Comparative evaluation of four endodontic biomaterials and calcium hydroxide regarding their effect on fracture resistance of simulated immature teeth



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Abstract

Aim Single-session apexification treatment with MTA is an alternative to the root-canal treatment of immature teeth. Since its results are far from ideal, research with MTA-derivative biomaterials continues; however, the number of studies is limited as of yet. This study aimed to compare the fragilities of *in vitro*-simulated immature teeth in single-session apexification with MTA and newly-developed calcium-silicate-based MTA derivatives.

Materials and methods Two hundred seventy human permanent upper incisors were randomly divided into study (n = 180), positive control ($Ca(OH)_2$) (n = 45), and negative control (NC-intact) (n = 45) groups. The study groups used ProRoot MTA (MTA-PR), MM-MTA, NeoMTA-Plus, and Biodentine (BD). Two-week, two-month, and one-year follow-ups data were recorded. A fracture resistance (FR) test was performed at the end of each period. The results of the biomaterials at the different follow-up timepoints were statistically analysed and compared.

Results The two-week FR medians were significantly lower in MM-MTA, NEO, MTA-PR, and $Ca(OH)_2$ groups ($p<0.001$, $p<0.001$, $p=0.003$, and $p<0.001$. respectively), compared to NC. The two-month FR medians were significantly lower in BD, MM-MTA, NEO, MTA-PR, and $Ca(OH)_2$, ($p=0.005$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively) compared to NC. The one-year FR medians were significantly lower in the BD, MM-MTA, NEO, MTA-PR, and $Ca(OH)_2$ groups ($p=0.002$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively) compared to NC. For all groups, FR at the one-year mark was significantly lower compared to the two-week and two-month marks ($p<0.001$, $p<0.001$). Statistics: All groups were compared with each other regarding fracture resistance at two weeks, two months, and one year. Also, for each group follow-up data on fracture resistance were compared.

Conclusions Since FR was significantly higher at the two-week and the two-month mark compared to the one-year mark for each material, filling the root canal completely with biomaterials used in our study leads to tooth denaturation over time, with effects like $Ca(OH)_2$.

KEYWORDS Immature teeth, Trauma.

Introduction

It is critical to retain the teeth that have lost their pulp or have become infected for any reason, performing a root-canal treatment for these teeth [Tanalp et al., 2012]. However, root canal treatment of immature teeth is difficult as they have not yet completed their root development and have weak dentin walls and a broad and open apex [Lawley et al., 2004; Wilkinson et al., 2007]. A broad apical aperture results in more difficult apical blocking and can bring about the risk of overflow of the materials used, leading to the risk of foreign body reaction in the periapical tissues [Rafter, 2005]. The space in the apical region needs to be closed before completion of the root canal treatment to avoid these complications. Today, the widely accepted and practiced method for this purpose is the "multi-session apexification treatment", which is to form a barrier in the apical region using calcium hydroxide [Sheehy and Roberts, 1997], and the "single-session apexification treatment" , which uses Mineral Trioxide Aggregate (MTA) and other similar biomaterials [Witherspoon and Ham, 2001; Rafter, 2005]. Even though high success rates have been reported regarding calcium hydroxide, which has been used for a long time in apexification treatment, there are important disadvantages to this method [Rafter, 2005]. These disadvantages are the questions concerning prolonged treatment, the requirement of high motivation of the patient as the treatment requires several sessions, the difficulties in patient follow-up, the risk of contamination between the sessions, the decreased root resistance against fracture, the porous structure of the formed calcified dental bridge, and the formation of a water-proof barrier on the apical tip [Dominguez Reyes et al., 2005; Andreasen et al., 2002; Binnie and Rowe, 1973; Tronstad et al., 2000; Shabahang, 2013]. The most prominent disadvantage is the prolonged contact with $Ca(OH)_2$ causing protein denaturation leading to increased root fragility. This effect can cause tooth fractures and, thus, failure in the long run, even in the case of successful apexification [Andreasen et al., 2002; White et al., 2002]. The purpose of a single-session apexification treatment, which resolves many disadvantages of multi-session apexification, is to block the apical region of the immature tooth with a biomaterial which can create a water-proof barrier, completing the treatment in a single session [Jyothi, 2012]. The success rate of MTA, which

is commonly used for this purpose, is reported to be 81-100% [El-Meligy and Avery, 2006; Simon et al., 2007; Pace et al., 2007; Witherspoon et al., 2008]. On the other hand, MTA has certain disadvantages such as the prolonged hardening period, the involvement of toxic content, difficulty in placing the material in the application area, coloring observed in the tooth over time, difficult extraction after placement, and high cost [Torabinejad et al., 1995; Duarte et al., 2005; Monteiro Bramante et al., 2008; Boutsioukis et al., 2008; Belobrov and Parashos, 2011; Lenherr et al., 2012]. Additionally, it was stated that MTA could cause protein denaturation in the tooth like calcium hydroxide, and, as a result, may lead to the reduced fracture resistance of the material [Holland et al., 1999].

Because of the above-mentioned disadvantages, the use of various materials containing tricalcium silicate as an alternative to MTA have been suggested, developed by changing the conventional ingredients of MTA in recent years [Jeeruphan et al., 2012; Macwan and Deshpande, 2014]. The novel material, Biodentine, also called "dentin replacement cement" (Septodont, Saint Maur des Fausses, France), is a new calcium silicate-based, biocompatible and non-toxic material [Jang et al., 2014; Attik et al., 2014; Jung et al., 2014; Zanini et al., 2012]. It contains tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide, and ferrous oxide [Sawyer et al., 2012]. Tricalcium silicate and dicalcium silicate form the primary and secondary cores, respectively, and the radiopacity is provided by zirconium oxide instead of bismuth oxide [Camilleri et al., 2012; Camilleri, 2015; Dammaschke, 2012]. NeoMTA Plus (Avalon Biomed Inc. Bradenton, FL, USA) is a material that contains Portland cement, tricalcium silicate, dicalcium silicate, calcium sulfate, silica, and 30% tantalum oxide for radiopacity [Camilleri et al., 2012; Govindaraju et al., 2017]; its particle size is smaller compared to conventional MTA [Camilleri et al., 2013]. Micro-Mega Mineral MTA (MM-MTA) (Micro-Mega, Besancon, France) is a water-insoluble and biocompatible root-canal filling material that contains tricalcium silicate, Portland cement, bismuth oxide, tricalcium aluminate, magnesium oxide, calcium sulfate dihydrate, and chlorine. The material contains calcium carbonate to make the manipulation of MTA easier [Tanalp et al., 2013; Kum et al., 2014]. MM-MTA is available in a capsule form to be used in a high-frequency capsule mixer, thus assuring mixing of the correct powder:liquid ratio and ensuring the homogeneity of the material [Khalil et al., 2015; Tanalp et al., 2013]. It has similar biocompatibility and radiopacity of MTA, and it was reported to have a perfect adhesion to the dentin (Micro-Mega, Besancon, France).

There are very few studies investigating the short- and long-term effects of these novel materials developed as alternatives to MTA on the fragility of the teeth when used for single-session apexification [Topcuoglu et al., 2015; Elnaghy and Elsaka, 2016; Karapinar-Kazandag et al., 2016; Çiçek et al., 2017].

This study aims to comparatively evaluate the effects of conventional MTA and newly developed calcium-silicate based MTA derivatives following application as root-canal filling in in vitro simulated immature teeth on subsequent short- and long-term tooth fragility in teeth that received calcium hydroxide treatment, and in healthy teeth that did not receive any treatment.

Materials and methods

This study was approved by the Ethical Committee of the Medical Faculty (27.04.2015-10/15).

The study was conducted on 270 human permanent upper incisors which were not decayed, fractured, or broken. Before the experiment, all the residue on the roots were removed using a scaler and were soaked in 0.1% thymol solution for disinfection. The samples included in the study were chosen using the digital radiography method (Digora Optime, Soredex, Turkey); it was subsequently confirmed that there was only one canal in each tooth, and no intra-canal calcification or resorption was present. Also, the sizes of the teeth were measured mesiodistally and buccolingually using a caliper, and the teeth with similar sizes were included in the study (BL: 5.74 ± 0.5 mm, MD: 6.07 ± 0.5 mm) [Topcuoglu et al., 2015].

270 teeth were randomly included into 4 study, 1 positive and 1 negative control groups, and they underwent the following procedures.

Study Groups (n = 180)

Under water-cooling, the roots of the teeth were cut using diamond discs, resulting in 12 mm long standard roots. Using a water-cooled high-speed aerator and with the help of diamond round and fissure drills (KG Sorensen, Zenith Dentaş ApS, Denmark), the endodontic cavities were drilled open. After the root pulps were extirpated using barbed broaches (Dentsply Maillefer, Ballaigues, Switzerland), the canals were broadened to 0.05 mm tapered F5 with the crown-down technique the using Protaper (Dentsply Maillefer, Ballaigues, Switzerland) rotatory system. Afterwards, using a No. 6 Peeso reamer (Mani Inc., Tochigi, Japan), the apex was passed 1 mm through to form standard-width open apices. During preparation, before each tool switch, 5 ml of 5.25% sodium hypochlorite, 5 ml of distilled water, 5 ml of 17% EDTA, and 5 ml of distilled water were used for irrigation. After the roots were dried using paper points (Dentsply Maillefer, Switzerland), the root-canal cavities were filled with a calcium hydroxide paste (Prevest DenPro Limited, India), and the openings were closed with a temporary restoration material (Cavit, 3M ESPE, Seefeld, Germany). The teeth were left to incubate for one week at 37 °C and at 100% humidity. After the incubation period, the temporary filling was removed, and the calcium hydroxide paste was removed from the canal using 5 ml 5.25% sodium hypochlorite, 5 ml 17% EDTA, and 5 ml distilled water.

The samples were then randomly divided into four groups of 45 teeth filled with the relevant material in each group: ProRoot MTA (MTA-PR), MM-MTA, Neo MTA Plus, Biodentine (BD).

The filling pastes were prepared in accordance with the manufacturers' recommendations. The apices of the teeth were placed on Simon glass, and the prepared materials were filled into the canals using lentulo spirals, pluggers, and spreaders to prevent overflow of filling material from the opening at the apex; the success of fillings was confirmed through radiograms. Then, the samples in the study groups were covered with moist cotton pellets, and the samples were kept in the incubator at 37 °C and at 100% humidity until their hardening periods were completed.

Positive control (calcium hydroxide) group (n = 45)

The teeth in this group were prepared and incubated in the same manner as the study groups. After the one-week long incubation, the root-canals were filled with calcium hydroxide.

Negative control (untreated teeth) group (n = 45)

The teeth that were included in neither the study groups nor the positive control group were included in the negative control group (n=45), and these samples did not undergo any process.

Subgroups

At the end of the hardening period in the study groups and following the placement of calcium hydroxide in the positive control group, the permanent restorations of the teeth were done using a self-etch bonding system (3M Adhesive 200T, St. Paul, MN, USA) with A2 nano-composite (Filtek Z350 XT; 3M ESPE, St. Paul, MN, USA).

Afterward, the study and control groups were randomly divided into sub-groups of equal sizes (n = 15) that would have three different follow-up periods (two weeks, two months, one year). During the periods determined for each sub-group, the teeth were kept in an incubator at 37 °C and at 100% humidity.

Fracture resistance test (universal test device).

After the determined waiting periods were over, the root surfaces of the teeth removed from the incubator were covered with 0.2-0.3 mm thick polyvinyl siloxane (Coltene/Whaledent AG, Altstatten, Switzerland) to simulate the periodontal ligament [Rees, 2001]. Afterward, the teeth were buried in cylindrical acrylic blocks of 1.5 cm diameter and prepared by using PVC molds, leaving 2 mm between the enamel-cement border and the acrylic resin and with the long axis of the tooth being parallel to the external border of the acrylic block. The samples were placed in the Universal Test Device (Instron Corp., Canton, MA, USA), to measure the fracture resistance. The 4-mm diameter cutter tip was placed at an angle of 45° to the middle-third of the palatine surface of the tooth. One mm/min force was applied to the tooth until it was fractured. The peak scissoring forces at the time of fracture were recorded in Newton (N) units.

Data evaluation and statistical analysis

All groups were compared with each other regarding fracture resistance at two weeks, two months, and one year. Also, for each group, the samples kept for two weeks, two months, and one year were compared among themselves regarding fracture resistance.

The analysis of the data was done using the IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY, USA) package software. The conformity of the fracture resistance measurements with normal distribution was tested with the Kolmogorov-Smirnov test, and the homogeneity of the variances was tested using the Levene test. The descriptive statistics were expressed in medians (interquartile range). The significance of the difference between groups regarding the fracture resistance was tested using the Kruskal-Wallis test. When the results of the Kruskal-Wallis test indicated significance, Conover's multiple comparison test was used to determine the reason(s) behind the differences. Unless indicated otherwise, $p<0.05$ was considered as statistically significant for the results. However, for all possible multiple comparisons, a Bonferroni correction was applied to control Type I errors.

Results

The fracture resistance results for the teeth at two weeks, two months, and one year for the study and control groups [median (min-max)] are shown in Table 1. The statistical differences are indicated using parentheses.

When the materials were compared regarding fracture resistance at the end of the second week, statistically significant differences were found ($p<0.001$). Compared to the negative control group (intact teeth), the median fracture resistances were found to be significantly lower in the MM-MTA, NEO, MTA-PR, and $Ca(OH)_2$ groups ($p<0.001$, $p<0.001$, $p=0.003$, and $p<0.001$ respectively). No statistically significant differences were found between the negative control group (intact teeth) and the BD group. Also, it was determined that the fracture resistance was significantly higher in the BD group when compared to the $Ca(OH)_2$ group ($p=0.006$). No statistically significant differences were found among the other groups according to the Bonferroni correction ($p>0.0167$).

When the samples were compared regarding fracture resistance at the end of the second month, it was determined that the BD, MM-MTA, NEO, MTA-PR, and $Ca(OH)_2$ groups were significantly less resistant to fracture compared to the negative control group (intact teeth) ($p=0.005$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). In addition, it was determined that the median fracture resistance of the BD group was significantly higher compared to the MM-MTA and $Ca(OH)_2$ groups ($p=0.012$ and $p<0.001$), and that the median fracture resistance of the MTA-PR group was significantly higher compared to the $Ca(OH)_2$ group ($p=0.005$). No statistically significant differences were determined among the other groups according to the Bonferroni correction ($p>0.0167$).

When the samples were compared at the end of the first year, the median resistances to fracture of the BD, MM-MTA, NEO, MTA-PR, and $Ca(OH)_2$ groups were found to be significantly lower compared to the negative control group (intact teeth) ($p=0.002$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). Also, the median fracture resistances of the BD, MM-MTA, NEO, and MTA-PR groups were found to be significantly higher when compared to the median fracture resistance of $Ca(OH)_2$ ($p<0.001$). No statistically significant differences were detected among the other groups according to the Bonferroni correction ($p>0.0167$).

The fracture resistance results [median (min-max)] for the study and control groups at the two week, two month, and one year marks are shown in Table 2. The statistical differences are indicated using parentheses.

The statistical analysis revealed that, for all groups, the fracture resistance at one year was significantly lower compared to the two week and two month timepoints ($p<0.001$, $p<0.001$). There was no statistically significant difference between the teeth at the two week and the two month timepoints (BD:

	2 Weeks	2 Months	1 Year
BD	1027.3 (395.5-1512.8)	833.8 (613.4-1413.1)	508.4 (172.2-971.4)
MM-MTA	611.2 (412.7-1339.0)	667.7 (464.7-982.8)	321.8 (136.2-820.4)
NEO	813.7 (279.6-1092.5)	735.2 (424.7-1120.2)	454.2 (140.4-715.1)
MTA-PR	919.3 (385.9-1830.5)	816.0 (730.6-1350.9)	475.2 (182.5-892.6)
$Ca(OH)_2$ (PC)	498.7 (112.8-1198.4)	636.0 (405.0-920.2)	85.4 (40.2-137.7)
Intact (NC)	1532.7 (887.4-1862.2)	1537.4 (896.8-1865.0)	937.9 (760.4-1090.3)

TABLE 1 The fracture resistance values of the study groups at follow-up periods [median (min-max)] and the statistical comparison between groups (Newton/N).

	BD	MM-MTA	NEO	MTA-PR	$Ca(OH)_2$ (PC)	INTACT (NC)
2 Weeks	1027.3 (395.5-1512.8)	611.2 (412.7-1339.0)	813.7 (279.6-1092.5)	919.3 (385.9-1830.5)	498.7 (112.8-1198.4)	1532.7 (887.4-1862.2)
2 Months	833.8 (613.4-1413.1)	667.7 (464.7-982.8)	735.2 (424.7-1120.2)	816.0 (730.6-1350.9)	636.0 (405.0-920.2)	1537.4 (896.8-1865.0)
1 Year	508.4 (172.2-971.4)	321.8 (136.2-820.4)	454.2 (140.4-715.1)	475.2 (182.5-892.6)	85.4 (40.2-137.7)	937.9 (760.4-1090.3)

TABLE 2 Fracture resistance values of the groups and comparisons according to the follow-up period [median (min-max)] (Newton/N).

p=0.295, MM-MTA: p=0.940, NEO: p=0.764, MTA-PR: p=0.340, $Ca(OH)_2$: p=0.442, NC: p=0.679).

Discussion

It has been reported that the fracture incidence, either spontaneously or following a traumatic injury, is over 60% in the cervical part of immature teeth [Katebzadeh et al., 1998; Carvalho et al., 2005; Cvek, 1992]. For this reason, choosing the treatment method and the material during root-canal treatment is critical for increasing the resistance and improving the prognosis of the tooth [Stuart et al., 2006]. On the other hand, the material used for apexification can play a key role in the prognosis of the tooth as a result of its short- and long-term effects on fracture resistance.

Our study revealed that the teeth to which calcium hydroxide was applied were 67%, 58%, and 90% less resistant to fractures at two weeks, two months, and one year, respectively, when compared to the intact teeth. These findings are compatible with the results of the studies regarding the effect of calcium hydroxide on fracture resistance in various follow-up periods [Marending et al., 2009; Zarei et al., 2013; Kawamoto et al., 2008; Twati et al., 2009; Andreasen et al., 2006; Andreasen et al., 2002; Topcuoglu et al., 2015; Tuna et al., 2011; Elnaghy and Elsaka, 2016; White et al., 2002]. Andreasen et al. [2002] followed up the application of $Ca(OH)_2$ on root canals for two weeks; one, two, three, six, and nine months; and one year. They determined that $Ca(OH)_2$ does not have any significant effect in the first month. However, it starts to cause a serious decline of resistance in the root-canal after the second month, and the fracture resistance decreases to 50% after the first year. Similarly, Zarei et al. [2013] concluded that $Ca(OH)_2$ treatments longer than one month reduce fracture resistance. Twati et al. [2009] indicated that the elasticity modulus of dentin decreased after six months.

Our study determined that the fracture resistance of the ProRoot MTA Group was significantly lower than that of the group of intact teeth for all timelines in the study (Table 1 and 2). This result might be related to calcium release from MTA over time, similar to calcium hydroxide [Sáez et al., 2017] or the neutralisation of the acidic structure of dentin with the high alkalinity of MTA after hardening, which causes the dissolution of the bioactive matrix components of the mineralised dentin tissue [Tomson et al., 2007], resultantly causing protein denaturation in the tooth [Holland et al., 1999]. Another reason may be the dissolution of the mineralised dentin collagen [Andreasen et al., 2006] and the subsequent deterioration of the hydroxyapatite structure [White et al., 2002; Tronstad et al.; 1981, Nerwich et al., 1993]. When the literature is reviewed, many studies comparing the fracture resistances of $Ca(OH)_2$ and MTA concluded that, similarly to $Ca(OH)_2$, MTA reduces fracture resistance at various periods such as two weeks [Madarati et al., 2010], five weeks [White et al., 2002], 100 days [Andreasen et al., 2006] and one year [Tuna et al., 2011]. However, the number of studies that evaluate and compare the short- and long-term effects of MTA on the fracture resistance of teeth is limited. Hatibovic-Kofman et al. [2008] found in their study that ProRoot MTA reduced fracture resistance at two months, but resistance improved in the long run. On the other hand, White et al. [2002] determined that the fracture resistance of teeth with MTA treatment was 33% less resistant to fractures and that this result was significant. Another study that found supporting findings compared the fracture resistances of intact teeth and teeth with WMTA application; it was shown that intact teeth have a higher fracture resistance after one year [Elnaghy and Elsaka, 2016]. In our study, we found that, even though there was no significant difference between $Ca(OH)_2$ and ProRoot MTA in the second week, at the second month and one year timepoints, the MTA-PR Group had a significantly higher fracture resistance. Similarly, Tuna et al. [2011] reported that $Ca(OH)_2$ applications cause more root fragility compared to MTA in the long run. Çiçek et al. [2017] found that, after four weeks, the fracture resistance of the WMTA group was significantly higher than that of the $Ca(OH)_2$ Group. Another study compared samples with ProRoot MTA and $Ca(OH)_2$ for one year and found that the MTA group was 33.33% more resistant than the $Ca(OH)_2$ Group, and that this result was statistically significant [Hatibovic-Kofman et al., 2008]. Our study similarly indicated that the ProRoot MTA Group was 30% more resistant than the $Ca(OH)_2$ Group at the end of the one-year follow-up period. The probable reason for MTA causing a smaller reduction in fracture resistance than $Ca(OH)_2$ was that the calcium ions released by MTA were less when compared to calcium hydroxide. Indeed, Saez et al. [2017] published a supporting study in which they compared the amount of calcium released by both materials, and, at the end of the study, they found that calcium hydroxide released more calcium than MTA after 60 days, and that there is a positive correlation between the calcium concentration and pH. When the effect of high pH on collagen denaturation is considered [Andreasen et al., 2002; White et al., 2002], it can be concluded that the mentioned findings are a result of this phenomenon. Additionally, regarding the hardness values of MTA and calcium hydroxide [Torabinejad et al., 1995], we presume that the harder nature of MTA helps in supporting the tooth during force conduction, thus increasing the fracture resistance.

The number of studies evaluating to what extent the fracture resistance is affected when other MTA-derived biomaterials are used is very limited. Topçuoğlu et al. [2015] found that, after two weeks, the fracture resistance of teeth which had undergone Biodentine treatment was less when compared to intact teeth. Another study concluded that Biodentine does not have a significantly lower fracture resistance compared to WMTA after one year and that both materials significantly lead to the reduction of fracture resistance compared to intact teeth [Elnaghy and Elsaka, 2016].

Since there are no studies evaluating the short- and long-term

effects of $Ca(OH)_2$, ProRoot MTA, and Biodentine, and subsequently comparing the results with each other and with intact teeth, this has created some questions regarding the starting point of the effect on fracture resistance and how it changes.

Our study found that at two weeks there were no significant differences between the fracture resistance of Biodentine and of intact teeth, whereas at the two-month and one-year marks, the values were found to be significantly lower for Biodentine. The higher fracture resistance for Biodentine in the short-term might be related to the similarity of the flexion endurance of Biodentine with dentine [Dammaschke, 2012]. However, the calcium release over time, similar to MTA and $Ca(OH)_2$ [Natale et al., 2015], might have resulted in collagen denaturation and increased fragility. The literature indicates that Biodentine does not negatively affect the integrity of the collagen matrix in the short term (two weeks); however, it can cause dentin denaturation in the long run [Leiendecker et al., 2012]. In our study, for all periods, Biodentine showed a higher fracture resistance compared to ProRoot MTA; however, this difference was not found to be statistically significant. Another study found that the Biodentine group (38.29 MPa) had a higher fracture resistance than the MTA Angelus group (36.72 MPa). However, similar to our study, this result was not statistically significant [Elnaghy and Elsaka, 2016]. It was reported that, as a result of the difference between the particle size of ProRoot MTA (2.44–3.05 μm) and the diameter of dentine tubules (0.9–2.5 μm) [Komabayashi and Spångberg, 2008; Garberoglio and Brännström, 1976], the material cannot fully penetrate the dentine tubules [AM et al., 2014]; however, Biodentine manifested adhesion with the dentine tubules by tag-like structures [Atmeh et al., 2012]. This effect might have led to the higher measured fracture resistance in our study of teeth where Biodentine had been applied.

In our study, ProRoot MTA, NeoMTA Plus, and MM-MTA all having a significantly lower fracture resistance compared to intact teeth indicates that biomaterials reduce the fracture resistance of teeth in both the short and the long term. However, as these biomaterials are relatively new, there are no studies in the literature investigating their effects on fracture resistance. The teeth in the NeoMTA Plus and MM-MTA groups having a significantly lower fracture resistance compared to intact teeth might be the result of protein denaturation resulting from reduced pH, like the other biomaterials. On the other hand, our study indicated that the fracture resistance was higher for MM-MTA and NeoMTA when compared to $Ca(OH)_2$. This difference was significant at one year for MM-MTA and for all timepoints for NeoMTA. This result can be explained by a lower calcium release of these biomaterials in the long run when compared to $Ca(OH)_2$ [Siboni et al., 2017; Luczaj-Cepowicz et al., 2015; Khalil et al., 2015]. The fact that there were no significant differences between the MM-MTA, NeoMTA Groups and the ProRoot MTA group in all follow-up periods can be explained by their similar alkalinities. Indeed, it was reported that both materials are similar to ProRoot MTA regarding Ca release and pH [Khalil et al., 2015]. Considering that alkalinity is the principal factor in the reduction of fracture resistance, that these materials show similar effects is an expected result. Our study did not find any statistically significant difference between the NeoMTA Plus and Biodentine groups at any follow-up period. We suggest that, besides the pH similarities of the materials [Siboni et al., 2017], NeoMTA having small particles like Biodentine allowing the material to effectively penetrate the dentin tubules and exhibit high bonding strength [Türker et al., 2016], together with showing similar fracture resistance after application of sodium hypochlorite [Govindaraju et al., 2017] might be contributing to the result.

Conclusion

When the change in fracture resistance over time was evaluated for each material, the fracture resistance was statistically significantly higher at the two-week and two-month follow-up, compared to the one-year period. This suggests that filling the root canal completely with the biomaterials used in our study leads to denaturation of the tooth over time, showing similar effects to $Ca(OH)_2$. Further research should be conducted regarding this issue.

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