THE EFFECT OF CHLORHEXIDINE AND DIMETHYL SULFOXIDE ON LONG-TERM SEALING ABILITY OF TWO CALCIUM SILICATE CEMENTS IN ROOT CANAL

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ABSTRACT

Aim To evaluate the long-term effect of chlorhexidine (CHX) and dimethyl sulfoxide (DMSO) on the sealing ability and biomineralization of two different calcium silicate cements (CSC) in root canal.

Methodology Sixty human third molar root canals were obturated with ProRoot MTA or Biodentine. Before obturation the canals were irrigated with saline (control), 2% CHX or 5% DMSO. Microleakage was tested after three days and after six months. After additional six months (12 months after root filling) the roots were cut into 2 mm thick dentine discs. The discs were stored in artificial saliva for one year. The bond strength was measured with the push-out method, and the failure mode was evaluated with a stereomicroscope. The most apical disc of each tooth was used for Vickers hardness test.

Results No significant differences between the groups was found in initial microleakage. The leakage increased significantly during the 6-month storage in all groups except in Biodentine-CHX group and Biodentine-DMSO group. CHX and DMSO irrigation significantly increased the leakage with ProRoot MTA with time, but there was no statistically significant difference compared to the ProRoot MTA-control group at six months' time point. CHX significantly reduced the push-out bond strength of ProRoot MTA. With Biodentine irrigation with CHX or DMSO resulted with significantly higher push-out strength compared to the Biodentine control group. Fracture analysis showed statistically significant difference in the distribution of the fractures between the groups, but neither CHX nor DMSO change the fracture pattern statistically significantly. With Vickers hardness test ProRoot MTA with and without DMSO as the final irrigant showed significantly higher dentin hardness than any Biodentine-group.

Conclusion Considering that aging increased the leakage in all groups except with Biodentine-DMSO and the differences in the push-out strength and surface microhardness data, it appears that the time-related biomineralizing effect of MTA and Biodentine does not improve sealing to dentin. CHX significantly reduced ProRoot MTA bond strength and increased pure adhesive failures with both cements.

INTRODUCTION

Calcium silicate based cements, so-called mineral trioxide aggregate (MTA) and materials modified from it are commonly used when tight but non-resin based seal between dental pulp tissue and restorations or root canal and periradicular tissue is needed. One common feature to all the products on the market is that they all contain tricalcium silicate (Ca_3SiO_5) and radiopacifying agent (Camilleri 2015). The first commercially available hydraulic calcium-silicate cement (hCSC), ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland), is composed of Portland cement and bismuth oxide as radiopacifying agent. Biodentine (Septodont, Saint-Maur-des-Fossés Cedex, France) is another commonly used hCSC, with zirconium dioxide to increase radio-opacity. These materials are widely used for repairing root and furcal perforations, in ortho- and retrograde root end fillings, pulp capping and even to obturate the whole root canal. They can be used in wet environment, and some even require wet environment for setting (Gancedo-Garavia & Garcia-Barbero 2006, Li *et* al 2014). The bioactivity, if considered as a property to induce mineral precipitation to the adjacent tissues, of hCSC is an important issue in the ability of possible remineralization of the dentin (Vallittu *et* al 2018). Calcium silicate based materials can nucleate apatite on their surface and possibly stimulate tissue repair (Prati & Gandolfi 2015) and even remineralize dentin (Li et al. 2017a,b). However, biological and other fluids may have different effects on the setting of the materials (VanderWeele *et* al 2006, Reyes-Carmona *et* al 2009, Reyes-Carmona *et* al 2010). Considering the widespread clinical use of hCSCs in endodontics, the information of the effect of different root canal irrigants on them is surprisingly scarce.

Chlorhexidine (CHX) is commonly used final irrigant in endodontics because of its antimicrobial properties and adhesion into root canal dentin (substantivity) (Basrani & Lemonie 2005, Basrani *et* al 2003, Basrani *et* al 2002). CHX also decreases the activity of collagenolytic enzymes, matrix metalloproteinases (MMPs) in radicular dentin (Santos *et* al 2009, Tay *et* al 2006). This effect could have a positive influence on the sealing ability and adhesion of the root canal filling material. CHX improves the long term adhesion to dentin with composite fillings (Tjäderhane 2015) and it may also at least moderately improve the immediate (Lindblad *et* al 2010) and the long-term (Shafiei & Memarpour 2010, Cecchin *et* al 2011, Lindblad *et* al 2012) post adhesion to root dentin. CHX is said to improve the sealer wettability (Ferreira de Assis *et* al 2011, Prado *et* al 2011). On the other hand, CHX does not seem to affect the immediate or long-term sealing ability of endodontic sealers (Bodrumlu *et* al 2010, Sharifian *et* al 2010). The data on the effect of CHX irrigation on the

behavior of hCSC in root canals is inconsistent (Guneser *et* al 2013b, Elnaghy 2014, Bayram *et* al 2015).

Dimethyl sulfoxide (DMSO) is a solvent that has long history in industry and pharmacology. Recent studies show that even low DMSO concentration improves both immediate and long-term adhesive bond strength in dentin (Stape *et* al 2018a, Stape *et* al 2018b, Salim Al-Ani *et* al 2018b, Tjäderhane *et* al 2013, Stape *et* al 2015, Stape *et* al 2016a, Stape *et* al 2016b, Guo *et* al 2017). The effect may be related to the increased dentin wettability even up to 36% (Mehtala *et* al 2017) DMSO acts as a MMP-enzyme inhibitor (Tjäderhane *et* al 2013) and by that may preserve the bond strength (Stape *et* al 2016a, Stape *et* al 2016b). DMSO also decreases the immediate and long-term microleakage of endodontic sealers, although the difference is minor (Lindblad *et* al 2019), and may increase the long-term bond strength of root canal post (Shafiei *et* al 2016). The influence of DMSO to hCSC is not known.

The aim of this study was to determine the effect of CHX and DMSO on the immediate and long-term sealing ability and adhesion of ProRoot MTA and Biodentine by measuring the microleakage, push-out bond strength and fracture modes in root canals. In addition, the potential effect of hCSCs on root canal dentin after aging was evaluated using Vickers hardness test. We hypothesize that neither CHX nor DMSO would affect the immediate or long-term microleakage or adhesion of either cement. The second hypothesis was that the hCSCs do not affect the microhardness of the dentin.

MATERIAL AND METHODS

Sixty human third molars extracted as a part of normal treatment in the University Student Health Care Centre in Tampere and Oulu, and the Unit of Specialised Oral Care in the City of Helsinki, Finland were used for the study with the patients' consent and approval from the Ethical Committee, Faculty of Medicine, University of Oulu. Teeth were stored in 0.2% sodium azide at 4ºC until used. Only molars with one straight separate root were selected. Crowns were removed at the cemento-enamel junction and the selected root was separated with a diamond disc. The length of the roots was adjusted to 9-10 mm. The root canals were prepared with Gates burs (Dentsply Maillefer, Ballaigues, Switzerland) no 3, 4 and 5 to a final 1.3 mm diameter through the apex. After preparation the roots were irrigated with 3 ml of 3% sodium hypochlorite (ChlorCid, Ultradent, Salt Lake City, UT, USA) followed by 3 ml of 18% ethylenediaminetetraacetic acid, (EDTA,

Ultradent) to remove the smear layer. The roots were randomly divided to two groups (30 roots for each group). Both groups were further randomly divided to three subgroups, two experimental groups and a control group (10 roots for each group). Before obturation the canals of the experimental groups were irrigated either with 2% CHX (Consepsis, Ultradent) indicated for the final irrigation of root canal, or 5% DMSO (Sigma-Aldrich, St Louis, MO, USA) for 60 s and dried with paper points. 5% DMSO concentration was chosen because of its superior wettability effect (Mehtala *et* al 2017) and the best preservation of dentin adhesive bond strength and interface integrity after aging (Salim Al-Ani *et* al 2018a). The canals of control groups were irrigated with sterile saline for 60 s and dried with paper points before obturation. The apex was closed with Coltoflax impression material (Coltoflax,Colténe/Whaledent, Altstätten, Switzerland) during the irrigation. The roots were filled either with ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland) or Biodentine (Septodont, Saint-Maur-des-Fossés Cedex, France) using amalgam carrier and a plugger, holdig the root against a glass sheet, leaving 1 mm of the coronal part of the canal unfilled for temporary filling material (Cavit G, 3M ESPE, Neuss, Germany). The materials were mixed and used according to each manufacturer's protocols. After filling the roots with ProRoot MTA a cotton pellet moistened with saline was placed on the coronal 1 mm of the canal and sealed with Cavit G. The roots filled with Biodentine were left on the table for 12 min to set and then sealed with Cavit G. All samples were stored at 100% humidity at 37ºC until tested. The microleakage was initially tested after 3 days. The temporary fillings were removed before the leakage was tested. After initial fluid filtration test, the temporary filling was replaced and the specimen were further stored at 100% humidity at 37ºC until the leakage was tested again after 6 months. After additional 6 months in 100% humidity (12 months after root filling), the roots were cut with an IsoMet Low Speed Saw (Buehler, Lake Bluff, IL, USA) into 2 mm discs. Three discs per tooth were obtained. The discs were stored in artificial saliva at 37ºC for one week, and then the push-out test was performed for two discs of each tooth. The most apical disc of each tooth was used for Vickers hardness test.

Fluid filtration test

Microleakage was measured by using a fluid filtration method as described by Bouillaguet et al. (Bouillaguet *et* al 2008). The apical part of the root was glued with cyanoacrylate glue (Flex Gel, LOCTITE Super Glue, Henkel, Düsseldorf, Germany) into a silicone tube connected to the device recording the fluid flow (Flodec, De Marco Engineering, Geneva, Switzerland). The tube was filled with distilled water under constant hydrostatic pressure of 6.89 kPa (10 psi) (Raina *et* al 2007)

(Wedding J.R. *et* al 2007). If any tube leakage were observed during testing, the measurement was stopped, the leaks were sealed and the measurement was repeated. The water pressure was applied to each root for 30 minutes and the fluid flow was recorded constantly with three seconds time interval.

Push-out test

The push-out test for measuring shear strength of the materials to dentin was performed by pushing the obturation material from the apical side of each disc using a universal testing machine (Lloyd LRX, Lloyd Instrument Ltd., Fareham, UK) with a custom-made jig and 1.0 mm/min cross-head speed. The force required to debond or break the material was registered and the point of failure was observed from the loading curve. To analyse the fracture modes, all discs were examined from both sides with a stereomicroscope (magnification 16 x), and digitally photographed.

Surface microhardness

The effect of obturation materials on dentin hardness was evaluated using surface microhardness test (Vickers hardness, VHN) with automated tester (Shimadzu Micro Hardness Tester HMV-G21, Shimadzu Corp., Tokyo, Japan). The most apical sections, not used for the push-out tests, of five randomly selected teeth in each group were tested with 1.961 N (200 g) force and 10 s holding time. Six to eight points were tested immediately (30-50 µm) under the dentin-obturation material border and at the respective normal reference dentin at about midroot. For each sample, a mean value of material-affected and reference dentin was calculated. Since the sections represented apical thirds of the teeth and the differences in apically advancing sclerosis between the samples could not be completely ruled out, the relative hardness was calculated for the material-affected dentin of each tooth using the normal dentin as reference (100%), and these relative hardness values were used to represent the sample.

Statistical analysis

Statistical analyses were performed with SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests demonstrated that all groups did not follow the normal distribution, and Levene test indicated that the data was heteroscedastic for all the measured parameters (leakage, push-out strength and microhardness). Therefore, non-parametric Kruskal-Wallis and Mann-Whitney tests were used to analyse the significance of the differences between the groups. Wilcoxon Signed Ranks test was used to analyse the significance of the differences in

leakage between the immediate and 6 months aged samples within the groups, and the differences in Vickers hardness values between the material-affected dentin and control dentin**.** Pearson Chi-Square test was used to analyse the differences in fracture modes. The statistical unit for all measurements was the root, and the level of significance was set into $p<0.05$.

RESULTS

Microleakage

The immediate and 6-month leakage values are presented in Figure 1. The immediate leakage was low (between 394 and 681 nl/30 min) in all groups, with no significant differences between the groups. The leakage increased significantly during the 6-month storage compared to the immediate values in all groups except in Biodentine-CHX group and Biodentine-DMSO group. The lowest leakage after 6 months was observed in the Biodentine-DMSO group, which was comparable to the ProRoot MTA-control group ($p>0.05$). Even though both CHX and DMSO irrigation significantly increased the leakage with ProRoot MTA with time, there was no statistically significant difference compared to the ProRoot MTA-control group at six months' time point, mainly due to high variation in leakage in both experimental irrigant groups.

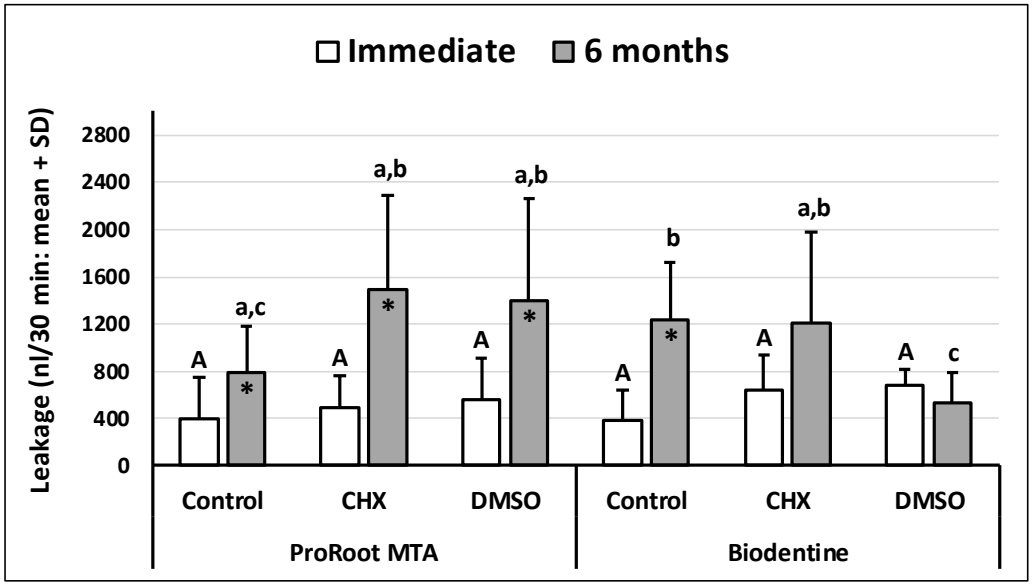

Figure 1. Leakage values (mean + SD) in different groups and time points. Different upper case letters indicate statistically significant differences between the groups at the immediate testing, and lower case letters at the 6-month testing (Kruskal-Wallis and Mann-Whitney tests, $p < 0.05$). *

indicates statistically significant difference from the immediate testing within the group (Wilcoxon signed rank test, $p < 0.05$).

Push-out

The 6-month push-out strength values are presented in Figure 2.ProRoot MTA with DMSO as the final irrigant and ProRoot MTA control showed the highest 6-month push-out bond strength, which was significantly reduced with CHX irrigation. With Biodentine, irrigation with CHX or DMSO resulted with significantly higher push-out strength compared to the Biodentine control group.

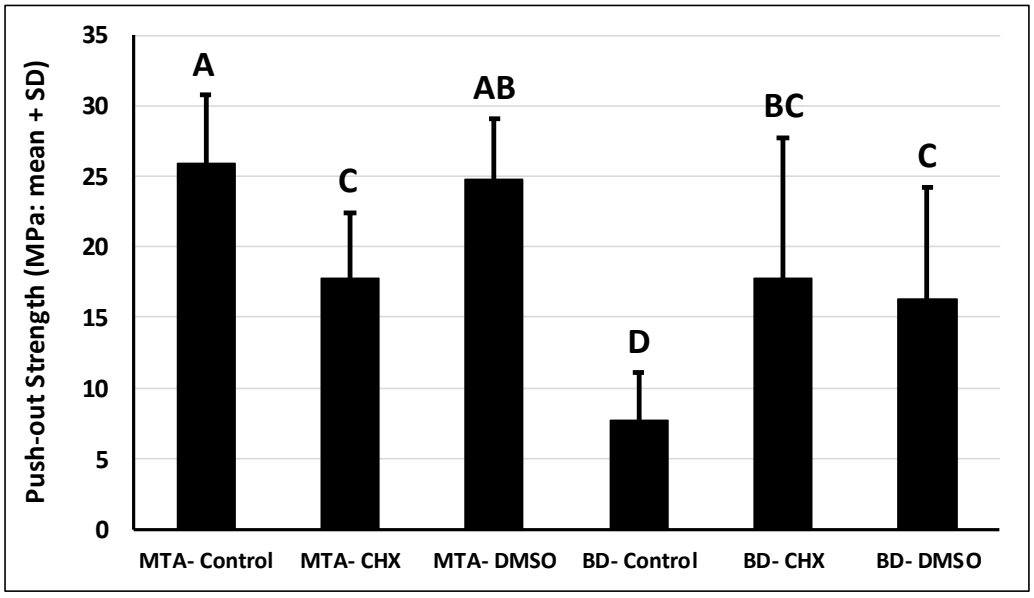

Figure 2. Push-out strength (mean + SD) in different groups. Different upper case letters indicate statistically significant differences between the groups (Kruskal-Wallis and Mann-Whitney tests, $p < 0.05$).

Fracture analysis

Fracture modes are presented in Figure 3. There was a statistically significant difference in the distribution of the fractures between the groups (Pearson Chi-Square test, $p<0.05$). With both ProRoot MTA and Biodentine, DMSO irrigation slightly reduced the purely adhesive fractures and increased the cohesive dentin fracture component. On the contrary, CHX irrigation increased the

purely adhesive fractures both with ProRoot MTA and Biodentine (by 26% and 38%, respectively) compared to the control group.

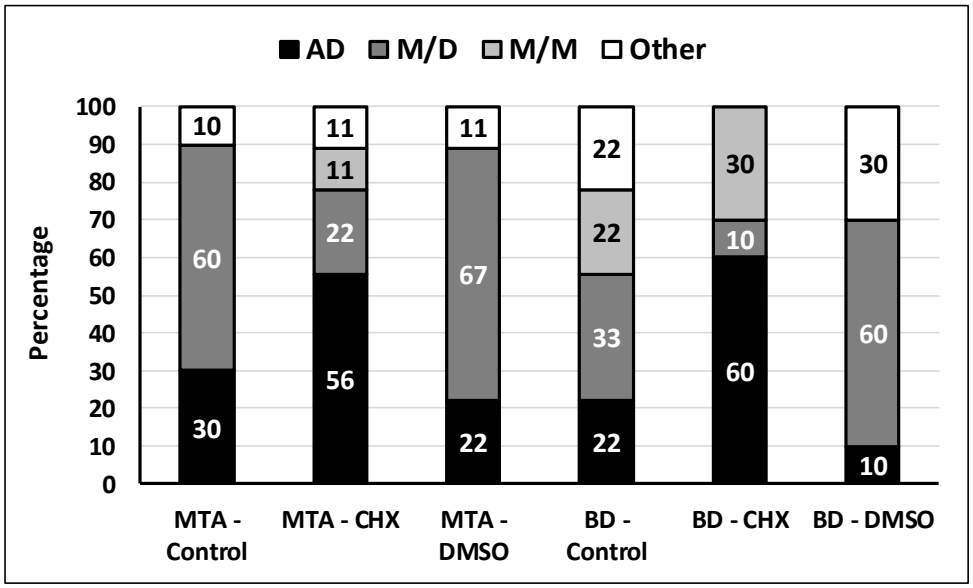

Figure 3. Push-out fracture mode percentages in different groups. AD: purely adhesive fracture; M/D: mixed fracture with adhesive and cohesive in dentin; M/M: mixed fracture with adhesive and cohesive in material.

There was no correlation between the root canal leakage and push-out bond strength when the whole data was included (**Figure 4**). The only subgroup to show statistically significant correlation was the MTA-DMSO group (Spearman correlation coefficient -0,783, p = 0.013).

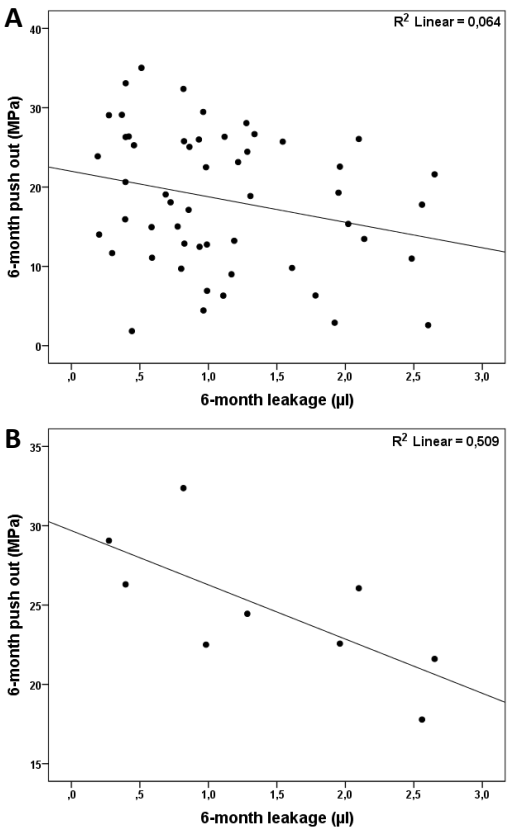

Figure 4. Correlation between the leakage and push-out bond strength **A)** for the whole data (Spearman correlation coefficient -0.236, p = 0.090) and **B)** for the MTA-DMSO group that was the only group with statistically significant correlation (Spearman correlation coefficient -0,783, p = 0.013).

Surface microhardness

The dentin surface microhardness values (VHN) are presented in Figure 5. In all groups except Biodentine-DMSO the dentin immediately under the interface was significantly harder than in the control are of the same tooth. ProRoot MTA with (127.1 ± 8.1%) and without (120.4 ± 4.1%) DMSO as the final irrigant showed significantly higher dentin hardness than any Biodentine-group. ProRoot MTA with CHX irrigation (123.0 ± 12.6%) did not show statistically significant difference to any other group except to Biodentine DMSO group (100.8 ± 10.4%), which was significantly lower than any other group.

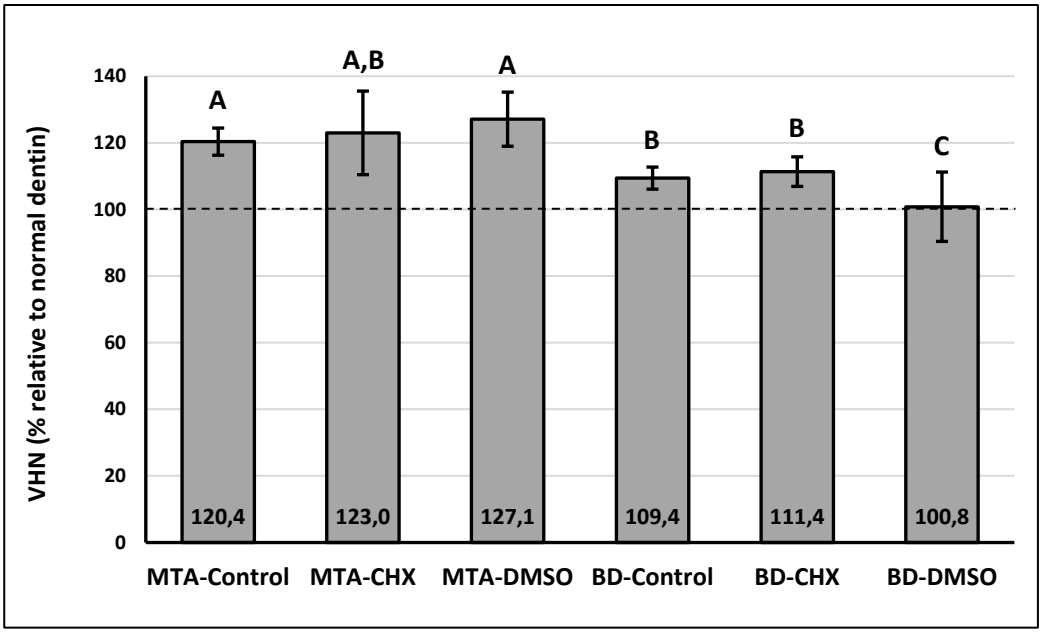

Figure 5. The effect of the obturation materials and irrigants on dentin microhardness immediately below the dentin-root canal border. The bars indicate the relative hardness (mean ± SD) compared to the respective normal dentin within the sample (dashed line: intrinsic control, 100%). The values at the base of the bars present the mean for each group. Different upper case letters indicate statistically significant differences between the groups (Kruskal-Wallis and Mann-Whitney tests, $p < 0.05$).

DISCUSSION

The present study investigated the effect of CHX and DMSO on the microleakage, push-out bond strength and fracture mode of ProRoot MTA and Biodentine. In addition, we wanted to see the effect of both cements to root dentin hardness immediately below the dentin-root canal border to express the possible interface biomineralization. The fluid filtration test was chosen to measure the leakage because of the quantitative data that is collected by the computer, thus minimizing the operator error. One important benefit of this method also is that the specimen could be measured again after the aging (Verissimo & do Vale 2006, Miletic *et* al 2002). The other tests, push-out and surface microhardness tests are commonly used tests in material testing.

Minor leakage existed initially and increased significantly with aging in all groups, including the control group, except in the Biodentine-CHX and Biodentine-DMSO groups. The highest microleakage was found in the aged ProRoot MTA-CHX group, although it was not significantly

different from the other groups at six months' time point except when compared to Biodentine-DMSO. This result is in concert with previous studies by us and others, where final irrigation with CHX results with the highest root canal sealer microleakage scores in aged samples (Lindblad *et* al 2019), and negatively affects the bond of white MTA (Elnaghy 2014) . The results indicate that CHX does not have the same kind of effect on preserving the dentin-obturation interface as it has with adhesive resins to coronal dentin (Carrilho *et* al 2007a, Carrilho *et* al 2007b). The CHX inhibition of dentin endogenous enzymes may not be as important in root canals as it might be with adhesives.

DMSO improves the adhesive bonding to coronal dentin (Stape *et* al 2018a, Stape *et* al 2018b, Stape *et* al 2015, Stape *et* al 2016b, Salim Al-Ani *et* al 2018b, Tjäderhane *et* al 2013) along with decreased leakage (Salim Al-Ani *et* al 2018b, Stape *et* al 2018b, Tjäderhane *et* al 2013, Stape *et* al 2015). Although DMSO (Mehtala *et* al 2017) (and also to minor extend CHX (Prado *et* al 2011)) increase dentin wettability and water is vital for hCSCs from the setting to their bonding and capability of forming new hydroxyapatite with root canal dentin (Prati & Gandolfi al 2015) (Camilleri 2015), in the present study the DMSO-induced preservation of the interface integrity was seen only with Biodentine-DMSO group. These findings indicate that leakage-free integrity of the interface between hCSC and root dentin may be material-dependent and is not universally achievable just by increasing the wettability.

The highest push-out strength was found with ProRoot MTA control and ProRoot MTA-DMSO, which also showed less purely adhesive failures in fracture analysis. CHX significantly reduced ProRoot MTA bond strength and increased pure adhesive failures with both cements, which is in concert with other studies (Elnaghy 2014, Guneser *et* al 2013b, Hong *et* al 2010, Holt *et* al 2007) and our leakage data. CHX inhibits the calcium hydroxide crystal formation on the surface of MTA (Hong *et* al 2010) indicating disturbance in MTA setting. In endodontics CHX is often used as the last irrigant and hCSC is placed immediately after irrigation. The lowest long-term leakage and the adhesive failure rates with both cements was seen with DMSO, indicating that probably the superior wettability induced by DMSO (Mehtala *et* al 2017) contributes to the interface integrity.

Contrary to ProRoot MTA, Biodentine-control had the lowest bond strength, which was significantly increased by both CHX and DMSO. In accordance to our study, Guneser et al have shown that CHX does not negatively affect the bond strength of Biodentine (Guneser *et* al 2013a). On the other hand, CHX significantly increased the adhesive failures with Biodentine as also with ProRoot MTA, so the actual effect on the adhesion still remains unclear. The findings in fracture analysis supports previous study (Majeed & Al Shwaimi 2017) that ProRoot MTA may be harder

than Biodentine, as we found more cohesive material fractures in Biodentine (except Biodentine-CHX) than in ProRoot MTA groups. This might at least partly also explain the push-out measurements, the absence of cohesive MTA fractures and the negative correlation between the leakage and push-out values in MTA-DMSO group.

The hCSCs ability to increase dentin mineralization with different irrigants was tested by the surface microhardness measurement. In accordance with a recent study (Cardoso *et* al 2018), dentin hardening was seen in every group (except Biodentine-DMSO, where the difference was not significant) compared to the control dentin which could indicate remineralization of the dentin. Since the leakage demonstrated almost completely opposite pattern, and there was no correlation between the push-out and leakage values, the data indicates that the interface biomineralization does not necessarily lead to lower leakage, questioning the assumption of leakage diminishing by remineralization suggested previously (Reyes-Carmona *et* al 2009, Reyes-Carmona *et* al 2010). The microhardness near the border of hCSC was higher than the hardness of the midroot control area. The dentin hardness has been explained to depend on the mineral content of the dentin (Seyedmahmoud *et* al 2017), which indicate the possible release of minerals e.g. calcium from hCSCs to dentin causing biomineralization. Dentin hardness was significantly higher in ProRoot MTA-control and ProRoot MTA-DMSO than Biodentine, indicating that ProRoot MTA's ability to increase biomineralization may be better than that with Biodentine.

There was no correlation between the root canal leakage and push-out bond strength when the whole data was examined, and MTA-DMSO was the only group with statistically significant correlation. Therefore, the increase in bond strength does not necessarily mean improved sealing. This study also shows that none of the testing methods used in this study could alone determine the superiority of either two hCSCs or irrigants used in this study. Since the same samples were used to analyze the immediate and long-term leakage, the immediate bond strength could not be evaluated. However, there is a consensus that aging improves hCSC bond strength (Neelakantan *et* al 2018) assumed to be caused by biomineralization at the dentin-obturation interface.

Conclusion

Based on the results of our study the first hypothesis that neither CHX nor DMSO would affect immediate or long term microleakage or adhesion of either cement was rejected. The second hypothesis that the hCSCs do not affect the microhardness of the dentin was also rejected. Considering that aging increased the leakage in all groups except with Biodentine-DMSO and the

differences in the push-out strength and surface microhardness data, it appears that the time-related biomineralizing effect of MTA and Biodentine does not improve sealing to dentin. Moreover, the leakage data does not directly reflect the push-out bond strength. Therefore, care should be taken when interpreting experimental data and their clinical relevance.

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