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RESEARCH ARTICLE

EVALUATION OF THE BIOCOMPATIBILITY AND BONE HEALING OF TWO DIFFERENT MINERAL TRIOXIDE AGGREGATES ON RAT MANDIBULAR TISSUE

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ABSTRACT

Introduction: MTA has been recommended primarily as a root end filling material, but it has also been used in pulp capping, pulpotomy, apical barrier formation in teeth with open apexes, repair of root perforations, and root canal filling.

Objectives: This study is designed to evaluate the biocompatibility and bone healing of MM-MTA in comparison to MTA.

Materials and Methods: This study was conducted on 30 rats by making osteotomies on both sides of the mandibles of the rats. Fifteen rats were sacrificed after one week to evaluate the short term inflammatory response and bone healing and the other fifteen were sacrificed after three weeks to evaluate the long term inflammatory response and bone healing. Specimens were collected for histopathological examination under light microscope and histomorphometric examination.

Results: Grey ProRoot MTA showed significantly lower inflammatory reaction than MM-MTA, and no statistical significant difference was recorded between the two materials in the histomorphometric analysis.

Conclusions: MM-MTA and Grey ProRoot MTA showed comparable biocompatibility when evaluated in vivo. Although the results are supportive for the Grey ProRoot MTA showing more biocompatibility compared to MM-MTA, both materials are considered to be equivalent in the ability of the formation of bone.

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INTRODUCTION

Most endodontic failures occur as a result of leakage of irritants from pathologically involved root canals into the peri-radicular tissues. Therefore a repair material should provide a good seal to an otherwise un-obturated root canal or improve the seal of an existing filling materials. An adequate apical seal is a major factor for improving endodontic success (Ng *et al.,* 2008). One of the most important criteria for an ideal endodontic material is its sealing ability and marginal adaptation (Torabinejad *et al.,* 1996). MTA was developed because the existing materials did not have the ideal characteristics for the orthograde or the retrograde root end fillings (Parirokh *et al.,* 2010). Since its introduction in 1993, MTA has been widely used in various endodontic procedures including pulp capping, root end filling, and perforation repair (Lee *et al.,* 1993, Bodrumlu2008, Hashem *et al.,* 2008, Kogan *et al.,* 2006).

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Recent studies show that MTA have superior effectiveness and a high success rate in apical microsurgery (Von Arx *et al.,* 2012). Materials used in endodontics are frequently placed in intimate contact with the periodontium and thus must be non-toxic and biocompatible with the host tissues. There are several in vitro and in vivo tests to evaluate the biocompatibility of the dental materials, they include testing the general toxicity profile of potential materials in a cell culture, implantation tests and usage tests in experimental animals according to the accepted clinical protocols. A number of biocompatibility and mutagenicitystudies have shown that MTA is a biocompatible material (Kim *et al.,* 2008, Bodrumlu2008, Abbasipour *et al.,* 2009, Gorduysus *et al.,* 2007). In fact the results of a meta-analysis on MTA biocompatibility showed that MTA is more biocompatible than super EBA, IRM and silver amalgam (Fernandez-Yanez Sanchez *et al.,* 2008). MTA is even considered biologically active by increasing cytokine production from human osteoblasts and inducing matrix formation for subsequent bone growth (Al-Rabeah *et al.,* 2006).Although MTA has many favorable properties, it has some disadvantages. One of the major disadvantages of the

material is its long setting time, which, in turn necessitates multiple visits by the patients. It is also difficult to mix MTA to obtain an optimal consistency to deliver it to the specific site and to condense it adequately. Many researches have focused on these limitations (Ding *et al.,* 2008, Lee *et al.,* 2011). Recently MM-MTA has been introduced. It has the same indications for use and is composed of similar materials and exhibits comparable technological characteristics to the conventional MTA. According to the manufacturers description MM-MTA is an insoluble, biocompatible, excellent adhesion to the dentine and radio-opaque endodontic material. It consisted of special capsules containing MM-MTA powder and liquid, automatic mixing is achieved quickly with a vibrating mixer or an amalgamator. In addition the resulting blend is extremely homogenous with transformation properties which are always optimal and reproducible. The addition of calcium carbonate to the formula considerably reduces the setting time (20 minutes) and also allows filling in the same session. It doesn't contain any pigments or coloring agents so it will not stain the teeth. Therefore this study was designed to evaluate the biocompatibility and bone healing of MM-MTA compared to Grey ProRoot MTA on rat mandibular tissue (Celik *et al.,* 2014).

MATERIALS AND METHODS

This study was a prospective comparative experimental animal study conducted on 30 white albino rats where an osteotomy (1mm diameter and 1 mm deep) was made under constant sterile saline irrigation with an inverted cone bur to prepare undercuts to retain the material on both sides of the mandible, in which the biocompatibility of micromega-mineral trioxide aggregate (MM-MTA) MicroMega MTA (MMTA; MicroMega, Besanchon, France)was compared to grey ProRoot MTA (PMTA; Dentsply, Tulsa, OK) conventional mineral trioxide aggregate (MTA) in the mandibles of rats.

Grouping

Group 1: Thirty rats received MM-MTA in the right side of the mandible.

Sub group 1a: Fifteen rats were sacrificed after 1 week to evaluate the short term inflammatory response and bone healing.

Sub group Ib: The other fifteen rats were sacrificed after 3 weeks to evaluate the long term inflammatory response and bone healing.

Group II:
Thirty rats received Grey Proroot MTA in the left side of the mandible.

Sub group IIa: Fifteen rats were sacrificed after 1 week to evaluate the short term inflammatory response and bone healing.

Sub group IIb: Fifteen rats were sacrificed after 3 weeks to evaluate the long term inflammatory response and bone healing.

Surgical procedure
General anesthesia was performed through intra-muscular injection of ketamine hydrochloride (Ketlar 0.5%) v, 20 mg / kg body weight, and xylazinevi (1 mg / kg body weight). After routine disinfection, and preparation of surgical field by betadine, an extra-oral submandibular incision was performed at the inferior border to raise a full thickness skin flap. The masseter muscle was incised and dissected from periosteum to expose the body of the mandible. By using low– speed round bur number 2, with copious irrigation, a surgical bony defect of ≈ 1 mm. in diameter and depth was done. The materials were mixed, delivered, and compacted into the prepared osteotomies according to the manufactures recommendation. MM-MTA was placed in the right side of the mandible and the Grey ProRoot MTA was placed in the left side**.** . After implantation, the soft tissues were held in place with a 3-0 polyglycolic acid resolvable suture material, and a fluid tight seal was obtained with histoacryl (El-Sweify*et al.,* 2008).

Post-operative care

The rats were put on soft diet eating cucumber and lettuce because of the surgery until the termination was done.

Scarification

Rats were randomly divided into 2 groups each consisting of 15 rats. After 1 week, fifteen rats were sacrificed to evaluate the short term inflammatory response .After 3 weeks the other Fifteen rats were sacrificed to evaluate the long term inflammatory response and bone healing Each half of the mandibles were selected for light microscopic examination, and image analysis by histomorphometry.

Histopathological examination (Bashkar 2011)

Specimens will then be fixed with 10 percent buffered formalin for 48 hours, dehydrated in ascending concentrations of ethyl alcohol (from 50 to 100), infiltrated with xylene and embedded in paraffin wax. Serial sections of the prepared paraffin blocks will be cut by microtome to (5mm) thickness. The specimens will be stained by:

Hematoxyline and eosin (H&E) (for general examination).
Specimens will then be observed under light microscope for the presence of inflammatory cells and will be categorized according to the following ordinal scale (Moretton *et al.,* 2000).

0:none, no detectable inflammation
1:mild scattered inflammatory reaction
2:moderate, localized inflammatory reaction
3:severe, diffuse and intense inflammatory reaction

Histomorphometric analysis (Parfitt1988)

Morphometric analysis will be carried out from experimental samples of heamatoxylin and eosin-stained section. Images will be viewed and recorded using Olympus microscope equipped with digital camera, using computer program matlab software (image j). The image of each slide of all groups will be captured using x 10 objective lens (Barr =200um) with

numerical aperture if a high resolution (16 bit digital camera, 1280x1024 pixel) for calculating the bone volume.

Statistical analysis

Data were fed to the computer and analysed using IBM SPSS software package version 20.0 (Kotz *et al.,* 2006). Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

The used tests were

1- Chi-square test: For categorical variables, to compare between different groups.

2- Monte Carlo correction: Correction for chi-square when more than 20% of the cells have expected count less than 5.

3- Student t-test: For normally quantitative variables, to compare between two studied groups.

RESULTS

Histological results

After one week: Subgroup Ia: the sections prepared from the right side of MM-MTA group revealed an osteotomy completely filled with granulation tissue and an inflammatory reaction of scale 3. The inflammatory cells were severe, diffuse and intense (Fig. 1).

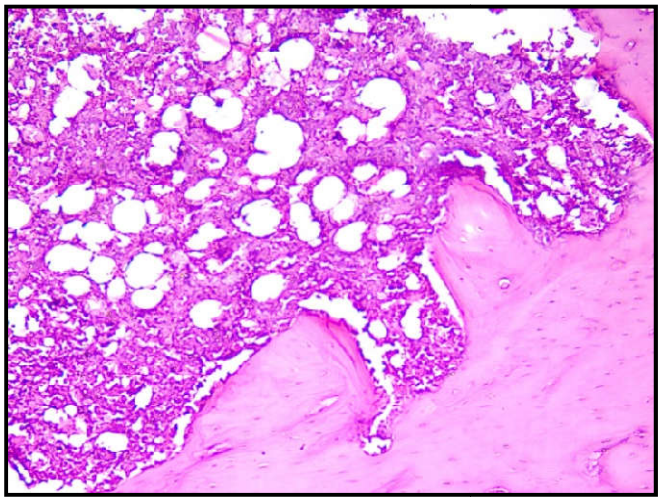

Fig. 1. LM of subgroup Ia showing an inflammatory reaction of scale 3 with small spaces of the dissolved material H&E stain × 100

Other specimens recorded an inflammatory reaction of scale 2. The inflammatory cells were localized and moderate (Fig. 2).

After one week: Subgroup IIa: most of the sections prepared from the left side containing the Grey ProRoot MTA revealed an osteotomy filled with granulation tissue with mild scattered inflammatory reaction of scale 1 (Fig 3). While the remaining other specimens showed moderate, localized inflammatory reaction of scale 2 (Fig 4).

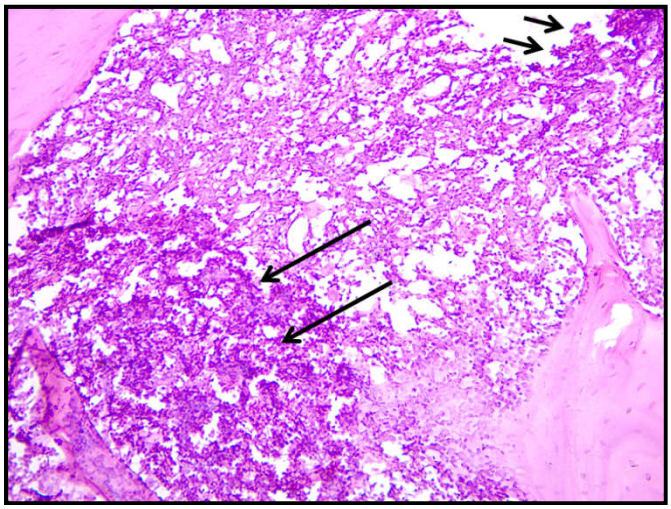

Fig. 2. LM of subgroup Ia showing the granulation tissue filling the site of the experiment. The inflammatory cells exhibited an inflammatory reaction of scale 2. The cells were moderate and localized. H&E stain × 100

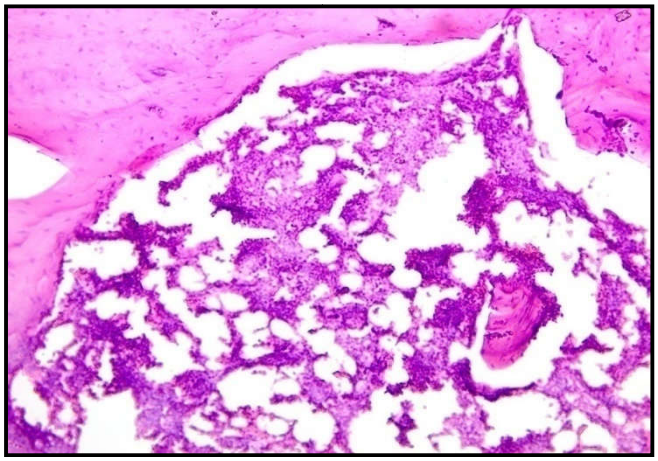

Fig. 3. LM of subgroup IIa showing granulation tissue with mild scattered inflammatory reaction of scale 1 and larger areas of the dissolved material. H&E stain × 100

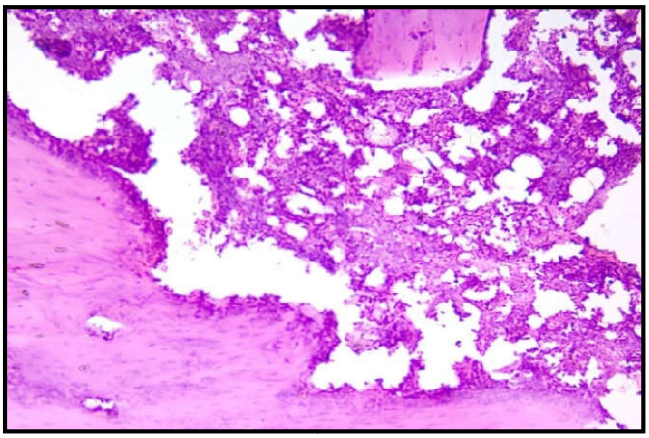

Fig. 4. LM of subgroup IIa showing the granulation tissue filling the osteotomy. The inflammatory cells were moderate and localized (scale 2). H&E stain × 100

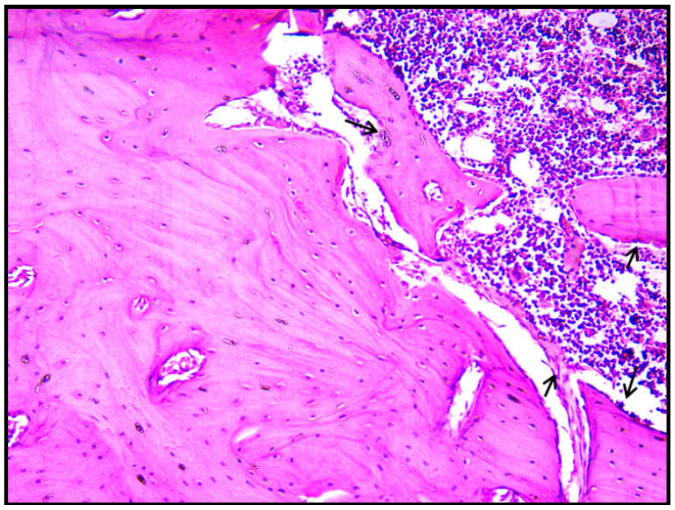

Fig. 5. LM of subgroup IIa showing the granulation tissue filling the osteotomy with deep violet inflammatory cells (scale 3). Note the newly formed bone in the experimental site (arrows) H&E stain × 100

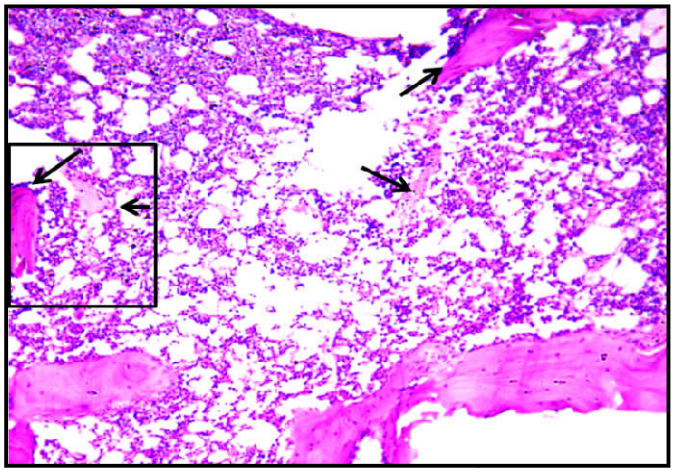

Fig. 6. LM of subgroup IIa showing moderate localized inflammatory reaction of scale 2, with new bone formation (arrows) H&E stain × 100

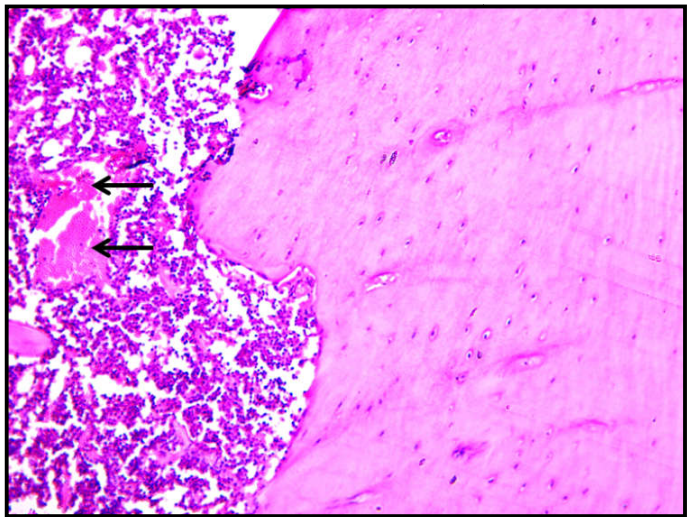

Fig. 7. LM of subgroup IIa showing mild, scattered inflammatory reaction of scale 1 with small organic bone deposition within the granulation tissue (arrows) H&E stain × 100

After three weeks: Subgroup Ib: Results obtained from sections prepared from the right side of the mandible showed a decrease in the number of specimens with the inflammatory reaction of scale 3. They also revealed new bone spicules (Fig 5). Most of the specimens showed an inflammatory reaction of scale 2. New bone formation joining the old bone was revealed (Fig 6).

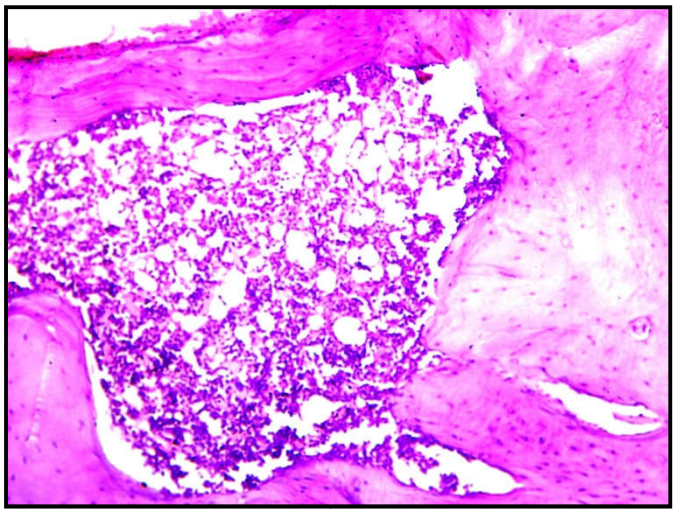

Fig. 8. LM of the subgroup IIb showing moderate, localized inflammatory reaction of scale 2 (deeply stained). Slight pink homogenous bone matrix within the osteotomy was revealed. H&E stain × 100

After three weeks

Subgroup IIb: most of the specimens prepared from the left side of the mandible showed mild, scattered inflammatory reaction of scale 1. The osteotomy was filled with the granulation tissue and small organic bone (Fig 7).
While the remaining specimens showed inflammatory reactions of scale 2 with the granulation tissue and homogenous bone matrix in between (Fig 8).

Statistical analysis

Histological analysis

Table 1 shows that the right side of the mandible containing the MM-MTA is less biocompatible with a statistically significant result compared to the left side containing the Grey ProRoot MTA using Chi square test.

Table 1. Comparison between the right (MM-MTA) and the left (Grey ProRoot MTA) side of the mandible according to inflammation in the first week

Inflammation	Right (n= 15)		Left (n= 15)		χ^2	^{MC}p
	No.	%	No.	%		
1 week score						
0	0	0.0	0	0.0	26.278*	<0.001*
1	0	0.0	12	80.0		
2	4	26.7	3	20.0		
3	11	73.3	0	0.0		

Table 2. Comparison between the right (MM-MTA) and the left (Grey ProRoot MTA) side of the mandible according to inflammation in the third week

Inflammation	Right (n= 15)		Left (n= 15)		χ^2	^{MC}p
	No.	%	No.	%		
3 weeks score						
0	0	0.0	0	0.0	28.775*	<0.001*
1	0	0.0	14	93.3		
2	10	66.7	1	6.7		
3	5	33.3	0	0.0		

Table 3. Distribution of the studied cases according to bone volume in 3 weeks

Cases No.	Bone volume in 3 weeks	
	Right side	Left side
1	0.1450	0.1775
2	0.2672	0.1897
3	0.2666	0.2808
4	0.1999	0.3613
5	0.1835	0.2388
6	0.2935	0.3031
7	0.1055	0.2400
8	0.3383	0.4076
9	0.2535	0.1995
10	0.2986	0.2944
11	0.3980	0.3421
12	0.2999	0.4583
13	0.5210	0.2959
14	0.5425	0.3459
15	0.3570	0.2999

Table 4. Comparison between the right (MM-MTA) and the left (Grey ProRoot MTA) side of the mandible according to bone volume in three weeks

	Right (n= 15)	Left (n= 15)	t	P
Bone volume in 3 weeks				
Min. – Max.	0.11 – 0.54	0.18 – 0.46	0.062	0.951
Mean ± SD.	0.30 ± 0.12	0.30 ± 0.08		
Median	0.29	0.30		

Table 2 shows that the right side of the mandible containing the MM-MTA is less biocompatible with a statistically significant result compared to the left side containing the Grey ProRoot MTA using Chi square test.

Histomorphometric analysis

After three weeks bone was formed and was calculated using T-test. The bone volume of the two sides of the mandible is shown in Table 3. Table 4 shows that there is no statistical difference between the two sides according to bone volume at the three weeks interval using T-test.

DISCUSSION

Biocompatibility can be described as a biomaterial's ability to function as a medical device within the human tissue and to carry out a specific task in the presence of an appropriate host response. These materials must not cause an unacceptable degree of harm to the body and must not carry any risks (Williams, 2008). The present study is an in vivo experimental study as those conducted by several authors such as Simsek *et al.,* 2015, McNamara *et al.,* 2010, El Sweify *et al.,* 2008 and Rahimi *et al.,* 2012. McNamara *et al.,* 2010 demonstrated performing intraoral osteotomies in rats on both sides of the mandible. They found that implantation studies in rats are shown to be an acceptable experimental model for their research and provide data for comparison of surgical methodology and outcome. When compared to the present study, osteotomies were performed through an extra oral approach, as that was performed by El Sweify *et al.,* 2008. In this work, the extra-oral approach was preferred as it provides a wider surgical field with easier accessibility and placement of the material into the bone. When compared to the intra-oral approach implemented by McNamara *et al.,* 2010, it was found that it provided a limited area for implantation. despite that, the extra-oral approach usage is less reported in the literature. Moreover, other authors such as Semsik *et al.,* 2015, used a sub-cutaneous approach for implantation of Micromega MTA, Biaggregate, and biodentin in contrast to the extra oral surgical approach adopted by the present study. The sub-cutaneous approach carries the advantage of being an easier approach than the surgical one. On the other hand, the former only allows testing degrees of inflammation, but does not allow histomorphometric analysis as the present study. Concerning the site of bone chosen, the rats' mandibles were targeted as the site of experimentation as the study performed by McNamara *et al.,* 2010.The mandible was selected to simulate the type of bone MTA is in contact with clinically, compared to evaluation of sites on long bones (as rats' femurs) as experimented by Rahimi *et al.,* 2012. The Mandibular location was chosen for an adequate bulk of bone to remain around the osteotomy (McNamara *et al.,* 2010).

The present study showed that MM-MTA resulted in more inflammation than Grey-pro root MTA, the difference in inflammation caused by the two materials was found to be statistically significant, according to statistical analysis of a scoring scale, such as the one used by McNamara *et al.,* 2010. The statistically significant difference in inflammation between Grey pro root MTA and MM-MTA was not reported before in the literature, as this is the first in vivo study to compare between these two materials. We deduced that Grey pro root MTA has more biocompatibility than MM-MTA when placed in rat mandibular tissue, as the former causes significantly less inflammation than the latter. This might be attributed to the presence of some trace metals such as beryllium (0.73 ppm), cadmium (0.21 ppm), and chromium (2.42 ppm) which are known to be a category 1A carcinogen but were not detected in Bioaggregate. These minor amounts of metal oxides appeared to be safe according to Kum *et al.,* 2014. Our results go with Margunato et al 2015 who aimed to compare the effect of ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK), Biodentine (Septodont, Saint Maur des Fossés, France), and MM-MTA (Micro-Mega, BesançonCedex, France) on the cell viability, hard tissue deposition capacity, and osteogenic differentiation of human bone marrow stem cells (hBMSCs) derived from mandibular bone.this study concluded that MTA is less toxic and more efficient to stimulate mineralization processes compared with Biodentine and MM-MTA. Although Margunato used White Pro root MTA and conducted an in vitro study instead of an in vivo one (Margunato *et al.,* 2015).

The biocompatibility of MM-MTA was found to be equivalent to Bioaggregate for the first time by Chang *et al.,* 2014, moreover, Simsek *et al.,* 2015, reported the same results when comparing MM-MTA with Biodentin and Bioaggregate but inflammation declined faster with the use of Biodentin. However, Jörn *et al.,* 2006, conducted a histological study in dogs comparing Grey pro root MTA and an experimental calcium phosphate cement. Moreover, they also performed histological examination following the same ordinal scale to grade the inflammatory response. The histological evaluation showed that no furcation in either group was free of inflammatory cells, thus thisstudy couldn't confirm the very high biocompatibility of the Grey pro root MTA (Jörn *et al.,* 2006). Concerning the bone healing, the present study showed that there is no statistical difference between the Grey pro root MTA and the MM-MTA. The Alveolar bone was measured using the image analysis system by calculating: Bone volume/total volume [(BV/TV; %) = bone hits/total hits × 400], which represented bone volume (BV; mm3) per total tissue volume (TV; mm3); the measurements and the mean of these measurements was calculated.This goes with the results of the in vitro study conducted by Margunato et al 2015, where the recently introduced MM-MTA showed comparable osteogenic activity on hBMSCs with both Biodentine and White Pro root MTA. Moreover, Rahimi *et al.,* 2012 evaluated new bone formation around the implanted Grey pro root MTA and calcium enriched mixture in rat femur using a different methodology. They concluded that there is no statistical difference between the two materials. In addition Pellicioni *et al.,* 2004conducted an invitro study where, Grey pro root MTA was compared with super EBA cement and silver amalgam. They found that Grey pro root MTA showed a good interaction with bone forming cells.

Conclusion

MM-MTA and Grey ProRoot MTA showed comparable biocompatibility when evaluated in vivo. Although the results are supportive for the Grey ProRoot MTA with a statistical significant result showing less biocompatibility compared to MM-MTA. Both materials are considered to be equivalent in the ability of the formation of bone with no statistical difference between them. Extra-oral surgical approach was proven to be a better method compared to the intra-oral approach.

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