RESEARCH ARTICLE

A Scanning Electron Microscopic Study on Effect of Blood and Artificial Salivary Contamination on Marginal Adaptation of Mineral Trioxide Aggregate, When Used as a Retrograde Filling Material: An *In Vitro* Study

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Abstract

Aim and objective: The present study was conducted to evaluate the marginal adaptability of mineral trioxide aggregate (MTA) as a root-end filling material when manipulated using two different IV fluids intended for pediatric usage; in the presence of blood and salivary contamination.

Materials and methods: Sixty single-rooted teeth were selected. Conventional endodontic root canal preparation was performed on all specimens followed by root-end resection and retrograde cavity preparation. The roots were randomly divided into two groups ($n = 30$). In the specimens of group I, fresh blood was used as a contaminant and in group II artificial saliva was used as a contaminant. In both groups, MTA (e-MTA, Kids-e-Dental®) manipulated using either Ringer's lactate IV fluid ($n = 15$) or Tetraspan IV fluid ($n = 15$) was used for root-end filling in blood or artificial saliva-coated retrocavities. Furthermore, these roots were placed in beakers pooled with fresh phlebotomized blood or artificial saliva. After incubating for 48 hours, the roots were divided longitudinally to expose the retrofilled cavities and were then sputter-coated with gold-platinum dust. To assess the marginal adaptation of MTA to radicular dentin "maximum gap width" and "gap perimeter" were measured in images obtained from scanning electron microscopy of root specimens. SPSS 21 was employed for statistical analysis at ($p < 0.05$). Mann–Whitney *U* test and ANOVA were used for analyzing the data obtained.

Results: The gap width was more among samples exposed to blood ($p < 0.05$) than artificial saliva. No significant difference was reported in the gap perimeter when cavities were filled with MTA mixed with either IV fluids ($p > 0.05$).

Conclusion: Exposure to blood during setting had a negative effect on gap width when retrocavities were filled with MTA using Tetraspan. No effect was seen on the arch perimeter in retrocavities filled with MTA mixed with Ringer's lactate or Tetraspan.

Clinical significance: For avoiding failure, it is critical to select a biocompatible root-end filling material with high sealing ability. Hence, by doing the same, the clinical situation can be simulated.

Keywords: Biocompatibility, Intravenous fluids, Mineral trioxide aggregate, Retrograde filling.

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Introduction

Mineral trioxide aggregate (MTA), introduced by Mahmoud Torabinejad in 1993 is a biocompatible class II medical device for root canal filling as recommended by the US-FDA. It was initially available as a gray variety and later a tooth-colored version commonly referred to as "white MTA" was available from 2002 onward.[1–3]

Over the years, research on MTA has made its application possible in various clinical situations like pulp therapy, apexification, bleaching of discolored teeth, repairing vertical root fractures, root perforations, and resorptive defects; orthograde root canal filling in addition to its use as a suitable root-end filling material.[4,5]

Among a glut of retrograde filling materials like GIC, amalgam, composite resin, and others, MTA has shown the most promising results owing to its biocompatibility, ability to form a mineralized tissue barrier, less apical leakage, enhanced marginal adaptation, and the unique possibility of being used in a humid environment.[1,5,6]

Another less than ideal property of MTA is its handling characteristics. According to the manufacturer's recommendations, when MTA is manipulated using sterile water, it produces a grainy, sandy mixture making its compaction and delivery to the required site cumbersome. To overcome this limitation, common reagents like chlorhexidine gluconate, local anesthesia solution, calcium

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chloride, etc., have been proposed as additives for MTA.[7] In a clinical setting, saline is often used to manipulate MTA when sterile water is not available or exhausted. Similarly, Ringer's lactate and Tetraspan are commonly available intravenous fluids in a pediatric health care setup. To our knowledge, both Ringer's lactate and Tetraspan IV fluids have never been used to reinforce MTA. Hence, these two IV fluids were tested in the present study to manipulate MTA.

In most clinical situations while performing a root end surgery, MTA comes directly in contact or may be mixed with blood, saliva, or tissue fluids leading to biologic contamination.[8] Thus, the present study aimed to check the effect of blood and artificial salivary contamination on marginal adaptability of MTA as a retrograde filling material, when MTA was manipulated using Ringer's lactate and Tetraspan IV fluids.

Materials and Methods

The present study was carried out in the Department of Pedodontics and Preventive Dentistry, Darshan Dental College and Hospital, Udaipur in accordance with Analytical Chemistry Department at Bhabha Atomic Research Centre, Mumbai after obtaining institutional ethical clearance.

Sixty fully developed, freshly extracted, caries-free human teeth were selected. Periodontally compromised, single-rooted intact teeth without fracture or craze lines and fully developed apices were included, whereas teeth having cervical caries or developmental anomalies, immature or fractured teeth, and deciduous teeth were excluded from this study.

In our study, the technique adopted from Milani et al.[7] was followed. After clearing teeth of extrinsic stains, debris, blood, or saliva, they were stored in 5% sodium hypochlorite for a week and then in saline until use. Decoronation was done up to the CEJ and the working length of roots was determined 0.5 mm short of the total root length or when a 2% stainless steel #15 K-file was visible at the apical foramen of the root. Chemico-mechanical preparation of the roots was performed up to #40 and step-back preparation was done up to #70 K-file with copious 5% NaOCl and saline irrigation. The root canals were dried, coated with a sealer, and obturated using gutta-percha points.

Then, apical 3 mm root resection was performed using a diamond disk followed by apical 3 mm retrograde cavity preparation with conventional round burs. The roots were coated with two layers of acid-resistant nail varnish except for the resected root-end surface. The roots were then divided into group I and group II where MTA (e-MTA, Kids-e-Dental) was exposed to fresh phlebotomized blood obtained from the first author, and artificial salivary contamination, respectively; and further subdivided as group IA (MTA + Ringer's lactate), group IB (MTA + Tetraspan), group IIA (MTA + Ringer's lactate), and group IIB (MTA + Tetraspan) where MTA was manipulated using either Ringer's lactate or Tetraspan IV fluids. These roots were placed in a glass beaker containing fresh blood or artificial saliva depending on the groups to which they belonged. The roots were then incubated at 37°C and 100% relative humidity for 48 hours. Later, roots were resected longitudinally, parallel to their long axes, and transported in air-tight containers for SEM analysis.

Root specimens were placed on an aluminum stub and sputtered with gold-platinum dust under ion-beam sputtering (*Quorum Technologies®*, *Kent*) and further subjected to an electron projection chamber for scanning electron microscopy (*Vega3 TESCAN®*, *Czech Republic*).

To assess the marginal adaptation of MTA to dentine, the following variables were measured by a blind observer:

Maximum Gap Width

This was the maximum distance between MTA and cavity walls measured directly at MTA–dentine interface at magnification (×400 to ×2200 μm).

Gap Perimeter Analysis

"*Gap Perimeter*" was measured as the ratio of the gap perimeter to the perimeter of retrocavity margin at magnification (×100 to ×600 μm) and was rated on a score criterion as follows:
Score 1: Less than or equal to ¼ of the cavity margin.
Score 2: More than ¼ but less than or equal to ½ of the cavity margin.
Score 3: More than ½ but less than or equal to ¾ of cavity margin.
Score 4: More than ¾ of the cavity margin.

Results

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Descriptive statistics such as mean, median, and standard deviation for continuous variables and frequency along with percentages of categorical variables were calculated. Mann–Whitney *U*-test checked the maximum gap width between MTA–blood and MTA–artificial saliva interface and ANOVA was used for comparing two groups. A Chi-square test was employed to test the gap perimeter between the two groups. A level of $p < 0.05$ was considered statistically significant.

Maximum gap width and gap perimeter were found to be significantly higher in blood-contaminated specimens (Figs 1 and 2) when compared with artificial saliva-contaminated specimens (Figs 3 and 4).

In Table 1, when intragroup comparison for maximum gap width was made, retrocavities filled with MTA–Tetraspan IV fluid showed a significantly higher gap width than MTA–Ringer's lactate IV fluid. In Table 2, when intragroup comparison for gap perimeter was made, both groups had a similar gap perimeter score with a non-significant statistical difference.

However, in Tables 3 and 4, when intergroup comparison was made for maximum gap width; retrocavities filled with MTA–

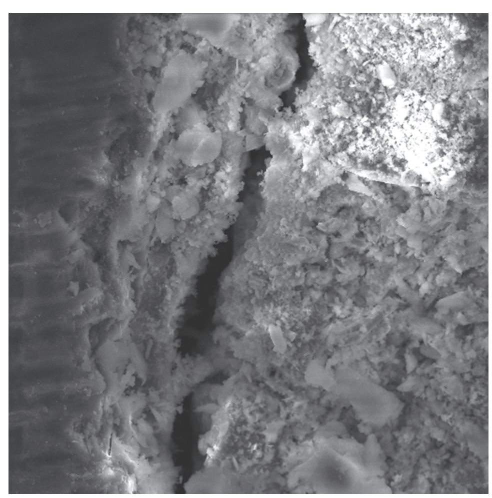

Fig. 1: SEM image shows the marginal gap at the MTA–dentine interface when MTA was manipulated using Ringer's lactate IV fluid in presence of blood contamination

Tetraspan IV fluid in the presence of blood contamination showed the highest gap width when compared with all other groups.

Discussion

The primary aim of conventional root canal treatment is the elimination and future exclusion of all microorganisms from the root canal. However, if conventional root canal treatment is impossible or has failed an alternative approach like periapical surgery is necessary which entails apicectomy and retrograde filling.[9] The first report of such a procedure appears to be made by a French-man Desirabode in 1843.[10,11]

The endodontic periapical surgery embraces exposure, resection of root apex, preparation of root end cavity, and insertion of root-end filling material.[12,13] Coupled with magnification through surgical operating microscopes, refined principles of soft and hard tissue management, use of tissue regenerative root-end filling materials, enhanced wound closure, and postoperative management; surgical endodontics has emerged as a highly predictable and relatively painless procedure.[14]

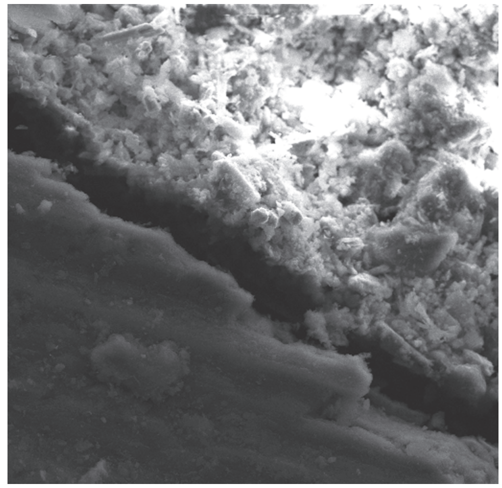

Fig. 2: SEM image shows the marginal gap at the MTA–dentine interface when MTA was manipulated using Tetraspan IV fluid in presence of blood contamination

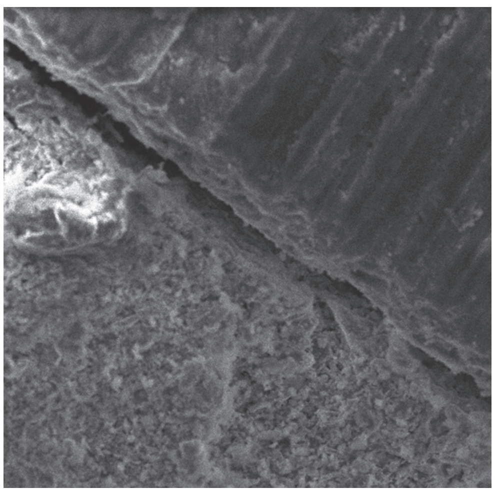

Fig. 3: SEM image shows the marginal gap at the MTA–dentine interface when MTA was manipulated using Ringer's lactate IV fluid in presence of artificial salivary contamination

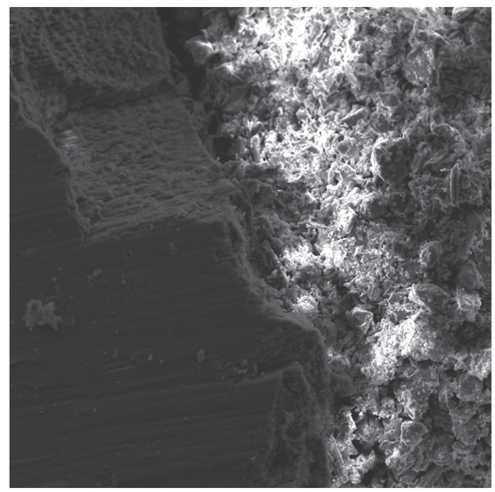

Fig. 4: SEM image shows the marginal gap at the MTA–dentine interface when MTA was manipulated using Tetraspan IV fluid in the presence of artificial salivary contamination

Table 1: Intragroup comparison of maximum gap width when cavities were filled with MTA mixed using Ringer's lactate and Tetraspan intravenous fluids

			Maximum gap width				
Groups		*N*	*Mean*	*Standard deviation*	*Median (IQR)*	*Standard error mean*	*p value*
Group IA Ringer's lactate IV fluid	Group I Exposed to blood contamination	15	2.34	1.48	2.88 (3.98)	0.38	0.461
Group IIA Ringer's lactate IV fluid	Group II Exposed to artificial saliva contamination	15	2.01	0.82	1.89 (2.77)	0.21	
Group IB Tetraspan IV fluid	Group I Exposed to blood contamination	15	4.00	1.60	3.99 (5.10)	0.41	0.016*
Group IIB Tetraspan IV fluid	Group II Exposed to artificial saliva contamination	15	2.53	1.08	2.65 (3.69)	0.27	

*Statistically significant

Table 2: Intragroup comparison of gap perimeter scores when cavities were retrofilled with MTA mixed with Ringer's lactate and Tetraspan intravenous fluids

Gap perimeter scores							
Groups		*N*	*Mean*	*Standard deviation*	*Median (IQR)*	*Standard error mean*	*p value*
Group IA Ringer's lactate IV fluid	Group I Exposed to blood contamination	15	1.66	0.81	1.00 (2.00)	0.21	0.808
Group IIA Ringer's lactate IV fluid	Group II Exposed to artificial saliva contamination	15	1.73	0.79	2.00 (2.00)	0.20	
Group IB Tetraspan IV fluid	Group I Exposed to blood	15	2.40	0.82	2.00 (3.00)	0.21	0.412
Group IIB Tetraspan IV fluid	Group II Exposed to artificial saliva	15	2.13	0.91	2.00 (3.00)	0.23	

Table 3: Intergroup comparison of maximum gap width in groups I and II

Maximum gap width				
Groups		*N*	*Mean*	*Standard deviation*
Group IA, IIA Ringer's lactate IV fluid	Group I Exposed to blood contamination	15	2.34	1.48
	Group II Exposed to artificial saliva contamination	15	2.01	0.82
Group IB, IIB Tetraspan IV fluid	Group I Exposed to blood contamination	15	4.00	1.60
	Group II Exposed to artificial saliva contamination	15	2.53	1.08
Total		60	2.72	1.46
p value			0.006*	
Post hoc pairwise comparison (IIA> IIB> IA> IB)			3 > 4 > 1 > 2	

*Statistically significant

Table 4: Intergroup comparison of gap perimeter scores in groups I and II

Gap perimeter scores				
Groups		*N*	*Mean*	*Standard deviation*
Group IA, IIA Ringer's lactate IV fluid	Group I Exposed to blood contamination	15	1.66	0.8165
	Group II Exposed to artificial saliva contamination	15	1.73	0.7988
Group IB, IIB Tetraspan IV fluid	Group I Exposed to blood contamination	15	2.40	0.8281
	Group II Exposed to artificial saliva contamination	15	2.13	0.9155
Total		60	2.72	0.8732
p value 0.072 NS				

In our study, root-end resection was performed using a diamond disk perpendicular to the long axes of the tooth followed by retrograde cavity preparation using a round bur in a high-speed, water-cooled airotor handpiece until a depth of 3 mm was achieved, in accordance with Gilheany et al.[15] and Bates et al.[16] who suggested that depth of retrograde filling and angle of resection are two important factors in direct control of the operator. Increased depth of retrograde preparation reduces apical microleakage and can be related to the fact that placement of a root-end filling material in a deeper cavity occludes many open apical dentinal tubules that are available for the passage of apical fluid. According to Gagliani et al.[17] and Malhotra and Hegde,[18] teeth should be apicectomized at 90° to their long axes and at a depth of 3 mm to exclude the presence of variations in root anatomy, lateral canals, and apical ramifications.

Until now many authors including Torabinejad et al.,[19] Bates et al.,[16] and Shipper et al.[8] have performed various *in vitro* and *in vivo* studies to conclude the use of MTA as a superior root-end filling material as compared to others. Hence, we chose MTA as the retrograde filling material. MTA was originally manufactured as Gray MTA but in 2002, a white-cream variation of the original formula of gray MTA was introduced as "white MTA".[20]

Mineral trioxide aggregate contains fine hydrophilic particles primarily of 75 wt% Portland cement, 20 wt% bismuth oxide, and 5 wt% calcium sulfate dehydrate or gypsum.[21] Belio-Reyes et al.[22] characterized crystalline minerals in MTA to be Bismite (19.8%), Haturite (51.9%), Larnite (23.2%), tricalcium aluminate (3.8%), and anhydrite (1.3%); and other trace elements like iron, nickel, copper, and strontium. Mineral trioxide aggregate is supplied as a powder-liquid package, which consists of MTA powder packed in individual pouches and sterile water packed in ampoules. Mineral trioxide aggregate should be prepared immediately before use. The powder should be stored in tight lid containers to prevent hydration.[20]

To date, many fluids like saline, local anesthetic solution, chlorhexidine, and sodium hypochlorite have been advocated to manipulate MTA for improving its working properties. The sterile water supplied with MTA often gets exhausted before the powder. Hence, in clinical settings, convenient and readily available fluids like saline, calcium chloride, sodium hypochlorite in liquid and gel form, KY jelly, and sterile local anesthetic solution are often mixed with MTA powder when sterile water is not available.[23,24]

In our study, Ringer's lactate and Tetraspan intravenous fluids have been used to manipulate MTA. The reason being that, in a Pediatric Healthcare setup, Ringer's lactate, and Tetraspan are safe, easily available, and the IV fluids of choice for fluid resuscitation in children and young adults since they belong to the categories of crystalloids and colloids.[25,26]

We checked marginal adaptation of MTA to radicular dentine in presence of blood and artificial salivary contamination. Blood and artificial salivary contamination were simulated because while performing periradicular surgery, root-end filling materials come directly in contact with these contaminants in virtual, specific clinical conditions.[22] Blood and artificial saliva were not wiped off the retrocavity walls to simulate worst clinical situations when MTA may get directly mixed or wiped off by these contaminants due to hemorrhage. The presence of such contaminants hampers the sealing ability of root-end filling materials.

The quality of apical seal obtained by retrograde filling materials has been assessed earlier using various methodologies.[27–30] However, all these techniques require the destruction of study samples to obtain measurements of leakage, thereby precluding measurements of changes in the apical seal over time in individual samples and indicate non-quantitative leakage. To overcome this disadvantage, Orucoglu et al.[28] introduced a computerized fluid filtration method to assess apical leakage.

But over the years, SEM images have played an important role in evaluating marginal adaptation of various retrograde filling materials. While viewing biological specimens under SEM, they need to be coated with a noble metal to reduce their conductivity and form high-resolution images. Clay et al.[31] stated that ion beam sputtering, deposits metals onto specimens and eliminates surface artifacts seen at high magnifications. Also, ion beam sputtering does not expose the specimen to a hot source, thus preventing its destruction due to heat.[32]

In the present study, images obtained from SEM assessed marginal adaptation of MTA to radicular dentine. Our idea of using SEM correlated well with Torabinejad et al.[12] who also used SEM to investigate the marginal adaptation of root-end filling materials and concluded that MTA and amalgam had fewer gaps than Super-EBA and IRM.

In our study, we used "maximum gap width" and "gap perimeter" as variables to assess the marginal adaptation of set MTA to radicular dentine. "Maximum gap width" was the maximum distance between MTA and retrocavity walls and "Gap Perimeter" was the ratio of the gap perimeter of MTA to the perimeter of the retrocavity margin calculated as scores designated between 1 and 4. This was similar to the scoring method used by Milani et al.[8] who evaluated the effect of exposure of blood on marginal adaptation of MTA.

When the "gap perimeter" was analyzed both, MTA manipulated using Ringer's lactate and Tetraspan IV fluid adapted equally well to root dentine in the presence of blood and artificial saliva.

When "maximum gap width" among retrograde filling and dentine was evaluated, it was observed that the presence of blood led to the formation of higher gap widths at the MTA–dentine interface when both IV fluids were used. This inferred that MTA manipulated using Tetraspan and Ringer's lactate IV fluids had a negative effect on marginal adaptation in the presence of blood, the former being more defying than the latter.

However, MTA manipulated using Ringer's lactate IV fluid had a positive effect on marginal adaptation to radicular dentine than MTA manipulated using Tetraspan IV fluid in presence of artificial salivary contamination.

The possible reason for this could be that in the presence of fluids like blood and artificial saliva, the hydration behavior of MTA was adversely affected and this interfered with MTA's crystallization.[25] According to Sarkar et al.,[33] Bozeman et al.,[34] and Rahimi et al.,[35] when MTA is placed in moist conditions it precipitates hydroxyapatite crystals on its surface. These hydroxyapatite crystals undergo nucleation and grow to fill in the gaps and spaces between MTA and the dentinal wall. As time passes, the reaction between the hydroxyapatite layer of MTA and the tooth's dentine leads to the formation of a chemical bond resulting in the formation of an apical seal at the MTA–dentine interface.

The findings of our study can be correlated well with those of Neekofar et al.,[36] Ustun et al.,[37] Ratih and Putri,[38] and Subramanyam and Vasanthrajan.[39] According to them, an interfacial gap created between MTA and radicular dentine in the presence of blood is because of air entrainment caused by hemoglobin and blood proteins like albumin which not only have adverse effects on marginal adaptability but also create porous voids, thus reducing the sealability of MTA and disocclusion of MTA and dentinal tubules.

The reason for better marginal adaptation of MTA in presence of artificial salivary contamination can be attributed to the fact that, since saliva is a phosphate-containing fluid, it interacts with MTA to form carbonated apatite on its surface similar to the moisture content in the blood. This can be conferred from the findings of Tay et al.[40] who suggested that calcium and hydroxyl ions are leached through outward diffusion from set Portland cements which creates a condition wherein calcium ions are continuously released to an environment of high alkalinity.

The results of our study were congruent with Gondim et al.[41] where ProRoot MTA exhibited better sealing ability when compared with Super-EBA and IRM. Harmoniously Bidar et al.[42] found that gray MTA showed better marginal adaptation than Portland cement and white MTA in decreasing order. A rapport to our study was found with Sanchez et al.[43] and Saini et al.[44] who found MTA to be close to an ideal root retro filling material in periapical surgery. The findings of Nekoofar et al.[45] were consistent with ours, who proposed that blood had a detrimental effect on MTA and suggested hemorrhage control in the surgical area if MTA was to be used.

Unanimously Hindlekar and Raghavendra[46] revealed that biodentine and MTA, both had similar sealing ability when compared with a lower seal produced by injectable GIC in blood and saliva contaminated cavities.

However, the results of the present study were in contrast with Montellano et al.[47] who performed a bacterial penetration study and found that contaminated tooth-colored MTA leaked more in the presence of saliva over their 30 day time interval. In another dissimilar study, Hasheminia et al.[48] compared the sealing ability of MTA with CEM and found CEM to be a more effective root-end sealant than MTA. Also, Ayatollahi et al.[49] concluded that CEM cement in dry and saliva contaminated canals provided a significantly better seal as compared to MTA.

In another neoteric study, Pandey et al.[50] comparatively evaluated the microleakage of MTA and Geristore in dry, saliva, and blood-contaminated environments and found Geristore equally effective as MTA in a dry environment but provided a better apical seal than MTA in saliva and blood-contaminated environments.

Conclusion

Mineral trioxide aggregate, being hydrophilic, can be manipulated using Ringer's lactate and Tetraspan. However, the presence of blood contamination at the junction of retrofilled MTA and root dentine had an unfavorable outcome in marginal adaptability of MTA when compared with artificial salivary contamination. Tetraspan, a hydroxyl ethyl starch (HES) containing intravenous fluid has a colloidal nature. Due to this fact, increased gap width and gap perimeter were observed at the MTA–dentine interface, especially in presence of blood than in presence of artificial saliva.

Our study was a bold and successful, *ex vivo* attempt to alter the original formulation of the liquid component of MTA. However, further *in vivo* studies need to be executed to confirm the findings of those obtained in our study.

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