International Journal of Adhesion and Adhesives 82 (2018) 206–210

Contents lists available at ScienceDirect

International Journal of Adhesion and Adhesives

journal homepage: www.elsevier.com/locate/ijadhadh

Effect of irrigant neutralizing reducing agents on the compromised dislocation resistance of an epoxy resin and a methacrylate resin-based root canal sealer *in vitro*

Pallavi Reddy[a,1], Prasanna Neelakantan[b,*,1], Kavitha Sanjeev[c], Jukka Pekka Matinlinna[d]

[a] Department of Conservative Dentistry and Endodontics, Army College of Dental Sciences, Secunderabad, India
[b] Discipline of Endodontology, Faculty of Dentistry, The University of Hong Kong, Hong Kong
[c] Department of Conservative Dentistry and Endodontics, SRM Dental College and Hospital, Chennai, India
[d] Dental Materials Science, Faculty of Dentistry, The University of Hong Kong, Hong Kong

ARTICLE INFO

Keywords:
Bond strength
Dislocation resistance
Epoxy resin
Methacrylate resin
Reducing agents
Sodium ascorbate
Sodium thiosulfate

ABSTRACT

Objectives: To determine the effect of two reducing agents (sodium ascorbate and sodium thiosulfate) on the dislocation resistance of a methacrylate and an epoxy resin based root canal sealer to sodium hypochlorite (NaOCl) treated root canal dentin.

Materials and methods: Single rooted teeth (n = 60) were instrumented with rotary instruments with an irrigation protocol of 3% NaOCl, 17% EDTA and 3% NaOCl in a sequence. Samples were randomly divided into three groups (n = 20) based on the final treatment: group 1 (saline), group 2 (5% sodium thiosulfate), group 3 (10% sodium ascorbate). Samples were then randomly divided into two subgroups (n = 10) based on the root canal sealer: A (a methacrylate resin sealer; EndoREZ); B (an epoxy resin sealer; AH Plus). The dislocation resistance was assessed using the push out bond strength test. The data were statistically analyzed by three-way ANOVA and a pair-wise comparison was done using the Bonferroni adjustment ($P = 0.05$).

Results: There was a significant interaction between the root thirds and the final treatment protocol for EndoREZ ($P < 0.001$) but not AH Plus ($P > 0.05$). The two experimental protocols significantly improved the adhesion (bond) strength of EndoREZ compared to saline ($P < 0.05$) with sodium thiosulfate producing significantly higher values than sodium ascorbate ($P < 0.05$). Both reducing agents did not improve the adhesion strength of AH Plus significantly when compared to the control ($P > 0.05$).

Conclusions: The bond strength of the methacrylate resin sealer EndoREZ is improved by the use of sodium thiosulfate and sodium ascorbate after irrigation with NaOCl, but these reducing agents do not improve the bond strength of the epoxy resin sealer AH Plus. Root canal sealers are differentially influenced by irrigant neutralizing (reducing) agents.

1. Introduction

While the primary objective of root canal irrigation is to remove microbial biofilms, organic tissue and accumulated hard tissue debris, it also alters the dentin substrate characteristics [1]. One important impact of such a process is the altered adhesion of filling materials to dentin [2,3]. It has been demonstrated that an irrigating protocol of sodium hypochlorite (NaOCl) followed by ethylenediaminetetraacetic acid (EDTA) offers optimal adhesion (bond) strength values for epoxy resin based and methacrylate resin based sealers [4,5]. The reported mechanisms for this enhanced adhesion have been the superior cleaning ability, to remove the organic and inorganic components of the smear layer to allow intimate adaptation of the root canal sealer to dentin walls [6].

Two types of resins are used as sealers in endodontic therapy: methacrylate resins and epoxy resins. Epoxy resin sealers such as AH Plus™ (Dentsply Sirona Endodontics, York, PA, USA) have been reported to bond chemically to the collagen of dentin [1,7]. On the other hand, methacrylate resin based sealers bond *via* a micro-mechanical bond by penetrating into the micro-porosities created within the dentin substrate by acidic primers [8,9]. A final rinse with NaOCl has been shown to be detrimental to the adhesion of both these materials. This is because NaOCl is proteolytic and can damage the naked collagen after removal of the hard tissue debris by EDTA [10], resulting in poor

* Correspondence to: Discipline of Endodontology, Faculty of Dentistry, The University of Hong Kong, 34, Hospital Road, Sai Ying Pun, Hong Kong.
E-mail address: prasanna@hku.hk (P. Neelakantan).

[1] Both these authors contributed equally to this manuscript and are co first authors.

https://doi.org/10.1016/j.ijadhadh.2018.01.018
Accepted 18 January 2018
Available online 31 January 2018
0143-7496/

chemical adhesion of epoxy resins to the collagen [1]. Moreover, the oxygen generated by NaOCl can inhibit polymerization of methacrylate resins [3]. Such a problem has been well documented in restorative dentistry, wherein oxidizing agents inhibit the polymerization of methacrylate resin composites used for restoring access cavities [11,12]. A recent work suggested that 5% sodium thiosulfate ($Na_2S_2O_3$) [3] and 10% sodium ascorbate ($C_6H_7NaO_6$) [12] applied for 10 min could possibly reverse such effects and enhance bonding of methacrylate resin based composites. It is not known whether such a mechanism would apply within the root canal system so as to reverse the compromised bond strength of resin based sealers (methacrylate and epoxy resins) after a final rinse of NaOCl.

The aim of this laboratory study was to investigate the effects of sodium thiosulfate or sodium ascorbate on the dislocation resistance of epoxy resin and methacrylate resin based root canal sealers from dentin, following root canal irrigation with sodium hypochlorite. The null hypothesis was that none of the agents have an influence on the dislocation resistance of both the materials tested.

2. Materials and methods

2.1. Specimen preparation

Extracted human single-rooted mandibular first premolars (n = 60) were collected, thoroughly cleaned and decoronated at the cemento-enamel junction using a water-cooled diamond saw. The roots were then radiographed at two angulations to confirm the presence of a single canal. The root lengths were standardized to 10 mm. Instrumentation of the root canals was done with ProTaper™ Next nickel titanium rotary instruments (Dentsply Sirona Endodontics, York, PA, USA) up to size X4. During instrumentation, irrigation was performed with 3% NaOCl (Parcan™, Septodont, Saint-Maur-des-Fossés Cedex, France) using a 5 mL disposable plastic syringe with a 31G side vented irrigation needle (Navitip 31G double sideport, Ultradent) placed passively into the canal to 1 mm short of the working length. The quantity and time of NaOCl used per canal was standardized to 5 mL and 15 min. This was followed by irrigation with 5 mL of 17% EDTA (Pulpdent, Watertown, MA, USA) for 1 min and then 5 mL of 3% NaOCl for 3 min. A pilot study demonstrated that a sample size of 10 per subgroup was required to show any statistically significant difference between the groups.

The samples were randomly divided into three study groups (n = 20) using a sealed envelope method, based on the final irrigation regimen: group 1 (saline), group 2 (5% sodium thiosulfate) and group 3 (10% sodium ascorbate). Sodium thiosulfate and sodium ascorbate were purchased from a commercial source (Sigma Aldrich, St. Louis, MO, USA). The volume of these agents was standardized to 5 mL with a contact time of 10 minutes. The canals were rinsed with 5 mL of distilled water and dried using paper points (Dentsply Sirona), and randomly allotted to one of the subgroups (n = 10) based on the root filling material: A (an epoxy resin root canal sealer; AH Plus™ Jet, Dentsply DeTrey, Konstanz, Germany) and B (a methacrylate resin based root canal sealer; EndoREZ, Ultradent Products). The sealers were placed into the root canals using a lentulospiral, which is a rotating spiral instrument designed specifically for placing cement-based materials into root canals. The quality of root canal filling was confirmed by taking radiographs at two angulations. Samples with voids or bubbles were discarded. Specimens were placed in an incubator at 100% humidity for 48 h to ensure complete setting of the sealer. All experimental procedures were performed by a single operator.

2.2. Measurement of dislocation resistance by push-out bond strength test

The roots were embedded in epoxy resin in a custom-made split-ring copper mould and 1 mm thick slices (± 0.04 mm) were obtained using a water-cooled precision saw (Ernst-Leitz, Wetzlar, Germany). For each root third, the first slice was selected from each tooth, resulting in 10 slices per root third. Each specimen was marked on its coronal surface with an indelible marker. The apical and coronal diameters of the obturated area were measured using an Olympus Camedia C-5060 digital camera (Tokyo, Japan) attached to a stereomicroscope (Global G6, St. Louis, MO, USA).

A compressive load was applied to each root section *via* a universal testing machine (LIoyd LRX-plus, Lloyd Instruments, Fareham, UK) at a cross-head speed of 1 mm/min using stainless steel plungers of different diameters, 1.10 mm (coronal), 0.8 mm (middle) and 0.5 mm (apical), positioned so that the plunger contacted only the filling material [13]. Since all chosen teeth were prepared using the same instrument sequence, the diameters of the root canals in the coronal (1.13 mm), middle (0.93 mm) and apical (0.60 mm) third in all the teeth were approximately the same, as observed in our pilot study and previously published study [14]. Furthermore, this step was calibrated and standardized previously in our experiments [14].

The push-out force was applied in an apico-coronal direction until bond failure occurred. The force was recorded using Nexygen™ data analysis software (Lloyd Instruments Ltd.). The maximum failure load was recorded in Newton (N) and the push-out bond strength (dislocation resistance) was calculated (in MPa) using the following formula [15]: Push-out bond strength (MPa) = N/A, where: N = Maximum load (N), A = Adhesion area of root canal filling (mm^2). The adhesion (bonding) surface area of each section was calculated as: $[(D1 \times D2)/2] \times \pi \times h$, where D1 and D2 are the greater and lesser diameters of the canal respectively and h is the thickness of the root slice (mm).

2.3. Data presentation and analysis

The main outcome variable in this study was the push-out bond strength/dislocation resistance (in MPa). Since the data were normally distributed, parametric statistical tests were applied. Three-way ANOVA was performed to investigate the interaction effects among the regions of the root, the surface treatment protocol, and the material, on dislocation resistance values. If the interaction effect existed, then two-way ANOVA was performed for each material. If there was no significant interaction, the effect was removed one-by-one and then one-way ANOVA was performed. The significant level was set as $P = 0.05$. For the pairwise comparisons, the Bonferroni adjustment was used.

3. Results

3.1. Overall interactions

There was a statistically significant interaction between the type of sealer, root segment and experimental protocols ($P < 0.001$) (Table 1). When the two-way ANOVA was applied, there was a significant interaction between the root segments and surface treatment protocol for EndoREZ ($P < 0.001$) except at the apical third between group 2 and 3 ($P > 0.05$), but not for AH Plus ($P = 0.319$).

3.2. Effect of treatment protocols and root filling material

Irrigation with sodium thiosulfate and sodium ascorbate improved the dislocation resistance of EndoREZ™, compared to group 1 (Saline) in all the root-thirds ($P < 0.001$). Sodium thiosulfate resulted in higher dislocation resistance values than sodium ascorbate in the coronal and middle third ($P < 0.001$). For AH Plus, there was no significant effect of the treatment protocol on the dislocation resistance in any of the root-thirds ($P = 0.61$). Neither sodium thiosulfate nor sodium ascorbate treatment significantly improved the dislocation resistance of AH Plus™, compared to the control (saline) in any of the root third ($P > 0.05$).

When specimens were treated with sodium thiosulfate (group 2), the dislocation resistance of EndoREZ™ was significantly higher than

Table 1
Push-out bond strength (MPa, means ± standard deviations) of an epoxy resin sealer (AH Plus) and a methacrylate resin based sealer (EndoREZ) after dentin surface treatment with two reducing agents (5% sodium thiosulfate and 10% sodium ascorbate) and control (saline) (n = 10).

Group	EndoRez			AH Plus		
	Coronal	Middle	Apical	Coronal	Middle	Apical
Group 1 (Saline)	0.9 ± 0.3^{a,A}	0.7 ± 0.2^{a,A}	0.5 ± 0.2^{a,A}	2.0 ± 0.2^{a,A}	1.8 ± 0.5^{a,A}	0.9 ± 0.3^{a,B}
Group 2 (Sodium thiosulfate)	5.6 ± 0.6^{b,A}	3.1 ± 0.5^{b,B}	1.9 ± 0.3^{b,C}	2.3 ± 0.3^{a,A}	1.9 ± 0.2^{a,A}	0.8 ± 0.3^{a,B}
Group 3 (Sodium ascorbate)	3.6 ± 0.4^{c,A}	1.5 ± 0.4^{c,B}	2.0 ± 0.4^{b,B}	2.0 ± 0.6^{a,A}	2.1 ± 0.3^{a,A}	0.9 ± 0.3^{a,B}

Within each group for each of the materials (EndoREZ and AH Plus), values with identical upper case superscript alphabet indicates no significant difference (P > 0.05); Between groups, for the same subgroup, values with identical lower case superscript alphabet indicates no significant difference (P > 0.05).

AH Plus in all of the root regions (P < 0.001). For the sake of clarity, these significant differences are not represented in the table. When specimens were treated with group 3, the dislocation resistance of EndoREZ™ was significantly higher than AH Plus™ in coronal and apical third (P < 0.001), while in the middle third, AH Plus™ had significantly higher dislocation resistance than EndoRez (P = 0.004). When irrigated with the control (saline), AH Plus™ had significantly higher dislocation resistance than EndoRez™ in all of the root-thirds (P < 0.05).

3.3. Effect of region of root canal

For EndoRez™, there was a significant difference in dislocation resistance values among the all regions of the root between the three groups whereas the difference was not significant in the apical third between groups 2 and 3 (P < 0.001). The coronal regions had the highest values (P < 0.001) in both groups 2 and 3 while the lowest values occurred in apical regions in group 2 and in middle regions in group 3 respectively. On the other hand, in group 1, there was no significant difference in the dislocation values among the coronal, middle and apical regions (P = 0.102).

For AH Plus™, one-way ANOVA showed that there was a significant difference in the dislocation resistance values among the coronal, middle and apical regions (P < 0.001). The apical regions had significantly lower values compared to the coronal and middle thirds (P < 0.001) but no significant difference in dislocation resistance values was found between coronal and middle regions (P = 0.332).

4. Discussion

Testing the adhesion of root filling materials is one way of studying the interactions between the materials and dentin substrate as well as substrate characteristics after exposure to different chemical agents [16]. Furthermore, a correlation has been established between the dentin bond strength and sealing ability for epoxy resin sealers [5], but not for methacrylate resin based sealers [17]. There has been considerable confusion in the literature with regard for terminology. While some authors define interfacial interaction between sealers and dentin as 'bond strength', one may argue that what is actually tested is an aspect of frictional retention [16,18]. That said, this paper will use the term dislocation resistance.

Sodium hypochlorite is a proteolytic agent and an oxidizing agent as it is primarily a disinfectant and a bleaching agent. The generation of oxygen inhibits polymerization of methacrylate resins [3]. The reduction in bond strength of methacrylate resin composites when the surface is treated with oxidizing agents such as NaOCl and hydrogen peroxide, has been well reported [3,19]. An irrigation protocol of NaOCl→EDTA→NaOCl was used in this study. Such a protocol has both advantages and disadvantages, and the endodontic literature lacks clarity about an 'ideal' irrigation protocol. The first rinse of NaOCl helps dissolve the pulp tissue and microbial biofilms, as well as achieve bacterial killing within the radicular space [20]. This is followed by the use of organic acids such as the tetravalent EDTA to remove the mineral component of the smear layer (also known as accumulated hard tissue debris) [21]. A final rinse with NaOCl is seen to be advantageous as this can now penetrate and achieve biofilm disruption as well as microbial killing within the dentinal tubules [22].

Prolonged NaOCl treatment may have a negative impact on the physico-mechanical properties of dentin: this happens *via* damage to the dentinal collagen [23]. However, such a damage has been shown to be dependent on the concentration of NaOCl, with 5.25% being more detrimental than 1.3% [10,24]. Using a combination of microscopic techniques, spectroscopy and mechanical testing, the authors demonstrated that collagen degradation of dentin is influenced by concentration and duration of use of NaOCl, immaterial of whether EDTA was used in the sequence. While the irrigation regimen in general, causes an increase in the apatite/collagen ration (indicating collagen degradation), at least 120 min of irrigation with 5.25% NaOCl was needed to bring about a significant effect.

Another problem with a final NaOCl rinse is the generation and release of oxygen bubbles. This can interfere with the polymerization of methacrylate resin based composites and adhesives [24]. That being said, agents that reverse the effect of sodium hypochlorite have been evaluated after bleaching with hydrogen peroxide and the literature is inconsistent in regards to the effects [12,25,26]. This is based on the claim that reducing agents such as sodium ascorbate or sodium thiosulfate, can reverse the oxidized state of the dentin substrate. Theoretically, such a mechanism should allow better adhesion of methacrylate resin based root canal sealers and there is evidence to prove the same [3,27]. However, it is unknown as to which of the two agents offers better results. It is also unknown if such a surface treatment will improve the dislocation resistance of epoxy resin based cements that have been shown to chemically bond to dentinal collagen [1]. To the best knowledge of the authors, this is the first *in vitro* study to test the effect of two antioxidants used for dentin conditioning after root canal irrigation, on an epoxy resin root canal sealer.

The results of the current study showed that the two reducing agents (sodium thiosulfate and sodium ascorbate) improved the dislocation resistance of the methacrylate resin based sealer compared to saline, but failed to reverse the reduction in dislocation resistance of the epoxy resin sealer, affected by NaOCl. Given this, the null hypothesis must be partially accepted. The reversal of compromised bonding for methacrylate resin based sealer could be because of removal of the oxygen bubbles generated by NaOCl [3]. Using micro-Raman spectroscopy and the push out bond strength test, Shrestha et al. demonstrated that sodium ascorbate could improve the degree of conversion and adhesion strength of a methacrylate resin based sealer RealSeal™ SE (SybronEndo, Orange, CA, USA) [27]. The results of the current study are in agreement. EndoREZ™ is a self-priming methacrylate resin sealer, albeit the main difference with RealSeal™ being the UDMA resin content and hydrophilic characteristic of EndoREZ™ [28,29]. EndoREZ™, a second-generation resin based sealer has been shown to have poor physical characteristics [30]. Nevertheless, this methacrylate resin based sealer was used because it does not require light activation, which in the opinion of the authors would induce another significant variable.

Interestingly, the dislocation resistance of AH Plus™ could not be reversed by the reducing agents used. This confirms that intact collagen is needed for the chemical adhesion of epoxy resin sealer to dentin and a final rinse of NaOCl may prevent such an adhesion by causing some degradation of the collagen. Based on the results of the present study, it may be hypothesized that following the loss of mineral encapsulation by irrigation with EDTA, the naked collagen may undergo some degradation at least at the surface even after a 3-min irrigation regimen. Such an ultrastructural characterization was beyond the scope of this work and this needs further analysis.

The dislocation resistance of both the root canal sealer materials showed a gradient with respect to the root thirds, with coronal third showing higher mean values than the middle and apical third. Such a difference is well reported in the literature [1,5]. The concentration of sodium ascorbate and sodium thiosulfate used was based on previous studies [3,12]. An important point to note is that sodium thiosulfate is used as the neutralizing agent for sodium hypochlorite in microbiological studies [31,32]. This process happens by a hypochlorite inhibiting mechanism [32] and it is unknown if treatment of dentin with thiosulfate will inhibit the extension of antibacterial activity of NaOCl into dentin. It is also unknown if the presence of excess hypochlorite ions plays a role in the reduction of bond strength of resin based sealers. This needs further analysis.

This study filled root canals only with the sealer rather than gutta-percha and sealer. This was based on previously published reports [1,5,14,17]. Both the sealers used in this study do not shrink and show a slight expansion in a humid environment [5,31]. Furthermore, filling the root canals only with the sealer truly reflects the bond strength between the sealer and dentin [5]. Our previous reports and pilot studies on the dislocation resistance of AH Plus have shown that failures are 100% of adhesive nature [1,5]. That said, previous studies have suggested that the tensile bond strength of AH Plus™ (about 60 MPa) and the relatively lower push-out bond strength values generated confirmed that the failures are undoubtedly adhesive in nature [5]. Therefore, a presentation of failure mode analysis was not deemed necessary in this work.

It has been shown that sodium ascorbate could also possess potential collagen cross-linking properties and might thus enhance the adhesion strength of methacrylate resins [33,34]. Furthermore, if used at an acidic pH, the demineralizing effect of sodium ascorbate can also bring about an increase in adhesion strength allowing for more effective penetration of the methacrylate resin into the dentinal tubules [28]. Such a claim may not hold true for epoxy resin sealers (such as AH Plus™) because it appears that tubular penetration of AH Plus™ does not contribute to its dislocation resistance [1,35] – strengthening the hypothesis that epoxy resins chemically bond to dentinal collagen [1,7]. The collagen cross linking property of sodium ascorbate also calls into need, the evaluation of dentin adhesion strength of resin based materials following long term storage with collagenolytic enzymatic challenge.

One limitation of this work is that the dislocation resistance of the resin based sealers was not assessed after ageing, since the initial aim was only to study if the reducing agents were able to improve the deterioration in dislocation resistance effected by sodium hypochlorite. Failures of resin based root canal fillings may be accelerated under ageing. Future studies need to be carried out in this direction. Furthermore, some future studies should also evaluate the effect of increased treatment time with the reducing agents on dislocation resistance of resin-based sealers.

5. Conclusions

- Both reducing agents (10% sodium ascorbate and 5% sodium thiosulfate) improve the adhesion strength of methacrylate resin based sealers to dentin irrigated with sodium hypochlorite.
- The adhesion strength of epoxy resin sealers is not improved by the neutralizing reducing agents.
- Sodium thiosulfate is more effective than sodium ascorbate in reversing the compromised bonding of methacrylate resin sealers.

Acknowledgment

The authors sincerely thank Ms. Samantha Kar Yan Li, Centralized Research Laboratories, Faculty of Dentistry, The University of Hong Kong for the data analysis.

References

[1] Neelakantan P, Sharma S, Shemesh H, Wesselink PR. Influence of irrigation sequence on the adhesion of root canal sealers to dentin: a Fourier Transform Infrared Spectroscopy and push-out bond strength analysis. J Endod 2015;41:1108–11.
[2] Leal F, Simao RA, Fidel SR, Fidel RA, do Prado M. Effect of final irrigation protocols on push-out bond strength of an epoxy resin root canal sealer to dentin. Aust Endod J 2015;41:135–9.
[3] Correa ACP, Cecchin D, de Almeida JF, Gomes BP, Zaia AA, Ferraz CC. Sodium thiosulfate for recovery of bond strength to dentin treated with sodium hypochlorite. J Endod 2016;42:284–8.
[4] De-Deus G, Namen F, Galan Jr. J, Zehnder M. Soft chelating irrigation protocol optimizes bonding quality of Resilon/Epiphany root fillings. J Endod 2008;34:703–5.
[5] Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J 2011;44:491–8.
[6] Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. Int Endod J 2012;45:42–8.
[7] Fisher MA, Berzins DW, Bahcall JK. An in vitro comparison of bond strength of various obturation materials to root canal dentin using a push-out test design. J Endod 2007;33:856–8.
[8] Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, et al. Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Part II: effects of mechanical cycling load on microtensile bond strengths. Dent Mater 2011;27:563–72.
[9] Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, et al. Bonding of universal adhesives to dentine–old wine in new bottles? J Dent 2015;43:525–36.
[10] Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod 2010;36:105–9.
[11] Khoroushi M, Kachuei M. Pull-out bond strength of a self-adhesive resin cement to NaOCl-treated root dentin: effect of antioxidizing agents. Restor Dent Endod 2014;39:95–103.
[12] Trindade TF, Moura LK, Raucci WN, Messias DC, Colucci V. Bonding effectiveness of universal adhesive to intracoronal bleached dentin treated with sodium ascorbate. Braz Dent J 2016;27:303–8.
[13] Pane ES, Palamara JE, Messer HH. Critical evaluation of the push-out test for root canal filling materials. J Endod 2013;39:669–73.
[14] Neelakantan P, Nandagopal M, Shemesh H, Wesselink P. The effect of root dentin conditioning protocols on the push-out bond strength of three calcium silicate sealers. Int J Adhes Adhes 2015;60:104–8.
[15] Nagas E, Cehreli ZC, Durmaz V, Vallittu PK, Lassila LV. Regional push-out bond strength and coronal microleakage of Resilon after different light-curing methods. J Endod 2007;33:1464–8.
[16] Moinzadeh AT, Jongsma LA, Wesselink PR. Considerations about the use of the "push-out" test in endodontic research. Int Endod J 2015;48:498–500.
[17] Moinzadeh AT, Mirmohammadi H, Hensbergen IA, Wesselink PR, Shemesh H. The correlation between fluid transport and push-out strength in root canals filled with a methacrylate-based filling material. Int Endod J 2015;48:193–8.
[18] Goracci C, Fabianelli A, Sadek FT, Papacchini F, Tay FR, Ferrari M. The contribution of friction to the dislocation resistance of bonded fiber posts. J Endod 2005;31:608–12.
[19] Feiz A, Mosleh H, Nazeri R. Evaluating the effect of antioxidant agents on shear bond strength of tooth-colored restorative materials after bleaching: a systematic review. J Mech Behav Biomed Mater 2017;71:156–64.
[20] Zehnder M. Root canal irrigants. J Endod 2006;32:389–98.
[21] Paque F, Boessler C, Zehnder M. Accumulated hard tissue debris levels in mesial roots of mandibular molars after sequential irrigation steps. Int Endod J 2011;44:148–53.
[22] Azim AA, Aksel H, Zhuang T, Mashtare T, Babu JP, Huang GT. Efficacy of 4 irrigation protocols in killing bacteria colonized in dentinal tubules examined by a novel confocal laser scanning microscope analysis. J Endod 2016;42:928–34.
[23] Marending M, Paque F, Fischer J, Zehnder M. Impact of irrigant sequence on mechanical properties of human root dentin. J Endod 2007;33:1325–8.
[24] Zhang K, Tay FR, Kim YK, Mitchell JK, Kim JR, Carrilho M, et al. The effect of initial irrigation with two different sodium hypochlorite concentrations on the erosion of instrumented radicular dentin. Dent Mater 2010;26:514–23.
[25] Hansen JR, Frick KJ, Walker MP. Effect of 35% sodium ascorbate treatment on microtensile bond strength after nonvital bleaching. J Endod 2014;40:1668–70.
[26] Gotti VB, Feitosa VP, Sauro S, Correr-Sobrinho L, Leal FB, Stansbury JW, et al. Effect

of antioxidants on the dentin interface bond stability of adhesives exposed to hydrolytic degradation. J Adhes Dent 2015;17:35–44.
[27] Shrestha D, Wu WC, He QY, Wei X, Ling JQ. Effect of sodium ascorbate on degree of conversion and bond strength of RealSeal SE to sodium hypochlorite treated root dentin. Dent Mater J 2013;32:96–100.
[28] Kardon BP, Kuttler S, Hardigan P, Dorn SO. An in vitro evaluation of the sealing ability of a new root-canal-obturation system. J Endod 2003;29:658–61.
[29] Zmener O, Pameijer CH. Clinical and radiographic evaluation of a resin-based root canal sealer. Am J Dent 2004;17:19–22.
[30] Kim YK, Grandini S, Ames JM, et al. Critical review on methacrylate resin-based root canal sealers. J Endod 2010;36:383–99.
[31] Rôças IN, Siqueira Jr. JF. Comparison of the in vivo antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. J Endod 2011;37:143–50.
[32] Kemp GK, Schneider KR. Validation of thiosulfate for neutralization of acidified sodium chlorite in microbiological testing. Poult Sci 2000;79:1857–60.
[33] Srinivasulu S, Vidhya S, Sujatha M, Mahalaxmi S. Shear bond strength of composite to deep dentin after treatment with two different collagen cross-linking agents at varying time intervals. Oper Dent 2012;37:485–91.
[34] Srinivasulu S, Vidhya S, Sujatha M, Mahalaxmi S. Effect of collagen cross-linkers on the shear bond strength of a self-etch adhesive system to deep dentin. J Conserv Dent 2013;16:135–8.
[35] De-Deus G, Brandao MC, Souza EM, Reis C, Resi K, Machado R, et al. Epoxy resin-based root canal sealer penetration into dentinal tubules does not improve root filling dislodgement resistance. Eur Endod J 2017;2. [7-7].