Original Study

IN VITRO COMPARISION OF TOOTH DISCOLORATION CAUSED BY *PRO-ROOT MTA*, *ORTHO MTA* AND *CEM CEMENT*

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ABSTRACT

Aim: Tooth discoloration is a major drawback of mineral trioxide aggregate (MTA). This study sought to compare tooth discoloration due to ProRoot MTA, Ortho MTA and calcium enriched mixture (CEM) cement.

Materials and Method: This in vitro study was conducted on 40 sound human premolars. The tooth crowns were cut 2mm below the cementoenamel junction. Pulp tissue was excavated and the teeth were immersed in sodium hypochlorite for 10 minutes and rinsed with saline. The tooth surfaces were covered with putty impression material except for the apical surface. A window was created in the putty on the buccal surface of the teeth corresponding to the size of the tip of the spectrophotometer. After baseline color measurement, the teeth were divided into four groups (n=10) of ProRoot MTA, Ortho MTA, CEM cement and control. The second color measurement was made after the application and setting of each material and the tooth end was sealed with light-cure glass ionomer cement. Subsequent measurements were carried out at two, four, eight, 12 and 16 weeks.

Result: Ortho MTA, CEM cement and ProRoot MTA caused clinically perceptible color change at 16 weeks ($\Delta E>3.3$). Color change in ProRoot MTA group was significantly greater than that in the control group ($P<0.05$). After ProRoot MTA, the greatest color change occurred in Ortho MTA, CEM cement and the control group, respectively.

Conclusion: Ortho MTA, CEM cement and ProRoot MTA all cause tooth discoloration. ProRoot MTA caused the greatest color change.

Key words: Dental Materials; Mineral Trioxide Aggregate; Tooth Discoloration

Introduction

Tooth discoloration caused by endodontic materials can compromise the esthetic results of treatment.[1,2] In general, pulpal bleeding, necrotic pulp tissue and endodontic materials are among the main causes of discoloration of traumatized and endodontically treated teeth.[2] Endodontic materials such as irrigating solutions, intracanal medicaments and sealers can cause discoloration due to their penetration into dentinal tubules.[2-5] Residual materials in the pulp chamber can also cause discoloration over time.[5] Tooth discoloration due to the presence of remnants is often seen in the cervical third of the crown because the enamel in this area is thinner than in other areas.[6]

Discoloration potential of many endodontic materials such as AH26, AH Plus, Sealapex, Endofill, Tubliseal and EndoREZ has been previously confirmed.[1,2,4,5,7] Gray MTA is a commonly used endodontic cement, which causes tooth discoloration. It appears that the radiopacifier in the formulation of gray MTA, which is bismuth oxide, is the main cause of discoloration by MTA. Naik and Hedge[8] in 2005 reported that 60% of pulpotomized primary molars with MTA showed discoloration after 24 hours.

White MTA was later introduced to overcome the discoloration caused by gray MTA; however, it also causes some degrees of discoloration. Watts *et al*,[9] in 2007 demonstrated that all white MTA samples showed discoloration after three days of exposure to phosphate buffered saline. In the clinical setting, MTA may be exposed to vital tissues containing blood vessels and it has been reported that presence of blood during setting of white MTA aggravates the discoloration.[4,10,11] ProRoot MTA is a formulation of white MTA with several advantages. However, it also possesses tooth discoloration potential as well as a long setting time,[12,13] difficult handling and high cost.[12,14] To overcome these shortcomings, the manufacturers attempted to produce calcium silicate-based cements. Jang *et al*,[15] in 2013 evaluated the discoloration following the use of white ProRoot MTA and showed increasing discoloration during 12 weeks at the dentin-cement interface. Valles *et al*,[16] in 2013 also indicated discoloration of white ProRoot MTA under light in an oxygen-free environment.

Ortho MTA was manufactured by the Bio MTA company in 2010 to overcome the limitations of other formulations of MTA. The manufacturer claims that this cementhas ashort setting time and causes minimal tooth discoloration due to elimination of Portland cement and heavy metals from its formulation and their replacement with nano materials.[17] Ortho MTA has a formulation close to that of ProRoot MTA with the difference that Ortho MTA does not contain calcium sulfate (gypsum). However, studies on tooth discoloration caused by Ortho MTA are scarce. Shokouhinejad *et al*,[18] in 2016 evaluated the discoloration of teeth caused by Ortho MTA. They found no significant difference in discoloration of ProRoot MTA and Ortho MTA in presence or absence of blood.

Calcium enriched mixture cement was recently introduced to the dental market, and has a different composition of calcium compounds while yielding results similar to MTA even when used as a pulp capping agent. Studies on tooth discoloration caused by CEM cement are limited. Arman *et*

al,[19] in 2015 compared the discoloration of white MTA and CEM cement and found no significant difference.

In vital pulp therapy, pulp capping agents are placed in the coronal part of the crown; thus, the discoloration potential of endodontic cements and pulp capping agents must be thoroughly studied. This study sought to assess and compare the discoloration of tooth crown following the application of ProRoot MTA, Ortho MTA and CEM cement for different periods of time.

Materials and Method

This in vitro, experimental study was conducted on 40 sound human premolars. The teeth were free from caries, restorations and cracks. The teeth were immersed in 5.25% sodium hypochlorite solution for one hour for disinfection and were then rinsed with saline. The teeth were stored in saline until the experiment. Sample size was calculated to be 10 samples in each group assuming five repetitions (since measurements were repeated during five weeks for each tooth), 0.50 correlation coefficient, 95% study power, error rate of 0.05 and variance of 40.45. The crowns were cut at 2mm below the cementoenamel junction using high speed handpiece under water spray.

Pulp tissue was excavated by a barbed broach, and organic residues on tooth surface were removed using a curette. The teeth were immersed in sodium hypochlorite solution for 10 minutes and were the rinsed with saline. All tooth surfaces except for the apical surface were covered with putty impression material. A window was created in the putty covering the buccal surface corresponding to the size of the tip of Vita Easy Shade spectrophotometer for color measurements. Baseline color measurement was then carried out. The teeth were then divided into four groups (n=10) of ProRoot MTA, Ortho MTA, CEM cement and control.

Preparation of ortho MTA: Distilled water was added to the vials containing Ortho MTA powder (Bio MTA, Seoul, Republic of Korea) according to the manufacturer's instructions in such a way that 1mL of water was visible on the powder. The vial was then placed in auto mixer of Ortho MTA and mixed for five seconds. After mixing, excess liquid was removed by a cotton pellet.

Preparation of CEM cement: The powder and liquid were poured on the mixing pad (Yektazist Dandan, Tehran, Iran). The liquid was gradually added to the powder and mixed by a spatula for 20 seconds.

Preparation of ProRoot MTA: ProRoot MTA powder and liquid (Dentsply, Tulsa Dental, Tulsa, OK, USA) were mixed on a glass slab in 3 to 1 ratio and gently mixed for one minute.

The pulp chamber was retro-filled with the respective materials according to the manufacturers' instructions by an endodontist. Adequate time was allocated for primary setting of the materials and then the cavity was sealed with light-cure glass ionomer cement (GC corporation, Tokyo, Japan). Second color measurement was done immediately after sealing. Subsequent measurements were performed after two, four, eight, 12 and 16 weeks. All color measurements were made at the same site. During the entire study period, the teeth were immersed in saline in separate containers. Saline was refreshed every two weeks.

Color assessments were done using Compact Easy Shade spectrophotometer (Vita Easy shade Compact; Vita Zahnfabrik, Bad sackingen, Germany).The L*, a* and b* parameters were measured for each tooth. Each measurement was repeated three times and the mean value for each parameter was calculated. Total color change (ΔE) was calculated using the formula below:

$$E=\{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}\Delta$$

Where L* indicates lightness with values ranging from zero (black) to 100 (white), a* indicates greenness-yellowness in green-yellow axis (negative values indicate greenness and positive values indicate redness) and b* indicates blueness-yellowness in blue-yellow axis (negative values indicate blueness and positive values indicate yellowness).

The data were analyzed using SPSS version 20 (SPSS Inc., IL, USA). Repeated measures ANOVA was used to assess the effect of type of material on ΔE and ΔL. Tukey's test was used for pairwise comparisons. Sidak test was applied to compare the time points.

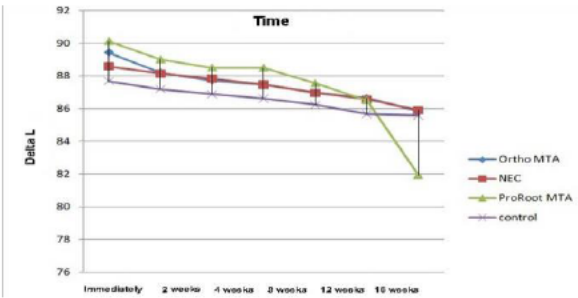
Results

Changes in L parameter:* The L* value decreased in all groups after the intervention compared to the baseline values. The maximum change in L* parameter occurred after 16 weeks in ProRoot MTA group, which was significantly greater than that in other groups (P=0.002). Comparison of the mean change in L* value in the four groups at different time points is presented in Table 1 (Figure 1).

Group	Ortho MTA	CEM cement	ProRoot MTA	Control	*p value
After setting	A -1.22 (±1.05)	-0.53(±0.72)	-1.12(±1.16)	A -0.38(±0.30)	0.15
2 weeks	A -1.72 (±0.98)	-0.91(±0.88)	-1.71(±1.16)	AB -0.70(±0.35)	0.021
4 weeks	-1.98 (±1.02)	-1.28(±0.96)	-2.08(±1.17)	B -1.00(±0.45)	0.041
8 weeks	-2.46 (±1.00)	A -1.80 (±1.00)	-2.62(±1.14)	-1.36(±0.41)	0.012
12 weeks	-2.78 (±1.01)	AB -2.22(±1.14)	-3.61(±1.36)	-1.90(±0.55)	0.005
16 weeks	-3.61 (±1.06)	B -2.86 (±1.10)	-8.19(±3.84)	-2.54(±0.48)	0.002
p value	0.001	0.001	0.001	0.001	-

* Comparison of the groups was done with one-way ANOVA Letters indicate groups without significant differences at differen time points using Turkey's test Baseline time point was before the application of MTA/cement

Table 1: Comparison of the mean (± SD) change in L parameter in the four groups at different time points.*



Graph 1: Chnages in L parameter over time

Comparison of changes in L* parameter over time revealed that the maximum changes in L* occurred in Ortho MTA group in the first and second time points. In all other time points, the greatest change in L* parameter occurred in ProRoot MTA group. Pairwise comparisons by Tukey's test showed significant differences in this regard between the first and third (P=0.021), first and fourth (P=0.041), first and fifth (P=0.012), first and sixth (P=0.005) and first and seventh (P=0.002) time points. Minimum change of L* at all time points occurred in the control group. All other pairwise comparisons revealed non-significant differences. [Table 2]

Time Point	First Material	Second Material	Mean Difference	Standard Error	p *
After Setting	ProRoot MTA	Ortho MTA	0.10	0.39	0.99
	ProRoot MTA	Control	-0.74	0.39	0.25
	ProRoot MTA	NEC	-0.59	0.39	0.44
	Ortho MTA	Control	-0.84	0.39	0.15
	Ortho MTA	NEC	-0.69	0.39	0.30
	Control	NEC	0.15	0.39	0.98
2 Weeks	ProRoot MTA	Ortho MTA	0.01	5.21	1.00
	ProRoot MTA	Control	-1.01	5.21	0.12
	ProRoot MTA	NEC	-0.80	5.21	0.16
	Ortho MTA	Control	-1.02	5.21	0.16
	Ortho MTA	NEC	-0.80	5.21	0.22
	Control	NEC	0.21	5.21	1.00
4 Weeks	ProRoot MTA	Ortho MTA	-0.1	5.21	1.00
	ProRoot MTA	Control	-1.08	5.21	0.11
	ProRoot MTA	NEC	-0.8	5.21	0.38
	Ortho MTA	Control	-0.98	5.21	0.19
	Ortho MTA	NEC	-0.7	5.21	0.60
	Control	NEC	0.28	5.21	1.00
8 Weeks	ProRoot MTA	Ortho MTA	-0.16	5.22	1.00
	ProRoot MTA	Control	-1.26	5.22	0.032
	ProRoot MTA	NEC	-0.82	5.22	0.35
	Ortho MTA	Control	-1.1	5.22	0.057
	Ortho MTA	NEC	-0.66	5.22	0.54
	Control	NEC	0.44	5.22	1.00
12 Weeks	ProRoot MTA	Ortho MTA	-0.83	0.47	0.31
	ProRoot MTA	Control	-1.71	0.47	0.005
	ProRoot MTA	NEC	-1.39	0.47	0.029
	Ortho MTA	Control	-0.88	0.47	0.26
	Ortho MTA	NEC	-0.56	0.47	0.64
	Control	NEC	0.32	0.47	0.90
16 Weeks	ProRoot MTA	Ortho MTA	-4.58	5.22	1.00
	ProRoot MTA	Control	-5.65	5.22	0.004
	ProRoot MTA	NEC	-5.33	5.22	0.021
	Ortho MTA	Control	-1.07	5.22	0.23
	Ortho MTA	NEC	-0.75	5.22	0.74
	Control	NEC	0.32	5.22	1.00

* Turkey's test p < 0 05 was considered statistically significant

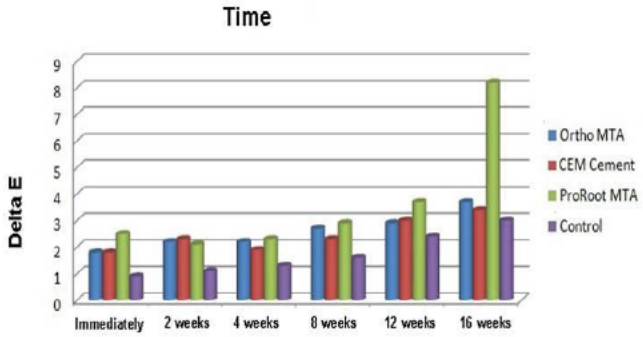
Table 2: Pairwise comparison of the changes in L parameter in the four groups at different time points*

Changes in ΔE: The maximum color change (ΔE) at 16 weeks occurred in ProRoot MTA group. The minimum color change after 16 weeks was noted in the control group followed by CEM cement group. The mean (± standard deviation) change in ΔE in the four groups at different time points is presented in Table 3 and Figure 2.

Group	Ortho MTA	NEC	ProRoot MTA	Control	*p value
After setting	1.86(±0.97)	A* 2.27(±0.82)	2.14(±1.05)	0.9040(±0.46)	0.002
2 weeks	2.25(±0.85)	AB 2.30(±1.00)	2.29(±1.04)	1.14(±0.34)	0.006
4 weeks	A 2.29(±0.93)	2.31(±0.91)	2.36(±1.12)	!.37(±0.47)	0.113
8 weeks	A 2.71(±0.95)	2.45(±0.97)	2.87(±1.19)	1.69(±0.48)	0.022
12 weeks	B 2.94(±1.04)	3.05(±1.04)	3.73(±1.37)	2.49(±0.38)	0.066
16 weeks	3.77(±1.12)	3.40(±0.87)	8.26(±3.78)	3.03(±0.72)	0.015
p value	0.001	0.001	0.001	0.001	-

* Comparison of the groups was done with one-way ANOVA Letters indicate groups without significant differences at different time points using Tukey's test Baseline time point was before the application of MTA/cement

Table 3: The mean (± standard deviation) change in ΔE in the four groups at different time points



Graph 2: Changes in E parameter over time

Comparison of ΔE values of the groups over time revealed the following results: Comparison of the first and second time points showed that maximum ΔE belonged to CEM cement group (P=0.002). Comparison of the first and third time points showed minimum ΔE in the control group and other groups had equal ΔE values (P=0.006). Comparison of the first and fourth (P=0.11), first and fifth (P=0.022), first and sixth (P=0.066) and first and seventh (P=0.015) time points showed maximum ΔE in ProRoot MTA group. [Table 4]

Discussion

Unesthetic appearance of teeth is a common concern for patients. Tooth color may change by use of dental materials.[20] Mineral trioxide aggregate is a commonly used endodontic cement, which causes tooth discoloration.[21] To overcome the shortcomings of gray MTA, Ortho MTA was introduced to the market by Bio MTA company in 2010. The manufacturer claims that this cementh as a short setting time and causes minimum discoloration of tooth.[17] On the other hand, CEM cement was recently introduced to dental market with applications similar to those of MTA. It has easier handling than MTA and can form hydroxyl apatite.[22] Moreover, it has shown superior results in pulp capping compared to MTA.[23] Since limited studies are available on tooth discoloration following the use of Ortho MTA and CEM cement, this study assessed tooth

discoloration caused by Ortho MTA and CEM cement in comparison with ProRoot MTA.

Time Point	First Material	Second Material	Mean Difference	Standard Error	p *
After Setting	ProRoot MTA	Ortho MTA	0.28	0.38	0.63
	ProRoot MTA	Control	1.24	0.38	0.037
	ProRoot MTA	NEC	-0.13	0.38	0.096
	Ortho MTA	Control	0.96	0.38	0.15
	Ortho MTA	NEC	-0.41	0.38	0.49
	Control	NEC	-1.37	0.38	0.02
2 Weeks	ProRoot MTA	Ortho MTA	0.04	5.27	1.00
	ProRoot MTA	Control	1.15	5.27	0.009
	ProRoot MTA	NEC	-0.01	5.27	1.00
	Ortho MTA	Control	1.11	5.27	0.034
	Ortho MTA	NEC	-0.05	5.27	1.00
	Control	NEC	-1.16	5.27	0.024
4 Weeks	ProRoot MTA	Ortho MTA	0.007	0.39	0.99
	ProRoot MTA	Control	0.98	0.39	0.08
	ProRoot MTA	NEC	0.37	0.39	0.79
	Ortho MTA	Control	0.91	0.39	0.11
	Ortho MTA	NEC	0.29	0.39	0.87
	Control	NEC	-0.61	0.39	0.42
8 Weeks	ProRoot MTA	Ortho MTA	0.16	5.22	1.00
	ProRoot MTA	Control	1.18	5.22	0.37
	ProRoot MTA	NEC	0.42	5.22	1.00
	Ortho MTA	Control	1.02	5.22	0.05
	Ortho MTA	NEC	0.26	5.22	1.00
	Control	NEC	-0.76	5.22	0.74
12 Weeks	ProRoot MTA	Ortho MTA	0.78	0.45	0.32
	ProRoot MTA	Control	1.24	0.45	0.047
	ProRoot MTA	NEC	0.68	0.45	0.45
	Ortho MTA	Control	0.45	0.45	0.75
	Ortho MTA	NEC	-0.10	0.45	0.99
	Control	NEC	-0.56	0.45	0.61
16 Weeks	ProRoot MTA	Ortho MTA	4.49	5.22	0.57
	ProRoot MTA	Control	5.23	5.22	0.014
	ProRoot MTA	NEC	4.86	5.22	0.088
	Ortho MTA	Control	0.74	5.22	0.99
	Ortho MTA	NEC	0.37	5.22	1.00
	Control	NEC	-0.37	5.22	1.00

* Tukey's test P<0 05 was considered statistically significant

Table 4: Pairwise comparison of ΔE in the four groups at different time points

In the current study, similar to many previous studies[10,21,24] extracted sound human teeth were used in order to better simulate the clinical conditions and compare the discoloration potential of teeth more accurately. Some researchers used composite resin to seal the root end.[18,21] However, in the current study, glass ionomer cement was used since glass ionomer can better seal dentinal tubules due to the bond of carboxylic ions to calcium ions of hydroxyl apatite and chemical bond to tooth structure.[25]

Several devices such asspectrophotometer[4,15,16,21,26-28] andcolorimeter[24] have been used for color measurement. Digital photographs taken with a high resolution camera and color analysis by Photoshop software can also be used for this purpose.[10,27] In the current study, a spectrophotometer was used. Spectrophotometer and spectroradiometer measure the light reflection index in the visible spectrum. Dozic *et al*,[29] in 2007 concluded that Easy Shade spectrophotometer and digital camera are the most reliable tools for color assessment. The CIE L*a*b* system was used in the current study to determine color change, which has been approved by the American Dental Association and is the most accurate and commonly used system for this purpose so far.[30] It quantifies the change in color parameters. Among the L*, a* and b* color parameters, ΔL* is more important and human eye better perceives the change in this parameter because the quality of rods, which are responsible for detection of black and white colors is higher than that of cones, responsible for colored vision.[31] Color change is clinically perceptible in values > 3.3.[27]

The results of our study showed that ProRoot MTA caused the greatest change in L* parameter. Ioannidis *et al*,[20] in 2013 assessed the discoloration of molar crowns following the application of white and gray MTA and noticed that both types of MTA cements decreased the L parameter. The greatest ΔE in our study was also noted in ProRoot MTA group, which was significantly higher than that of the control group. After ProRoot MTA, the greatest ΔE was noted in Ortho MTA group, which was not significantly different from that of the control group. In the CEM cement group, color change was clinically perceivable as well (ΔE > 3.3), but it was less than that in the remaining two groups. Although to the best of authors' knowledge, no previous study has compared the same combination of endodontic cements in terms of their discoloration potential, our findings were in line with the results of previous studies comparing ProRoot MTA with other cements.[4,10,19,32] Felman and Parashos[10] assessed tooth discoloration following exposure to white ProRoot MTA and demonstrated grayish discoloration in all samples. Although they used standardized digital photographs for assessment of tooth discoloration, their findings were in line with ours.

Valles *et al*,[16] in 2013 indicated that the discoloration caused by white ProRoot MTA was significantly greater than that of Portland cement and Biodentine. They concluded that a combination of light and anaerobic conditions (similar to the clinical setting) can aggravate the discoloration potential of calcium silicate-based cements. Camilleri[27] in 2014 evaluated the color stability of white ProRoot MTA and Portland cement exposed to different endodontic irrigating solutions and showed brownish discoloration following immersion of white MTA in

sodium hypochlorite while no such discoloration occurred in Portland cement samples. They concluded that bismuth oxide present in the composition of white ProRoot MTA is probably responsible for greater discoloration of this cement. The main reason for tooth discoloration due to the application of ProRoot MTA has yet to be fully elucidated. This discoloration may be related to metal ions in the composition of MTA such as aluminum, iron, magnesium and bismuth oxide. Another suggested theory for tooth discoloration is the interaction between bismuth oxide and dentin collagen.[6] Lower discoloration caused by Ortho MTA compared to ProRoot MTA may be attributed to the lower metal content of the former. The difference in metal content of the two formulations of MTA has been evaluated by Kum *et al*,[33] who showed that all metal ions had a lower concentration in Ortho MTA compared to ProRoot MTA except for zinc, which was higher in the composition of Ortho MTA. Discoloration due to the application of CEM cement may be due to its chemical formulation containing calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide and calcium chloride; however, the exact mechanism of this discoloration has yet to be fully understood.

Shokouhinejad *et al*,[18] in 2015 reported clinically perceivable ($\Delta E > 3.3$) color change in both ProRoot MTA and Ortho MTA groups after six months. Color change in Ortho MTA group was greater than that in ProRoot MTA but this difference did not reach statistical significance. The difference in the results of studies in this regard may be due to the type of device used for color measurement since Shokouhinejad *et al*.[18] used a spectroradiometer while we used a spectrophotometer. Moreover, they evaluated the discoloration potential of materials in presence and absence of blood. Dozic *et al*,[29] in 2007 concluded that Easy Shade spectrophotometer and digital camera are the most reliable methods for color measurement. Several methods have been suggested to prevent or minimize tooth discoloration caused by MTA. For instance, it has been reported that application of dentin bonding agents prior to filling the pulp chamber can prevent penetration of MTA components into dentinal tubules and lower the risk of discoloration.[24] In another study, internal bleaching was tested to correct the discoloration caused by MTA. Removal of discolored MTA was effective but internal bleaching was unsuccessful as long as the MTA was in place.[26]

Adequate sample size and selection of extracted human teeth for the experiment were among the strengths of this study. However, this study had an in vitro design. Thus, generalization of the results to the clinical setting must be done with caution since in vivo setting cannot be perfectly simulated in vitro. Future clinical studies are required to better elucidate the discoloration potential of ProRoot MTA and CEM cement in the oral environment.

Conclusion

Within the limitations of this in vitro study, the results showed that Ortho MTA, CEM cement and ProRoot MTA caused clinically perceptible discoloration after 16 weeks. The greatest change in L parameter occurred in ProRoot MTA group at 16 weeks. Total color change was also the greatest in ProRoot MTA followed by Ortho MTA and CEM cement groups, respectively.

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