

## Comparison of the Biocompatibility of Pro Root MTA, Retro MTA and MTA Plus Using an MTT Assay Study

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### Abstract

The purpose of this *in vitro* study is to evaluate and compare the biocompatibility of three commercially available root-ending materials, Pro Root MTA, Retro MTA, and MTA Plus, at different storage time after mixing with human periodontal fibroblast using a MTT assay method.

The set root-ending materials and varied concentrations of fresh samples (3, 6, 12, 25, 50 mg/ml) at different time were placed adjacent flasks of human periodontal fibroblast in DMEM medium within 96-well plates. Cellular viability was evaluated using a MTT assay after 24, 48, and 72 hr of the initial mixing. The results were analyzed with 1-way ANOVA. The cytotoxicity was chosen as the indication of the biocompatibility of these root-ending materials. The results showed that there was no significant difference between the cytotoxicity of Retro MTA, MTA Plus and Pro root MTA ( $P > 0.05$ ), neither for the fresh, nor the set samples indicating almost similar biocompatibility for two commercially-available root-ending materials of Retro MTA and MTA Plus compared to that of Pro Root MTA.

**Keywords:** Cytotoxicity; Retro MTA; MTA Plus; Pro Root MTA; MTT Assay

### Introduction

The study on the biocompatibility of root-end filling materials has received great deal of attention in dentistry because their possible toxic compounds may damage the surrounding tissues, as they will be placed in close contact with live tissues such as dental pulp remnants, periodontal ligament and alveolar bone. Along with being biocompatible, an ideal root-end filling material should properly adhere and seal the root canal system, and also be easily processed [1,2].

In 1993, Mineral trioxide aggregate (MTA), first developed at Loma Linda University, offered the ideal characteristics for orthograde or retrograde root-end fillings materials. MTA is a powder of small hydrophilic particles, consisting of tricalcium silicate, tricalcium aluminate, tricalcium oxide and bismuth oxide besides other mineral oxides [3], which sets in the presence of water [4]. Although MTA is primarily implemented as a root-end filling material, it also can be used in pulp capping, pulpotomy, apical barrier formation in teeth with open apices, repair of root perforations, and root canal filling [5,6].

For a long time, ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK) has been used worldwide for variety of clinical applications including apical barriers in teeth with immature apices, repair of root perforations, root-end filling, direct pulp capping, and pulpotomy [7]. During

the last decade, the cytotoxicity of the Pro-root MTA has been extensively studied and proven to be biocompatible [8]. However, the long setting time, high cost, and limited availability of this specific type of MTA have urged material scientists to develop new types of root-end filling materials to overcome these drawbacks. The alternative materials must meet the advantages of the Pro-root MTA, and also be more accessible, cost effective, and be set at shorter time.

Recently, new brands of mineral trioxide aggregate (MTA)-based products, including Retro MTA and MTA Plus, have been introduced to the market which are significantly cheaper than the Pro Root MTA. One of the fast-setting calcium silicate cements is Retro MTA, with an initial setting time of about 180 second (according to the manufacturer). The main components of this type of MTA are calcium carbonate, silicon oxide, aluminum oxide, and calcium zirconium complex [9]. In 2015, Chung, *et al.* studied the cytotoxicity of Retro MTA on human pulp-derived cells and compared it with that of ProRoot MTA using an *in vitro* investigation [9]. Regarding MTA Plus, which has the highest amount of MTA in its formulation, to our knowledge, there is no study on its biocompatibility and performing a cytotoxicity evaluation seems to be necessary.

Although a few studies evaluated and compared the biocompatibility of each of these products with that of Pro Root MTA, those findings are limited and not comprehensive. More studies must be performed on different living cells, especially on human cells, to ensure of these new endodontic materials are biocompatible as they are frequently used in close contact with living tissues and their toxic compounds may damage the surrounding tissues, interfere on the healing process or cause allergic reactions. Here in this study, we evaluated the cytotoxicity of these two new root-end filling materials and compared the results with that of Pro Root MTA to determine the biocompatibility of these new products.

### Material and Methods

The test materials in this study are Pro Root MTA (Dentsply, Tulsa Dental, OK, USA), Retro MTA (bioMTA, Seoul, South Korea), MTA Plus (Prevert-Denpro, Jumma, India). Samples were prepared according to the manufacturer's direction. The samples were divided into two groups. The first group included all materials in a freshly mixed state, whereas in the second group materials were placed in apex simulation models and were allowed to be set and incubated for 24 h at 37°C at 100 % relative humidity.

Extracts of the materials were made as follows: 5 ml of complete Dulbecco's Modified Eagles Medium (DMEM) was added to 1gr of test material in freshly mixed state (neat concentration). To observe a dose-response relationship, the extracts were serially diluted with complete DMEM to achieve the concentrations of 3, 6, 12, 25, and 50 mg/ml. 5-Fluorouracil was dissolved in complete DMEM and tested as positive control; complete DMEM placed into empty 96 well tissue culture plates for 24, 48, 72 h and was tested as negative control.

In this *in vitro* study, human gingival fibroblasts (HGFs) were extracted using the explants technique and were implemented to conduct the experiments. Cells were grown in RPMI 1640 cell culture, containing 10% (V/V) bovine fetal serum, 1% Penicillin, 1% Streptomycin, 1% antifungal, and 1% antibiotic. Then, the HGF cells were collected by washing with serum free DMEM before treatment using 5trypsin (0.1%), 1 EDTA (0.1%) solution in phosphate buffered saline for 7 - 10 min. The cells from the fourth collection were plated in a 96-well plate at a density of 5000 cells per well and allowed to attach for 24h to the DMEM plus supplements. Single cell suspensions of HGF cells were seeded in 96-well flat-bottomed plates, 5000 cells per well in complete DMEM, and incubated in a humidified atmosphere of air and 5% CO<sub>2</sub> at 34°C for 24h. The culture medium then was replaced by 200 µl aliquots of the test extracts or control media. After 24, 48, and 72h of extract incubation the cell cultures were removed and cellular viability was evaluated using MTT assay.

In order to perform MTT assay, a stock of MTT solution (5 mg/ml) was prepared as follows: 50 mg of MTT powder was added into 10 ml PBS. To prepare the final MTT solution, 1 ml of stock solution was added into 9 ml of PPMI, containing 5% FCS and antibiotic. After 24, 48, and 72h of close contact between the extracts and cells, the culture media containing the extracts were emptied on cells, 100 ml of the final MTT solution was added to each group and the plates were incubated for another 1h. After this period, MTT solution was removed

from the cells and 100 µl of dimethyl sulfoxide solution was added to each group to dissolve formazan crystals. After performing liquid pipetting for each group, optic density (OD) in 570 nm wavelength was read by ELISA reader (STAT FAX, 2100, USA). Optical absorption is positively related to the number of metabolically active cells. The data were analyzed using the one-way ANOVA and Tukey's test at 95% significance level.

**Results and Discussions**

To assess the biocompatibility of the set root-ending materials and varied concentrations of fresh samples (3, 6, 12, 25, 50 mg/ml), MTT Assay was performed at 24, 48, 72h time intervals. The results were analyzed using 1-way ANOVA. Table 1 represents the comparison of the cytotoxicity of Retro MTA and MTA Plus with that of ProRoot MTA. As can be seen in Table 1, there was no significant difference between the cytotoxicity of the test materials (P > 0.05), neither for the set samples, nor the fresh samples.

	Group	Time	Material	Mean	P-Value	Material	Mean	P-Value	
set	24 h		ProRoot MTA	0.900	0.45*	ProRoot MTA	0.900	0.490	
			MTA Plus	0.914		RetroMTA	0.831		
	48 h		ProRoot MTA	1.012	0.70*	ProRoot MTA	1.012	.641	
			MTA Plus	1.024		RetroMTA	.919		
	72 h		ProRoot MTA	0.785	0.09*	ProRoot MTA	0.785	0.100	
			MTA Plus	.821		RetroMTA	0.752		
	concentration	3 mg/ml	24 h	ProRoot MTA	1.298	0.85*	ProRoot MTA	1.298	0.869
				MTA Plus	1.330		RetroMTA	1.326	
			48 h	ProRoot MTA	1.288	0.72*	ProRoot MTA	1.288	0.795
MTA Plus				1.328	RetroMTA		1.316		
72 h			ProRoot MTA	1.273	0.96*	ProRoot MTA	1.273	0.863	
			MTA Plus	1.264		RetroMTA	1.299		
6 mg/ml		24 h	ProRoot MTA	1.309	0.13*	ProRoot MTA	1.309	0.804	
			MTA Plus	1.475		RetroMTA	1.280		
		48 h	ProRoot MTA	1.290	0.64*	ProRoot MTA	1.290	0.835	
			MTA Plus	1.344		RetroMTA	1.268		
		72 h	ProRoot MTA	1.302	0.23*	ProRoot MTA	1.302	0.975	
			MTA Plus	1.476		RetroMTA	1.298		
12 mg/ml		24 h	ProRoot MTA	1.314	0.76*	ProRoot MTA	1.314	0.261	
			MTA Plus	1.355		RetroMTA	1.436		
		48 h	ProRoot MTA	1.298	0.18*	ProRoot MTA	1.298	0.146	
			MTA Plus	1.449		RetroMTA	1.439		
		72 h	ProRoot MTA	1.325	0.76*	ProRoot MTA	1.325	0.268	
			MTA Plus	1.371		RetroMTA	1.464		
25 mg/ml	24 h	ProRoot MTA	1.386	0.71*	ProRoot MTA	1.386	0.451		
		MTA Plus	1.443		RetroMTA	1.562			
	48 h	ProRoot MTA	1.438	0.79*	ProRoot MTA	1.438	0.414		
		MTA Plus	1.426		RetroMTA	1.472			
	72 h	ProRoot MTA	1.287	0.16*	ProRoot MTA	1.287	0.165		
		MTA Plus	1.447		RetroMTA	1.427			
50 mg/ml	24 h	ProRoot MTA	1.487	0.32*	ProRoot MTA	1.487	0.231		
		MTA Plus	1.374		RetroMTA	1.422			
	48 h	ProRoot MTA	1.384	0.97*	ProRoot MTA	1.384	0.841		
		MTA Plus	1.391		RetroMTA	1.421			
	72 h	ProRoot MTA	1.368	0.89*	ProRoot MTA	1.368	0.661		
		MTA Plus	1.347		RetroMTA	1.416			

**Table 1:** Cytotoxicity analysis of Retro MTA and MTA plus in comparison with ProRoot MTA.

The cytotoxicity of end-root filling materials has been always an important concern for dentists, because these materials are usually in intimate contact with living tissue, and their toxic effects, especially in endodontic therapy, can cause degeneration of the periapical tissue and delay wound healing [10,11]. Hence, in the current *in vitro* study, the biocompatibility of two commercially abandon root-end filling materials, Retro MTA and MTA Plus, was evaluated by comparing their cytotoxicity with that of the well-studied ProRoot MTA, using a MTT assay. The MTT assay works based on measuring the capacity of mitochondrial dehydrogenizing enzymes in living cells to convert the yellow water-soluble tetrazolium salt into dark blue formazan crystals while the water insoluble product is stored in the cytoplasm of living test cells. The amount of the formed formazan is directly proportional to the mitochondrial enzyme activity [12,13].

In this study, human gingival fibroblasts (HGFs), extracted using the explants technique, were implemented to conduct the experiments. To evaluate dose-response effect in material toxicity studies serial dilution method was used after mixing materials to achieve effective dilutions for performing the tests [14]. Neat concentration (1 gram of test sample with 5 mL of culture media) was prepared based on the study by Ossorio., *et al* [15]. In the current study, the direct method was not utilized but the extracts of the test materials were used. This method (open apex model) provides the opportunity to simulate the clinical situation where root ending materials and the vital cells are not directly in contact. Also, using this method, the test materials can be easily sterilized by filtration. Furthermore, the effect of materials on cells that are both distant to and in contact with them can be readily examined. The use of extracts also simulates the immediate postsurgical root end environment in which toxic elements of the retro-filling material leach into the surrounding fluids in the bony crypt. Another advantage is extracts can be made in a series of concentrations to observe a possible dose-response relationship and determine the ideal concentration for the sensitivity of the cells tested [16,17]. It should be also noted that the sterilization of the test materials for direct contact testing introduces the possibility of changing the properties of the materials which is another reason for using extract method.

For evaluating toxicity, Retro MTA, MTA Plus, and ProRoot MTA were tested in both freshly mixed and set states. Generally, as freshly mixed materials release other materials during chemical setting reactions, they render more cytotoxicity. However, when the setting reaction complete, materials structure becomes chemically stable and may show less cytotoxicity. In this experiment, human periodontal fibroblasts were subjected to the extracts of materials which were serially diluted with complete DMEM to achieve the concentrations of 3, 6, 12, 25, and 50 mg/ml. Then, after 24, 48, and 72h of extract incubation, the cell cultures were removed and cellular viability was evaluated using MTT assay. The results did not show any meaningful differences with respect to cytotoxicity within different groups of

materials (P-value > 0.05). Similar chemical components of these root-end filling materials can be the most important reason for the observed behaviour [1,17]. However, the results from the cytotoxicity investigation of each of these materials in each state reveal that statistically there is a significant difference between the cytotoxicity of each material during time of measurements. Similar results were reported by Deus, *et al.* on the cytotoxicity of ProRoot MTA, MTA Angelus, and Protland Cement in which the human ECV 304 endothelial cell lines were subjected to the set state of the mentioned materials [18]. The findings of other research groups also support the results of the current study [9,17-21].

### Conclusion

The current *in vitro* study showed almost similar biocompatibility for the two commercially-available root-ending materials of Retro MTA and MTA Plus compared to that of ProRoot MTA.

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