A) WAngelus - WAG, (B) GAngelus - GAG, (C) WProRoot - WPR, (D) Aureoseal - AS, (E) Bioaggregate - BA, (F) Biodentine - BD, (G) WPortland cement - WPD, (H) GPortland cement - GPD and (I) GC Fuji VIII.

Multiple comparisons were made to determine statistical differences between the groups. Confident intervals of the six MTA and MTA-like calcium silicate cements were very similar and these materials showed gap size ranging from 5.62-7.28 µm. BioAggregate produced the smallest gap size amongst them (5.62±1.57 µm) while Biodentine produced the biggest gap the (7.28±2.07 µm) but there was not statistically significant differences between them (p>0.05).

On one hand there was such a difference (p<0.05) between them and Fuji VIII (9.37±2.48 µm), except for Biodentine. On the other hand significant difference existed between them and the two forms of Portland cement which produced a superior seal (2.53±1.11 µм and 4.02±2.16 µм).

Both the gray and white forms of MTA-Angelus (6.37±2.17 µм and 6.87±1.83 µм) showed a bigger gap size than the BioAggregate, wProRoot MTA (5.89±2.54 µм) and Aureoseal (6.25±2.66 µм). WPortland cement took a median position whereas there was no significant difference between it and gPortland cement, BioAggregate, wProRoot MTA and Aureoseal but there was significant disparity between it and gMTA-Angelus, wMTA-Angelus, Biodentine and Fuji VIII.

4. Discussion

There are several reports about gap size produced by routine MTA products as a root-end filling materials, but no any data about their marginal adaptation as a repairing materials for furcal perforations. The researches studying the properties and clinical application of new calcium silicate-based materials BioAggregate, Aureoseal and Biodentine are not so many and we did not find any comparative studies between them and the two basic MTA products evaluating their gap size. The application site is likely to influence the gap size between the material and dentinal walls, because MTA is compacted against a different kind of tissues. Nevertheless, due to the lack of information we included the published data about retrograde application of MTA in this discussion.

In 1995 Torabinejad et al reported that MTA (Tulsa, USA) used as a root-end filling material produced minimal gap size of 2.68±1.35 µм in comparison with the mean values of other restorative materials - amalgam, SuperEBA и IRM [6]. In 2004 Shipper et al using the original MTA at the same application site measured gap size in the range 0.523 – 0.750 – 1.190 µм. Badr reported gray ProRoot MTA with a mean gap of 2.141±0.530µм [9]. In a recent study Rosares-Leal et al measured minimal gap width of 0.1 µm [30]. Evaluating marginal adaptation of MTA-Angelus by means of scanning electron microscopy Xavier et al reported a gap size of 0.812 and 1.051 µм in two groups [7].

In aforementioned studies the gap size varies from 0.1 to 2.68 µм. In contrast, Bidar et al reported results that considerably deviated from these. According to them gMTA used as root-end material produced mean gap size of 19.8 µм, wMTA produced gap size of 14.8 µм and Portland cement - 26.5 µм [29].

Gap width measurements of the specimens done under the conditions in this study were not in agreement neither with those of Bidar et al, nor with the other cited authors. This study showed calcium silicate-based cements sealing furcal perforations to have a mean gap in the range of 5.62-6.87 µm. Consequently, among cited results found in literature ours take a median position.

Generally, microleakage is considered to be a quantitative manifestation of marginal adaptation of materials [3], but according to Xavier et al there is not a correlation between these two aspects of MTA sealing ability [7]. In contrast, Torabinejad et al comparing the two methodologies stated the existence of such a correlation [6].Therefore, we also added some data about microleakage of the Portland cement to the discussion, MTA-like cements and RMGIC used as perforation repair, root canal and root-end filling materials.

In a dye-extraction leakage study Hashem et al. reported that ProRoot MTA as furcation repair cement leaked less than MTA-Angelus [10]. Conversely, according to Bortoluzzi et al, MTA-Angelus leaked less than ProRoot MTA [31]. However Feris and Baumgartner did not specify any difference between the two products [4]. Similar sealing properties were found with the white MTA-Angelus, PC and MTA Bio in furcation area, though neither of them provided absolute impermeability of fluids [12].

In a dye leakage study Stefopoulos at al stated that wProRoot showed less apical leakage (1.16±0.22 SE) than the gray form (1.66±0.32 SE) when used as an apical barrier in teeth with simulated open apices [5]. Conversely, some investigators did not find statistical difference between the gray and white forms of MTA [4], [32].One of these studies, performed by Shahi et al, compared the sealing ability of both forms of MTA and Portland cement. There were no statistically significant difference between gMTA and wMTA or white and gray PC, but significant differences were observed between the MTA groups and the PC groups. PC was concluded to have better sealing ability than MTA [32]. Our results are in agreement of this data. In this study we also found absence of significant statistical difference in the gap size produced from wProRoot MTA, gMTA-Angelus and wMTA-Angelus. Meoreover, the two forms of Portland cement showed the least gap size.

According to some researchers the gray form of ProRoot MTA exhibits superior sealability than white MTA form [29], [33]. Matt et al used a linear dye leakage model with ProRoot MTA and demonstrated significantly less dye penetration with gray MTA compared to white MTA [33].GProRoot MTA was not included in our investigation because it was not available. The two form of Portland cement were included instead.

The comparative data about the sealing ability of MTA products are controversial. Therefore, on the basis of available information, it is not possible to conclude that one of the CSMs has superior sealing ability in comparison with others. BioAggregate, Aureoseal and Biodentine are relatively new materials, proposed for the same indications, and there is a little scientific information about them. In a recent study El Sayed and Saeed using dye penetration technique compared sealing ability of wProRoot MTA and BioAggregate. They concluded that BioAggregate showed higher sealing ability than MTA ProRoot with significant difference between them [34]. These findings are in disagreement with our results. In this study both common MTA products and new calcium silicate-based materials produced comparable gap size in the material-dentine interface.

In our study GC Fuji VIII was used as representative material from the group of resin-reinforced glass ionomer cements. This material showed the biggest gap size (9.37±2.48 µm) in comparison with MTA, MTA-like cements and Portland cements with a significant difference between them. This could be related to the fact that this material is autocured. The presence of some humidity at the apical side of perforations was likely to influence the setting time and gap size of the GC Fuji VIII, as well.

Xavier et al reported that Vitremer used as root-end material produced gap size ranging from 2.86 to 4.62 µм [7]. Rosares-Leal et al are in agreement with them – they found Vitrebond with the gap size of 2.5 µm [30].These findings are in disagreement with the mean values of the gap width produced from Fuji VIII in our study.

The type of SEM used for evaluation of marginal adaptation and methods for samples preparation are very important in SEM studies. In such a study, Shipper et al compared MTA with amalgam by using high- and low-vacuum conditions. The results demonstrated that the size of the gap between the root-end filling material and the margin of the root-end cavity is smaller under the low-vacuum microscope. They attributed this finding to the sample examination without standardized preparations and in moist conditions [11].

A clinically oriented *in vitro* model for simulating large furcal perforations was used in this study. Although *in vitro* tests cannot completely simulate *in vivo* conditions, material with excellent or very good *in vitro* adaptation to dentine with small gap width may achieve the best clinical sealing ability.

Under the *in vitro* conditions of this study all tested calcium silicate cements showed good marginal adaptation when used to seal large furcal perforations. The results indicated that no significant difference was found between the materials ($p>0.05$). This study also showed that MTA and CSMs produced less gap size than resin-modified glass ionomer cement GC Fuji VIII.

5. Conclusion

Under the condition of this study all MTA products and MTA-like calcium silicate-based materials showed gap size ranging from 5.62-7.28 µm without significant difference between them. Such a difference exists between them and Gray Portland cement (2.53±1.11 µм). Although the data from the current study showed that all CSMs provide good marginal adaptation to the dentinal walls, a comparative leakage investigation of these materials should be performed.

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