Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Comparison of curative effect between different retrograde filling materials in young permanent molar root canal therapy

Fengna Qi*, Honglei Sun, Li Zhang, Jingwei Zheng

Department of Oral Medicine, Hebei Eye Hospital, Xiangdu District, Xingtai, Hebei Province, China

ARTICLE INFO

Original paper

Article history:
Received: August 16, 2021
Accepted: December 13, 2021
Published: December 30, 2021

Keywords:
Mineral Trioxide Aggregate; Interleukin-1β; Portland cement; Intermediate restorative material

ABSTRACT

The first mandible and maxilla permanent molars are the first permanent teeth that grow next to the deciduous teeth and may decay due to carelessness. Their caries can spread to the pulpal tentacles and cause pulpal and periapical diseases. In the current study, we tried to compare the curative effect of different retrograde filling materials, i.e. white MTA, gray MTA, Portland cement, and IRM, in young permanent molar root canal therapy. Because IL-1β stimulates bone degradation by osteoclasts, IL-1β gene expression was also measured for further evaluation. For this purpose, 400 students (240 boys and 160 girls) aged 8 to 11 years referred to the Pediatric Dental Center for first permanent molar root canal therapy were selected during two years. After recording the demographic characteristics of each patient, the first permanent molar teeth were examined by a general dentist with Abslang and decayed teeth were considered to have both discolorations in their grooves and apparent opacity. The patients, who need root canal therapy, were divided into four groups. The first group was treated with gray MTA. The second group was treated with white MTA. The third group received Portland cement for root canal therapy. The fourth group was treated with IRM. Also, IL-1β gene expression was evaluated by the real-time PCR technique. Relative changes in gene expression in PBMC cells were performed using One Way ANOVA. SPSS 18 software was used to determine the correlation of gene expression in PBMCs. The results showed that there was no significant difference between the groups in terms of age ($p = 0.12$) and gender ($p = 0.24$). Also, the need for endodontic treatment in the mandible ($n = 278$) was higher than the maxilla ($n = 85$) and both jaws ($n = 37$). But there was no significant difference between the groups in terms of the need for endodontic treatment ($p = 0.32$). The results of Pearson correlation coefficients between studied groups in terms of IL-1β gene expression showed that gray MTA and white MTA were not statistically different, but MTAs were generally different from Portland cement and IRM, with higher IL-1β gene expression. In general, the results showed that the teeth in the vicinity of gray MTA and white MTA showed a more appropriate response than Portland cement and IRM, so the use of MTA and its preference over other materials is recommended. In the case of Portland cement, more studies are needed to reach a conclusion comparing this material with MTA.

DOI: http://dx.doi.org/10.14715/cmb/2021.67.6.40

Introduction

Dental caries are likely to be the most common infectious disease in the world (1). This condition is caused by sugar consumption and the effect of microorganisms and causes loss of calcified dental tissues. Advanced decay lesions involve pulp and peripheral tissues, and if they remain without treatment, they can cause pain and discomfort for the patient and ultimately to lose teeth (2). Permanent first molecular caries are prevalent in mouth problems, accounting for almost 59% of dental caries. First permanent molars are considered the most critical permanent teeth, maintaining jaw contact in the closed state (3). They are interfaces between the teeth and the navigator of jaw growth. Any damage to them causes a risk to the dental skeletal system. Also, due to the loss of permanent primary molars, temporal joint abnormalities have been reported, including pain during chewing, frequent headache, sinus pain, etc. (4).

The age of 6-13 years old is the time to grow permanent teeth. The dental arc at this age is very sensitive to environmental changes (5). Because of the great importance of these teeth, the first permanent molar teeth have been proposed by the World Health Organization as a sign and an indicator for predicting decay activity, oral health, and dental health, and determination of vulnerable groups to decay, in 1994

*Corresponding author. E-mail: qifengna0617@163.com

(5, 6). These teeth are the first teeth that grow along with deciduous teeth in the child's mouth, and usually, because of the lack of parental awareness, they are neglected and suffer from decay. The decay of these teeth due to the spread of pulp tentacle in fresh young teeth causes pulp disease and needs root therapy (7).

One of the main goals of root treatments is the complete regeneration of damaged PDL (periodontal ligament) and bone (8). Because the materials are used directly with live tissue, such as connective tissue and bone, the success of the treatment depends on the proper regeneration of these tissues (9). Therefore, these materials should have high relative compatibility and minimum cytotoxicity, and they can also stimulate repair in these areas (10). Many cells are related to these materials, but it may be more critical for fibroblast cells, osteoblasts, or cementoblasts (8). Despite numerous efforts, the ideal substance with all the above properties has not been made to build the perfect filler. Among the various materials introduced to fill the root or closure of the perforation, it could be indicated to Amalgam, IRM (Intermediate restorative material), Super Eba, composite, Glass Ionomer, MTA (Mineral Trioxide Aggregate), and Dentin Bonding Agent (9-11). Also, extensive researches on Portland cement are underway (12). Unfortunately, all the above materials cannot form a new bone, PDL, and cement. Histological studies show that the new cement is formed only in the presence of MTA, composite, and hydroxyapatite. Amalgam, IRM, and Super Eba are lacking this critical ability (11, 12).

One of the critical characteristics of ideal retrograde filling material is enough apical seal supply to prevent leakage of intra-channel stimulants into the apical peripheral tissue (13). Therefore, in the current study, we tried to compare the curative effect of different retrograde filling materials, i.e. white MTA, gray MTA, Portland cement, and IRM, in young permanent molar root canal therapy. Because IL-1β stimulates bone degradation by osteoclasts (14, 15), IL-1β gene expression was also measured for further evaluation.

Materials and methods

Study population

In this study, 400 male and female students aged 8 to 11 years referred to the Pediatric Dental Center for first permanent molar root canal therapy were selected during two years. To calculate the sample size according to the pilot, P = 11% was calculated, and according to the formula, the sample size and numbers ($\alpha = 0.09$ and $d = 0.03$) $n = 318$ were obtained. For more certainty, 400 people were studied. The sample consisted of 160 female students and 240 male students. The parents of the students were informed about this project and written consent was obtained from them to have their children in this study.

The patients were divided into four groups. The first permanent molar root canal in the first group was treated with gray MTA. The second group was treated with white MTA. The third group received Portland cement for root therapy. The fourth group was treated with IRM.

Evaluation of first permanent molar root canal

After recording the demographic characteristics of each student, the first permanent molar teeth were examined by a general dentist with Abslang. A more detailed examination was performed on the teeth suspected of decay, using a mirror and a catheter (Smic, China) in the light of a flashlight. Then, decayed teeth were considered to have both discolorations in their grooves and apparent opacity.

Suspected caries teeth were referred to the School of Dentistry for cold and heat tests as well as periapical radiographs. A piece of ice was placed on the tooth surface to perform the cold test after isolating the tooth with a cotton roll and drying it. If the pain was acute and prolonged, irreversible pulpitis was recorded. In the heat test, the isolated teeth were dried.

It was then tested with a catheter heated on a flame. It should be noted that the sharp and long pain was a sign of irreversible pulpitis. Subjects who tested positive for the test were referred to the radiology department for periapical radiography to confirm the need for endodontic treatment with parental consent. The patients, who need first permanent molar root canal therapy, were divided into four groups. The first permanent molar root canal in the first group was treated with gray MTA. The second group was treated with white MTA. The third group received Portland cement for root therapy. The fourth group was treated with IRM.

Evaluating IL-1β gene expression

To evaluate the expression level of IL-1β, Peripheral blood samples (5 ml) were obtained from a vein (at the start of the experiment, a week after treatment and 1 month after treatment) and collected in cell preparation tubes containing anticoagulants (heparin). Blood samples were then diluted with an equal volume of buffer phosphate (PBS) solution. To separate the cells, a centrifuge was used for 20 minutes. Finally, the cell pellet was washed with PBS solution.

According to the manufacturer's instructions, total mRNA was isolated from PBMC cells using an RNA extraction kit (Roche, Germany). The nanoDrop device was used to standardize the concentration of all extracted RNA samples and evaluate the purity and concentration of RNA. One microgram of RNA per sample was used for cDNA synthesis by the cDNA Synthesis Kit (Fermantase, Germany). The primers were designed by Oligo5 (WWW.oligo.net) for IL-1β and β-actin gene as a housekeeping gene, based on sequences registered in GenBank. In addition, the BLAST database search used the NCBI Database to monitor their specificity theoretically (Table 1).

Table 1. Characteristics of primers used for RT-PCR

Gene		Primer sequences (5'-3')	Accession No.	Product Length
IL-1β	Forward	TGGCAGAAGTACCTGAGCTCG	NM_000576.2	115bp
	Reverse	AGGTCCTGGAAGGAGCACTTC		
β-actin	Forward	AGACGCAGGATGGCATGGG	NM_001101.3	161bp
	Reverse	GAGACCTTCAACACCCCAGCC		

To evaluate the expression of the IL-1β gene in PBMC cells, a typical PCR method was performed for all samples in a final volume of 20ml with one unit of Taq DNA polymerase. The reaction mixture containing 2 5mM MgCl2, 0.5mM of each type of dNTPs, 1.5pmol primer, 2.5µl of Taq DNA polymerase (Roche, Germany), and 1µl of cDNA were used as a template in each RT-PCR reaction. To amplify IL-1β and β-actin genes, PCR was started at 95°C for 5 minutes and during 35 cycles at 95°C for 1 minute, 60°C for 40 seconds and 72 minutes, and then a final elongation step at 72°C for 10 minutes. Finally, PCR products using 2% agarose were observed in gel electrophoresis.

In addition, positive control amplification was used to evaluate the performance of the primers. Sybergreen nucleotide fluorogenic kit (Roche, Germany) was used to monitor cDNA amplification for real-time PCR. This was done by measuring the fluorescence intensity using a pair of primers using Real Real-time PCR (Corbett, Germany) for each sample in 10µl of the solution, including 2ml of Fast Start Master solution and 0.3µmol was performed. A total of 9µl of this reaction mixture was placed in a 0.1 ml vial, and 1µl of cDNA was added as a template. The thermal cycle consisted of an initial step of tempering at 95°C for 10 minutes, and then a replication program (initial primer binding, replication, and measurement) was repeated for 45 cycles. The elongation program is for 10 seconds, 60°C, and 72°C for 10 seconds by measuring the fluorescence at the end of each stage of the elongation phase. The third step involved a melting curve program. Melting curve analysis showed only one peak for each reaction, which was confirmed by electrophoresis of PCR products, and only one band with the expected size was observed.

Statistical analysis

The number of samples was determined by Minitab 16.1 software and the efficiency of each reaction was evaluated by Linreg software. Real-time PCR data were analyzed using the ΔΔCT method and Rest software (2005 and 2009). Relative changes in gene expression in PBMC cells were performed using one-way analysis of variance (One Way ANOVA) and compared with the Post Hoe Tukey method. SPSS 18 software was used to determine the correlation of gene expression in PBMCs. A p-value less than 0.05 ($p < 0.05$) is statistically significant in the present study.

Results and discussion

A total of 400 students with permanent molar root canal caries between 8 and 11 years with a mean age of 9.35 years participated. 240people (60%) were boys, and the rest were girls. These patients were divided into four groups of 100 people. The first group was treated with gray MTA. There were 54 boys and 46 girls in this group. The mean age in this group was 10.31 years. The second group was treated with white MTA. There were 63 boys and 37 girls in this group with an average age of 8.95 years. The third group received Portland cement for root therapy. The group included 59 boys and 41 girls with a mean age

of 9.15 years. The fourth group was treated with IRM. There were 64 male and 36 female patients in this group. The mean age in this group was 8.99 years. The one-way analysis of variance showed that there was no significant difference between the groups in terms of age (p = 0.12) and gender (p = 0.24) (Table 2). Also, according to the results, the need for endodontic treatment in the mandible (n = 278) was higher than the maxilla (n = 85) and both jaws (n = 37). But there was no significant difference between the groups in terms of the need for endodontic treatment (p = 0.32).

Table 2. Demographic and clinical characteristics of the patients

Trait		First group (n=100)	Second group (n=100)	Third group (n=100)	Fourth group (n=100)	P-value
Age (year)		10.31	8.95	9.15	8.99	0.12
Gender	Male	54	63	59	64	0.24
	Female	46	37	41	36	
Root Canal Therapy (Endodontic Treatment)	Mandible	65	73	69	71	0.32
	Maxilla	25	20	22	18	
	Both Jaws	10	7	9	11	

The expression of the IL-1β gene showed that at the start of the experiment, there were no significant differences between studied groups (p = 0.319) (Figure 1). But, after a week, the expression of this gene was increased in all studied groups. The most enhance belonged to the first group and the least enhance belonged to the fourth group. After a month, the expression of this gene was reduced in all four groups. But this decrease was more significant in the third and fourth groups.

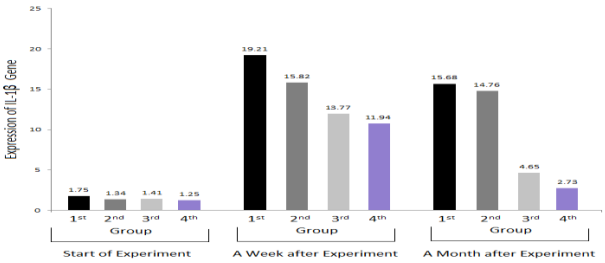

Figure 1. The expression of IL-1β at the start of the experiment, a week after the experiment, and a month after the experiment between studied groups

The results of Pearson correlation coefficients between studied groups in terms of IL-1β gene expression showed that gray MTA and white MTA were not statistically different, but MTAs were generally different from Portland cement and IRM, with higher IL-1β gene expression. A comparison of the two groups is given in Table 3.

Table 3. Pearson correlation coefficients between studied groups in terms of IL-1β gene expression

	Gray MTA	White MTA	Portland cement	IRM
Gray MTA	-	0.21^{ns}	0.56*	0.61*
White MTA	0.18^{ns}	-	0.41*	0.44*
Portland cement	0.48*	0.42*	-	0.12^{ns}
IRM	0.51*	0.49*	0.13^{ns}	-

The retrograde filling materials in this study were gray MTA, white MTA, IRM, and Portland cement. Gray MTA was introduced to the dental community in 1992 and approved by the FDA in 1996. Despite the many advantages of gray MTA, one of its drawbacks was its dark color, which causes problems in aesthetically essential areas. On this basis, a new type of white MTA has recently been introduced (16). The main components of white and gray MTA are similar and the main difference between white MTA is less concentration of AL_2O_3 (17). Because the main components of MTAs are very similar to Portland cement, some believe that Portland cement can be used instead of MTA (18). Limited studies have been performed comparing Portland cement with MTA. In this study, Portland cement was also used to compare the expression level of cytokine (IL-1β) in the presence of this substance with gray and white MTA. In recent years, IRM has been used extensively to fill root ends or repair perforations. However, the toxicity of this substance has been proven (19). For this reason, various researchers have used this substance as a negative control.

A suitable and new method to study the reaction of substances is to study their effect on the expression of cytokines in the body (20). Cytokines are low molecular weight proteins that cause the growth and differentiation of immune system cells (21). These biologically active materials have a wide variety of structural and functional diversity. Interleukin-1 (IL-1) is a large and important family of cytokines that perform a wide variety of biological functions in the body. IL-1α and IL-1β are two important subsets of the IL-1 family (22).

Studies showed the importance of cytokines in pulp and periapical diseases (23, 24). One of the most important cytokines involved in the pulp and periapical inflammatory response is IL-1β (24, 25).

This cytokine is secreted from a wide variety of cells (24). Yang *et al*. believe that the ability to produce and secrete IL-1β exists in every cell in inflammatory pulp and periapical lesions (26). However, the essential IL-1β-secreting cells include monocytes, macrophages, endothelial cells, osteoblasts, fibroblasts, and PMNs. IL-1β and TNF-α stimulate bone degradation by osteoclasts, collectively referred to as osteoclast-activating factor (OAF) (22). In humans, IL-1β performs the most OAF activity, indicating its high level of expression and pharmacological potency. IL-1β has also been shown to play an essential role in inducing an acute response (27).

On the other hand, it has been shown that IL-1β also plays a positive role and participates in the repair process. Lertchirakarn *et al*. (28) showed that IL-1β could increase collagenase expression in pulpal fibroblasts. In addition, proinflammatory cytokines have been shown to play a significant role in protecting the pulp from the spread of infection. In addition, the results of a study by Liu *et al*. (29) suggest that some of the actions induced by IL-1β and TNF-α play an essential role in periapical protection, especially in the first few days after stimulation. Barrientos *et al*. (30) believe that a large set of BMP (Bone morphogenic protein) mediators, including IL-6, IL-1β, and EGF (Epidermal Growth Factor), can inhibit the penetration, proliferation, and differentiation of progenitor cells into cells.

Bone resorption is an essential component of bone remodeling. This process is critical during the repair of bone loss (31). As soon as the osteoblasts receive the appropriate signal, they release soluble intermediates that stimulate bone resorption (31, 32). TNF-α, IL-1β, and PGF2 are among the osteoblast-derived proteins released into the bone matrix and are crucial bone resorption mediators (21). In this study, the expression level of the IL-1β gene in relation to gray MTA and white MTA was significantly higher than IRM and Portland cement. IL-1β secretion in the presence of gray and white MTA was not significantly different. Increased expression of the IL-1β gene may indicate activation of osteoblast cells in the presence of gray and white MTA.

Acknowledgements

None.

Interest conflict

None.

References

1. Pitts NB, Zero DT, Marsh PD et al. Dental caries. Nat Rev Dis Prim 2017; 3(1): 1-16.
2. Fleming E, Afful J. Prevalence of total and untreated dental caries among youth: the United States, 2015–2016. 2018
3. Nyvad B, Takahashi N. Integrated hypothesis of dental caries and periodontal diseases. J Oral Microbiol 2020; 12(1): 1710953.
4. Qiu W, Zhou Y, Li Z et al. Application of antibiotics/antimicrobial agents on dental caries. Biomed Res Int 2020; 2020.
5. Shi W, Tian J, Xu H, Zhou Q, Qin M. Distinctions and associations between the microbiota of saliva and supragingival plaque of permanent and deciduous teeth. PLoS One 2018; 13(7): e0200337.
6. Shi X, Mao J, Liu Y. Pulp stem cells derived from human permanent and deciduous teeth: Biological characteristics and therapeutic applications. Stem Cells Transl Med 2020; 9(4): 445-464.
7. Xiong Hc, Chen K. Comparative analysis of transcriptomes between apical pulp-derived cells from deciduous teeth and permanent teeth or dental pulp cells from exfoliated deciduous teeth. J Gene Med 2019; 21(6): e3098.
8. Maeda H. Mass acquisition of human periodontal ligament stem cells. World J Stem Cell 2020; 12(9): 1023.
9. Long J, Kreft J-U, Camilleri J. Antimicrobial and ultrastructural properties of root canal filling materials exposed to bacterial challenge. J Dent 2020; 93: 103283.
10. Souza MA, Rauber MGP, Zuchi N et al. Influence of final irrigation protocols and endodontic sealer on bond strength of root filling material with root dentin previously treated with photodynamic therapy. Photodiagnosis Photodyn Ther 2019; 26: 137-141.
11. Topçuoğlu HS, Topçuoğlu G. Postoperative pain after the removal of root canal filling material using different techniques in teeth with failed root canal therapy: a randomized clinical trial. Acta Odontol Scand 2017; 75(4): 249-254.

12. Camilleri J. Will bioceramics be the future root canal filling materials? Curr Oral Health Rep 2017; 4(3): 228-238.
13. Murata K, Washio A, Morotomi T, Rojasawasthien T, Kokabu S, Kitamura C. Physicochemical Properties, Cytocompatibility, and Biocompatibility of a Bioactive Glass Based Retrograde Filling Material. Nanomaterials 2021; 11(7): 1828.
14. Shiratori T, Kyumoto-Nakamura Y, Kukita A et al. IL-1β induces pathologically activated osteoclasts bearing extremely high levels of resorbing activity: a possible pathological subpopulation of osteoclasts, accompanied by suppressed expression of Kindlin-3 and Talin-1. J Immunol 2018; 200(1): 218-228.
15. Son HS, Lee J, Lee HI et al. Benzydamine inhibits osteoclast differentiation and bone resorption via down-regulation of interleukin-1β expression. Acta Pharm Sinica B 2020; 10(3): 462-474.
16. Kang S-H, Shin Y-S, Lee H-S et al. Color changes of teeth after treatment with various mineral trioxide aggregate–based materials: an ex vivo study. J Endod 2015; 41(5): 737-741.
17. Marciano MA, Camilleri J, Lucateli RL, Costa RM, Matsumoto MA, Duarte MAH. Physical, chemical, and biological properties of white MTA with additions of AlF3. Clin Oral Invest 2019; 23(1): 33-41.
18. Svetlozarova S. Comparison between white–colored and gray-colored mineral trioxide aggregate (MTA)–a review of the literature. Paper presented at: Varna Medical Forum, 2019.
19. Wu M-K, van der Sluis LW, Wesselink PR. A 1-year follow-up study on leakage of single-cone fillings with RoekoRSA sealer. Oral Surg Oral Med Oral Pathol Oral Radiol 2006; 101(5): 662-667.
20. Turin L, Torinesi R, Pastorelli G. Real-time PCR detection of the effect of postweaning on the expression of cytokines and NF-kB in piglets. J Biol Regul Homeost Agents 2019; 33(6): 1737-1745.
21. Chami VdO, Nunes L, Capelli J. Expression of cytokines in gingival crevicular fluid associated with tooth movement induced by aligners: a pilot study. Dental Press J Orthod 2018; 23: 41-46.
22. Komiya H, Shimizu K, Noma N et al. Role of neuron-glial interaction mediated by IL-1β in ectopic tooth pain. J Dental Res 2018; 97(4): 467-475.
23. Hirsch V, Wolgin M, Mitronin AV, Kielbassa AM. Inflammatory cytokines in normal and irreversibly inflamed pulps: a systematic review. Arch Oral Biol 2017; 82: 38-46.
24. TOLEDO AONd, COUTO AMd, Madeira MFM, Caldeira PC, Queiroz-Junior CM, AGUIAR MCFd. Cytokines and chemokines associated with Treg/Th17 response in chronic inflammatory periapical disease. Braz Oral Res 2019; 33.
25. Darvishi E, Aziziaram Z, Yari K et al. Lack of association between the TNF-α-1031genotypes and generalized aggressive periodontitis disease. Cell Mol Biol 2016; 62(11): 63-66.
26. Yang N-Y, Zhou Y, Zhao H-Y, Liu X-Y, Sun Z, Shang J-J. Increased interleukin 1α and interleukin 1β expression is involved in the progression of periapical lesions in primary teeth. BMC Oral Health 2018; 18(1): 1-6.
27. Huang H, Wang W, Lin P, Huang C, Chen C, Chen Y. The roles of autophagy and hypoxia in human inflammatory periapical lesions. Int Endod J 2018; 51: e125-e145.
28. Lertchirakarn V, Birner R, Messer HH. Effects of interleukin-1β on human pulpal fibroblast proliferation and collagen synthesis. J Endod 1998; 24(6): 409-413.
29. Liu C, Xiong H, Chen K, Huang Y, Huang Y, Yin X. Long-term exposure to pro-inflammatory cytokines inhibits the osteogenic/dentinogenic differentiation of stem cells from the apical papilla. Int Endod J 2016; 49(10): 950-959.
30. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen 2008; 16(5): 585-601.
31. Ikebuchi Y, Aoki S, Honma M et al. Coupling of bone resorption and formation by RANKL reverse signalling. Nature 2018; 561(7722): 195-200.
32. Akhlaghi M, Ghasemi Nasab M, Riasatian M, Sadeghi F. Soy isoflavones prevent bone resorption and loss, a systematic review and meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr 2020; 60(14): 2327-2341.