COMBINED EFFECTS OF SIMVASTATIN AND CALCIUM HYDROXIDE VERSUS MINERAL TRIOXIDE AGGREGATE IN DIRECT PULP CAPPING OF RABBIT'S INCISORS (HISTOLOGICAL STUDY)

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ABSTRACT

INTRODUCTION: Direct pulp capping (DPC) is a procedure to maintain the pulp vitality using a material with specific properties. Mineral trioxide aggregate (MTA) is considered as a gold standard material, but it has some drawbacks. In this study, a combination of simvastatin and calcium hydroxide (SIM-CH) was used as a DPC material as it was proved that simvastatin influenced odontoblast cells differentiation.
OBJECTIVE: To compare histologically the effect of the combination of SIM-CH to MTA as a DPC material in rabbits' incisor.
MATERIALS AND METHODS: 32 sound maxillary incisor teeth of 16 male New Zealand white rabbits were divided into; group I received MTA, and group II received (SIM-CH) as a DPC material. Class V cavities were done in the upper incisor teeth, then DPC materials were applied. The final restoration was applied. The animals were euthanized after 1, 2 weeks. Routine histological preparation was done, Scoring system was done to evaluate the newly formed dentin. The percentage of the surface area of the newly formed dentin was assisted by histomorphometric analysis.
RESULTS: Both groups showed new dentin formation. Group I reported somewhat more continuous layer of tertiary dentin than SIM-CH group. On the other hand, the dentin bridge of the SIM-CH group demonstrated more orderly organized tubules. There was a statistically significant difference regarding the histomorphometric analysis of the percentage of the surface area of the newly formed dentin of group I compared with group II ($p < 0.05$).
CONCLUSION: SIM-CH could be a remarkable alternative DPC material.
KEY WORDS: Direct pulp capping, mineral trioxide aggregates, simvastatin, calcium hydroxide.
RUNNING TITLE: SIM-CH versus MTA in direct pulp capping.

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INTRODUCTION

Direct pulp capping (DPC) is a procedure in which a bioactive material is applied directly on the exposed pulp to stimulate pulp cells to form dentin like bridge (1). The material of choice has been calcium hydroxide (CH) (2). However, it has many disadvantages, such as tunneling defects in dentin bridges, imperfect seal, less antibacterial effect (3, 4).
Mineral trioxide aggregate (MTA) is considered as a new gold standard pulp capping material because of its remarkable properties (4). Studies have demonstrated that MTA induced reparative dentin with thick dentinal bridging and minimal inflammation (5).

Besides, it has a superior biocompatible property with long-term effective seal (6). Unfortunately, it has limitations such as, its long setting time, difficult manipulation, cost, and crown discoloration (7, 8).
Nowadays trials are done to generate a low cost-effective pulp capping material which promotes dentin bridge formation (9).
Simvastatin is a low-cost cholesterol lowering drug which consists of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (10). Generally,

Statins are capable of enhancing endothelial function and decrease vascular inflammation (11).

In dentistry, few vivo studies have tackled the effect of simvastatin in pulp healing, In a study, simvastatin showed an inferior properties compared to calcium hydroxide (CH) as a direct pulp capping of human primary teeth (12). However, the combined effect of statins and α tricalcium phosphate was nearly equal to the effect of MTA in human teeth (13). Besides, the combined effect of simvastatin with appropriate dose and mineral trioxide aggregates (MTA) showed a superior positive effect as opposed to MTA alone (9).

In light of these reflections, this study did a novel combination of simvastatin and calcium hydroxide (SIM-CH) as a direct pulp capping material to enhance the efficacy compared to mineral trioxide aggregates (MTA) on rabbits' incisors.

The study was conducted with the null hypothesis that there is no significant difference between calcium hydroxide combined with simvastatin on healing of the pulp tissue compared to mineral trioxide aggregates.

MATERIAL AND METHODS

The experiment got the approval of the ethical committee on the guidelines of Alexandria University – Institutional Animal Care and Use Committee approval number (IORG0008839).

Rabbit Incisors Mechanical Pulp Injury Model

16 healthy male New Zealand rabbits of 4 months old (n= 32 teeth) having all maxillary and mandibular incisors free from caries and fracture, with approximate weight of 1.25-1.5kg were used in this study. Animals were obtained from the animal's house of Medical Research Institute, Alexandria University.

All operating procedures were done under general anesthesia and sterile conditions using intramuscular injection of xylazine (3 mg/kg) (Xyla-ject® by Adwia co. S.A.E., Egypt) and (20 mg/kg) ketamine (Ketamine10%® by Alfasan, Holland).

Direct Pulp Capping Procedures

After achieving the analgesic procedure successfully, all maxillary teeth were disinfected, and then isolated with rubber dam. On the labial surface of each tooth a class V cavity with the dimension of 1.5 mm 2 mm was done using a sterile round bur (0.10 ISO standards). Copious irrigation with sterile distilled water irrigation was obligatory during the cutting to prevent heat form inducing pulpal damage. When the pulp was almost reached, the pulp horn was exposed using the tip of an explorer till a drop of blood appears. Controlling the bleeding was achieved by applying sterile cotton pellets with 0.9% normal saline on the exposed pulp.

Pulp capping material was assigned in each quadrant according to the following:

- Group I (Right side – n=16 teeth) received MTA material.
- Group II (Left side - n=16 teeth) received the mix of simvastatin- calcium hydroxide (SIM-CH) figure 1.

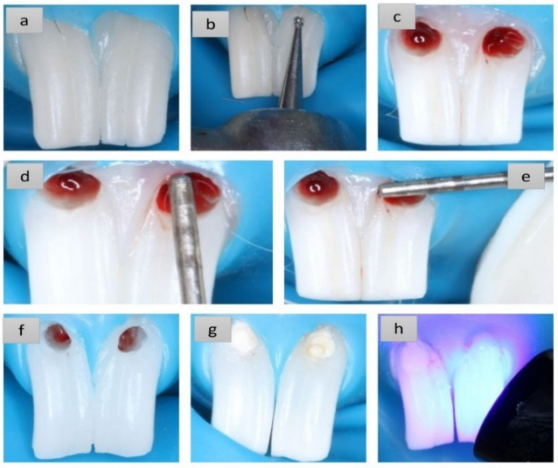

Figure (1): Photographs of tooth preparation and restorative procedures. (A) sound maxillary incisor teeth. (B) direction of cutting. (C) Mechanical pulp exposure on the labial surface. (D, E) measuring the cavity dimensions. (F) stopping of the bleeding (G)Site of pulp exposure capped with MTA on the right tooth, while SIM-CA on the left one. (H) restored with light cure resin-modified glass ionomer.

Preparation of the Materials

Group I: The MTA (Root dent, TehnoDent" Co.Ltd.Belgord region, Russia) powder was mixed with distilled water with regard to the manufacturer's instructions. The mixture directly covered the exposed pulp, and then the cavities were restored by light cure resin modified glass ionomer (GC Fuji II LC CAPSULE, GC corporation, Tokyo, Japan) as a coronal restoration.

Group II: Simvastatin (EGPI (Egyptian Group for Pharmaceutical Industries), Egypt) powder was mixed with calcium hydroxide (Prevest Denpro Limited, Bari Brahmana, Jammu - 181133 India) with ratio (1:1). The concentration was (100 mg simvastatin: 100 mg Calcium hydroxide/ 25microliter saline). Saline was measured using the Micro-Pipette, and then it was added to form a creamy mix. Finally, for better handling properties of the new material, 4 spoons using (Excavator double ended, Dentsply Maillefer, code B 0095, ISO SIZE 6x 57 58) of catalyst of calcium hydroxide paste cement (TechNew HYDCAL, Brazil0 was added. The coronal restoration was like group I.

- After completing the restorative procedure, rabbits were observed for any symptoms of pain or fever and the operative sites were checked on daily basis. To decrease pain, subcutaneous injection with a non-steroidal anti-inflammatory drug, Carprofen (2 to 4 mg/kg), was given daily for 3 days (14).

- The rabbits were euthanized after DPC at two observational time intervals of 1, 2 weeks (14, 15).

Histological Processing

Rabbits were euthanized at each time interval (14, 15) by intravenous injection with a lethal dose (100 mg/kg) of pentobarbital sodium. The rabbits were decapitated, and then the part of maxilla carrying the incisors teeth were dissected. Fixation was done for each segment contained the experimental teeth, investing bone and the surrounding soft tissues using 10% neutral–buffered formalin. Then they were washed, and decalcified with 5% trichloroacetic acid (16), dehydrated with ascending concentrations (50%, 70%, 90%, and 95%) of ethanol, cleared with xylene, and embedded in paraffin wax blocks (17). The blocks were cut with an average thickness of 5 µm. The direction of cutting was labiolingual in serial sections until the mid of the pulp exposure was reached. Numbers were given to the slides, and median sections were chosen. Slides were stained with H&E (Jones H&E staining kint ® by Venatana Medical systems, USA) to be examined by light microscope for histological evaluation.

Histological Analysis

Each section was analyzed by an experienced and calibrated oral biologist blinded to groups. The sections were re-examined after 7 days to confirm the given scores. Samples were evaluated using a light microscope (BX41; Olympus, Tokyo, Japan) connected to a high-resolution video camera. The mineralized tissue barrier formation was described according to the modified criteria of Islam, Toida (18), Quantity of Tertiary Dentin Formation was evaluated according to the modified criteria of Yang, Lee (19), morphology of dentinal tubules and extent of inflammation were graded on the report of the modified criteria of Likitpongpipat et al (20). The criteria and scores are listed in Table 1

Table (1): Showing Scores Used for Histological Assessment.

Scores	Characterization
The mineralized tissue barrier formation (18)	
1	A layer of scattered and foggy mineralized tissue deposition (initial).
2	partial/incomplete mineralized tissue barrier formation extending more than half of the exposure site but not completely closing it (partial).
3	Complete mineralized tissue barrier formation.
Quantity of tertiary dentin formation(19)	
1	Slight tertiary dentin formation along the pulp-dentin border.
2	Moderate tertiary dentin formation along the pulp-dentin border.
3	Heavy dentin formation along the pulp-dentin border, appearing as complete obliteration.
Morphology of dentinal tubules (2	
1	Non-detected.
2	Disorganized arrangement.
3	Orderly arrangement.
Extent of pulp inflammation (20)	
1	None detected
2	Restricted to the areas beneath the exposure site/dentine bridge or within some parts of the pulp chamber
3	Occupy the whole pulp chamber

Histomorphometry

The percentage of surface area of the newly formed dentin was evaluated at x100 magnification by Image J program (21) of the experimental groups.

Statistical Analysis

IBM SPSS software package version 24.0 was used to analyze the data. Qualitative data was demonstrated using number and percent. Chi-square test was used to compare between different groups regarding categorical variables. Quantitative data was reported using mean and standard deviation for normally distributed data. On the other hand, abnormally distributed data was described using median, minimum, and maximum. Independent t-test was used for normally distributed data, comparison between two independent population independent t-test. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

RESULTS

All groups reported well-formed new dentin. However, the percentage of the surface area of the newly formed dentin in group I in both intervals were higher than group II. So, there was a statistically significant difference regarding the histomorphometric analysis of the percentage of the surface area of the newly formed dentin of group I compared with group II in both interval ($p< 0.05$), and between 1st and 2nd weeks of each group ($p< 0.05$). There was no statistically significant difference was seen among groups in the other variables. In addition, no statistically significant difference between the 1st and 2ed week of the same group regarding the other studied variable. However, an inferior response of group I at the 1ST week regarding the number of samples of organized TD was noticed, when compared with group II group at the 1st and 2nd weeks. In other pulpal findings, group II was also inferior to group I in the inflammatory response. (Table 2, Table 3)

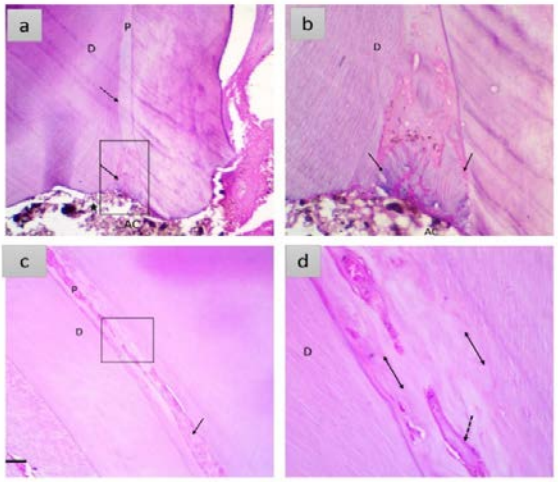

Figure (2): Light microscopic (LM) photo of (MTA) group of pulp tissue at 1st week. a. spicules of new dentin formation at the orifice (arrow) beneath pulp capping material (*). Apically, tertiary dentin almost obliterates the canal (dotted arrow). b. Higher magnification of the previous micrograph (a) inset showing scattered newly dentin formation of organized dentinal tubules (arrow). c. In the middle region, increase deposition of tertiary dentin on the periphery. Pulp is fibrotic (arrow). d. Higher magnification of the previous micrograph (c) inset showing complete dentin bridge(doubled arrow) of disorganized tubular pattern. Pulp shows hyalinization (dotted arrow). Pulp (P), Dentin(D), Access cavity (AC) {(a, c) x100 and (b, d) x400 magnification (all are H&E staining).

Table (2): Showing Comparison Between Different Studied Variables in Group I and II at 1st And 2nd Week.

		1st week		2nd week		t-test P1 value
		NO	%	NO	%	
Quantity of tertiary dentin formation	**Group I**					
	1	0	0	0	0	0.290
	2	3	37.5	2	25	0.864
	3	5	62.5	6	75	N.S.
	Group II					
	1	1	12.5	0	0	0.084
	2	3	37.5	4	50	0.958
	3	4	50	4	50	N.S.
	X^2	0.052		1.066		
	P2 value	0.974		0.586 N.S.		
The mineralized tissue formation	**Group I**					
	1	1	12.5	0	0	0.476
	2	3	37.5	3	37.5	0.788
	3	4	50	5	62.5	N.S.
	Group II					
	1	2	25	1	12.5	0.586
	2	3	37.5	4	50	0.745
	3	3	37.5	3	37.5	N.S.
	X^2	0.476		0.586		
	p value 2	0.788 N.S.		0.745 N.S.		
Morphology of dentinal tubules (DT)	**Group I**					
	1	3	37.5	1	12.5	1.5
	2	2	25	2	25	0.472
	3	3	37.5	5	62.5	N.S.
	Group II					
	1	2	25	1	12.5	0.66
	2	1	12.5	2	25	0.716
	3	5	62.5	5	62.5	N.S.
	X^2	1.033		0.0		
	p value 2	0.596 N.S.		1.0 N.S.		
Inflammation	**Group I**	4	50.0	5	62.5	0.41
	1	4	50.0	3	37.5	0.565
	2	0	0.0	0	0.0	
	3					
	Group II					
	1	3	37.5	4	50.0	0.41
	2	5	62.5	4	50.0	0.565
	3	0	0.0	0	0.0	
	X^2	0.41		0.41		
	p value 2	0.565		0.565		

X^2= Chi square test
P was significant if ≤ 0.05
N.S. = Not significant
X^2-test 1 = comparison between the 1st and 2nd week in the same group
X^2-test 2 = comparison between group I and II at the same time

Table (3): Showing Comparison Between Different Studied Variables in Group I And II At 1st And 2nd Week of The Histomorphometry Analysis.

		First week	Second week	P1 value
Surface area of the newly formed dentin	Group I			
	Range	34-57	48-53	2.14
	Mean	41.13	49.75	0.017*
	SD	9.86	1.83	
	Group II			
	Range	8-26	25-51	5.22
	Mean	18.50	35.25	0.001*
	SD	6.46	8.84	
	t-test	8.25	6.22	
	p2 value	0.001*	0.0013*	

T= student t-test
P was significant if ≤ 0.05
* Significant difference
N.S. = Not significant
t-test 1 = comparison between the 1st and 2nd week in the same group
t-test 2 = comparison between group I and II at the same time.

First Week

Group I

Continuous dentin bridge was found in half of the specimens (Fig 2.b, Fig 2.d). 62.5% of the specimens showed increased deposition of tertiary dentin along each other till obliterating the pulp (Fig 2.a). Dentin of organized tubular pattern was in 37.5% of the specimens (Fig 2.b). 50% of the specimens were free of the inflammation with no cases reported severe inflammation. The mean of the percentage of the dentin surface area was 41.13±9.86

Group II

Continuous dentin bridge was found in 37.5% of the specimens (Fig 3.c). 50% of the specimens showed increased deposition of tertiary dentin

along each other till obliterating the pulp (Fig 3.a) while 37.5% demonstrated increased deposition with no obliteration (Fig 3.d). Dentin of organized tubular pattern was observed in 62.5% of the specimens (Fig 3.b). 37.5% of the specimens were free of the inflammation with no cases reported severe inflammation. The mean of the percentage of the dentin surface area was 18.50± 6.46

There was a statistically significant difference in the percentage of dentin surface area between group I and group II in the first week ($P < 0.05$).

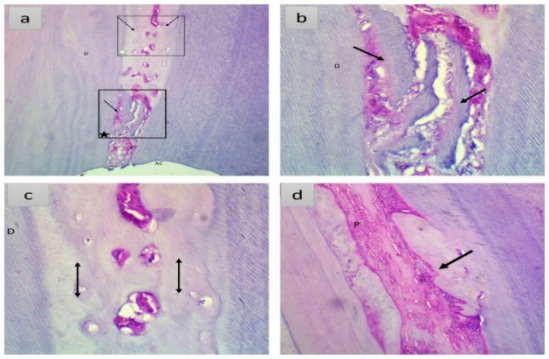

Figure (3): Light microscopic (LM) photo of (SIM-CH) group of pulp tissue at 1st week. a. spicules of new dentin formation at the orifice (arrow) lined by tertiary dentin that almost obliterates the canal (dotted arrow). Apically narrow pulp could be noticed. b. higher magnification of the rectangular box with black star in the previous micrograph (a) inset showing scattered newly dentin formation of organized dentinal tubules (arrow). c. higher magnification of the rectangular box with white star in the previous micrograph (a) inset showing complete dentin bridge (doubled arrow) formed by deposition of tertiary. Notice tertiary dentin is of osteodentin type. d. Deposition of tertiary dentin (arrow) on both sides with no areas of complete obliteration at mid region. Pulp (P), Dentin(D), Access cavity (AC).{(a, d) x100 and (b, c) x400 magnification (all are H&E staining).

Second Week

Group I

Continuous dentin bridge was found in 62.5% of the specimens (Fig 4.c, Fig 4.d). 75% of the specimens showed increased deposition of tertiary dentin along each other till obliterating the pulp (Fig 4.a. Fig 4.d). Dentin of organized tubular pattern was observed in 62.5% of the specimens (Fig 4.b). 62.5% of the specimens were free of the inflammation. The mean of the percentage of the dentin surface area was 49.75±1.83.

There was a statistically significant difference between 1st and 2nd week of group I in the percentage of the surface area of the newly formed dentin ($P < 0.05$).

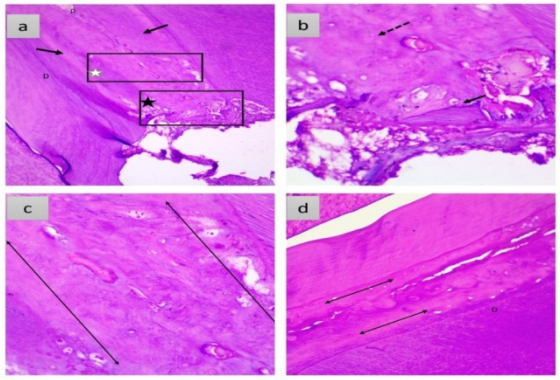

Figure (4): Light microscopic (LM) photo of (MTA) group of pulp tissue at 2nd week. a. Complete dentin formation at the orifice, and pulp space is almost obliterated by tertiary dentin coronally (arrow). b. Higher magnification of the rectangular box with black star in previous micrograph (a) inset showing scattered newly dentin formation of organized dentinal tubules (arrow) while the rest of dentin shows disorganized pattern (dotted arrow). c. Higher magnification of the rectangular box with white star in previous micrograph (a) inset showing complete dentin bridge(doubled arrow) formed by deposition of tertiary (arrow). d. Deposition of tertiary dentin which Almost obliterated the pulp (arrow) (d). Pulp (P), Dentin(D), Access cavity (AC). {(a, d) x100 and (b, c) x400 magnification (all are H&E staining).

Group II

Continuous dentin bridge was found in 37.5% of the specimens (Fig 5.c). 50% of the specimens showed increased deposition of tertiary dentin along each other till obliterating the pulp (Fig 5.a. Fig 5.d). Dentin of organized tubular pattern was observed in 62.5% of the specimens (Fig 5.b). 50% of the specimens were free of the inflammation, while inflammation restricted to the orifice was seen in 37.5%. The mean of the percentage of the dentin surface area was 35.25±8.84

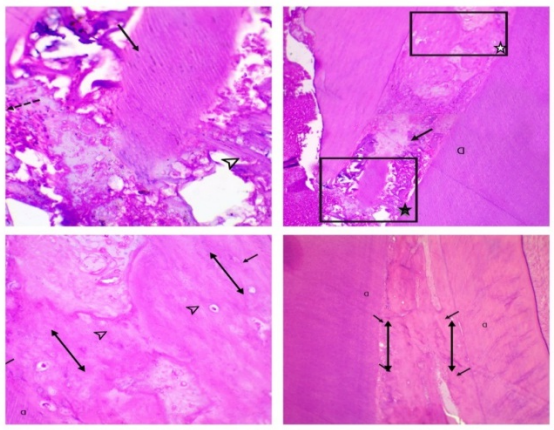

Figure (5): Light microscopic (LM) photo of (SIM-CH) group of pulp tissue at 2nd week. a. In the coronal reign incomplete dentin bridge (arrow) in contact to the pulp capping material (*). More apically, the pulp is almost occupied by tertiary dentin (arrow). b .higher

magnification of the rectangular box with black star in previous micrograph (a) inset showing incomplete bridge formation with regular dentinal tubules(arrow), and other areas of newly formed dentin bridging the space between the newly formed dentin and the original dentin (arrowhead) notice inflammatory cells (dotted arrow).higher magnification of the rectangular box of white star in previous micrograph (a) inset continuous dentin bridge (arrowhead & doubled arrow).d. Almost obliteration the pulp by deposition of tertiary dentin (arrow & double arrow) at mid region . Pulp (P), Dentin(D), Access cavity (AC). {(a, d) x100 and (b, c) x400 magnification (all are H&E staining).

There was a statistically significant difference between 1st and 2nd week of group II in the percentage of the surface area of the newly formed dentin ($P < 0.05$).

There was a statistically significant difference in the percentage of dentin surface area between group I and group II in the second week ($P < 0.05$).

DISCUSSION

Teeth with reversible pulpitis can be retained for longer time and at lower costs through applying DPC technique. The outcome over longer-term is highly influenced by the choice of the capping material (1). Therefore, finding a material with excellent properties has been an issue.

In this study, a comparison was done to discover the effect of a novel material, which consists of simvastatin mixed with calcium hydroxide (SIM-CH), to MTA as it is considered a reference material for DPC (22). As far as it is known, no study has tested this novel mixed material before.

In the current study, we investigated the quantity of the formed dentin, the continuity, and the morphology of dentinal tubules and the extent of the inflammation.

The findings indicated that there is no statistically significant difference between the tested materials in all tested parameters except for the percentage of the surface area of the newly formed dentin. There was a statistically significant difference regarding the histomorphometric analysis of the percentage of the surface area of the newly formed dentin of group I compared with group II and a statistically significant difference was seen in each group between 1ST and 2nd weeks. However, pulp capped with MTA formed a well-formed dentin that almost obliterated the canal. On the other hand, SIM-CH group demonstrated more orderly DTs, and more inflammation restricted to the areas beneath the exposure site.

Rabbits, especially New Zealand white species, were the animal of choice because their incisors are larger than rats. So, this will facilitate the restorative procedure. Besides, the structure of their teeth and jaw are similar to human ones (23). Histologically, the dental papilla inside the pulp is composed of mesenchymal tissue and odontoblasts which are producing primary dentine with typical dentinal tubules(24). Rabbits are suitable for the experiments because they meet international standards for dental research. They are nonaggressive. In comparison with larger animals, they are very cost-effective and available and animals (25). Besides, During dental treatment, vital pulp therapy is also applicable to rabbits' teeth and calcium hydroxide cement is perfectly used to treat exposed pulp (26).

In the present study, the evaluation period intervals were 1 and 2 weeks to prevent the prompt formation of secondary and tertiary dentin, and tooth wearing from negatively affecting the results of the study. Maxillary incisor teeth grow rapidly at an average rate of 2 mm per week to compensate tooth wearing (27).

The finding of this study revealed that group I (MTA) formed complete dentin bridge that almost obliterated the pulp chamber with tertiary dentin. This is in accordance with the finding of Muruganandhan J, et al (28).

This finding could be attributed to that the continued release of calcium ions during setting time of MTA material (29), although it is not component of MTA (30), has the ability to stimulate several signaling molecules, such as transforming growth factor-β (TGF-β), macrophage colony-stimulating factor (MCSF). Calcium ions also can form crystals that attract fibronectin, which controls cellular adhesion and differentiation (31). In addition, MTA can uncouple and activate growth factors nested in the proximal dentin (32) and also increase the secretion of TGF-β1 from pulp cells (33). TGF-β1 is the main role in reparative dentinogenesis as stimulate the migration of progenitor cells (34) and accelerate odontoblast cells differentiation (35).

The current study showed that there was non-significant difference between both groups in quantity and continuity of mineralized tissue formation.

CH can activate tissue enzymes which lead to tissue mineralization. This is done through the elevated pH of calcium hydroxide which activates alkaline phosphatase (36). It also solubilizes proteins from dentin matrix, such as bone morphogenic protein (BMP) and transforming growth factor-beta one (TBF-β1), due to high alkalinity. As a result, the release of these bioactive molecules is an important factor in dentin bridge formation (37).

Besides, a study done by Dianat, Mashhadiabbas (38) showed that complete dentin bridge was reported in all of the rats' teeth treated with 1.5% simvastatin. In addition, Pettiette, Zhong (39) concluded in his study that systemic administration of statins could stimulate odontoblastic activity as it was noticed that patients who were treated with statin showed a significant loss of vertical height of the pulp chamber and increased in

pulp calcification. Therefore, the results may be affected by the presence of simvastatin in group II.

The result of this study demonstrated that tubular dentin formation in SIM-CH group after one week period was higher than that of MTA group. Previous studies have confirmed that MTA has a superiority in forming tubular dentin with fewer tunnels (28, 40).

Okamoto, Sonoyama (41) have reported that statin enhances odontoblastic differentiation through stimulating dentin sialophosphoprotein (dspp) gene expression, and osteocalcin (OCN). Moreover, simvastatin boosted alkaline phosphatase (ALPase) activity and increased mineral nodule formation (42).

So, the early enhancement of the tubular dentin formation could be explained by the effect of simvastatin on gene expression of dentin sialophosphoprotein (dspp), and osteocalcin (OCN).

In this study, there was non-statistically significant difference between both groups about the inflammation. MTA group seemed to produce more samples free of inflammation.

In previous studies, MTA reported lower inflammatory response compared to CH(43, 44). Besides, according to Aeinehchi et al (2003)(45), MTA produced lower hyperemia than CH.

Simvastatin shows anti-inflammatory, antiviral and antimicrobial effects(46-48). It prevents the adhesion and extravasation of leukocytes in inflammation areas. As a result, the stimulation of T cells and inflammatory cytokines, such as IL-1β, IL-6 and tumour necrosis factor-α (TNF-α) decreases(49).

On the basis of the findings of this study, the null hypothesis that the novel combination between simvastatin and calcium hydroxide (SIM-CH) would show no significant difference in the histological success compared with MTA when used as direct pulp capping materials was accepted. However, there was a statistically significant difference regarding the histomorphic analysis of the percentage of the surface area of the newly formed dentin of group I compared with group II.

Although our findings represented SIM-CH as a novel DPC material, it should be considered that the present research was conducted in non-carious teeth with healthy pulp free of inflammation. Besides, although the greater similarity between rabbit and human pulp, pulp orifice is narrow to be exposed to the DPC materials.

Therefore, further clinical trials on human pulp are recommended to get the benefit of the large surface area of the pulp access, exposed to the material. Furthermore, carious teeth with pulpal inflammation are better to be used to confirm the current results. Different concentration of the SIM-CH should be tried.

CONCLUSIONS

SIM-CH could be a promising DPC material alternative to MTA in direct pulp-capping procedure.

CONFLICT OF INTEREST

The authors declared that they do not have conflict of interest.

FUNDING

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REFERENCE

1. Cushley S, Duncan H, Lappin M, Chua P, Elamin A, Clarke M, et al. Efficacy of direct pulp capping for management of cariously exposed pulps in permanent teeth: a systematic review and meta-analysis. Int Endod J. 2021;54:556-71.
2. Bresolin CR, Marques RS, Okamura B, Costa C, Moura-Netto C, Lara JS, et al. Efficacy of an iodoform-based filling material for pulpectomy of primary teeth–a 24-month non-inferiority randomized clinical trial. Int J Paediatr Dent. 2021.
3. Agrawal V. Calcium hydroxide: A miracle munition. Indian J Dent Res Rev. 2011;1:16-8.
4. Nosrat A, Seifi A, Asgary S. Pulpotomy in caries-exposed immature permanent molars using calcium-enriched mixture cement or mineral trioxide aggregate: a randomized clinical trial. Int J Paediatr Dent. 2013;23:56-63.
5. Dominguez MS, Witherspoon DE, Gutmann JL, Opperman LA. Histological and scanning electron microscopy assessment of various vital pulp-therapy materials. J Endod. 2003;29:324-33.
6. Mente J, Hage N, Pfefferle T, Koch MJ, Geletneky B, Dreyhaupt J, et al. Treatment outcome of mineral trioxide aggregate: repair of root perforations. J Endod. 2010;36:208-13.
7. Tran XV, Salehi H, Truong MT, Sandra M, Sadoine J, Jacquot B, et al. Reparative Mineralized Tissue Characterization after Direct Pulp Capping with Calcium-Silicate-Based Cements. Materials. 2019;12:2102.
8. Youssef AR, Emara R, Taher MM, Al-Allaf FA, Almalki M, Almasri MA, et al. Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. BMC Oral Health. 2019;19:133.
9. Dianat O, Mashhadiabbas F, Ahangari Z, Saedi S, Motamedian SR. Histologic comparison of direct pulp capping of rat molars with MTA and different concentrations of simvastatin gel. J Oral Sci. 2018;60:57-63.
10. Liu C, Wu Z, Sun Hc. The effect of simvastatin on mRNA expression of transforming growth factor-β1, bone morphogenetic protein-2 and vascular endothelial growth factor in tooth extraction socket. Int J Oral Sci. 2009;1:90-8
11. Wang CY, Liu PY, Liao JK. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. Trends Mol Med. 2008;14:37-44.

12. Aminabadi NA, Maljaei E, Erfanparast L, Aghbali AA, Hamishehkar H, Najafpour E. Simvastatin versus calcium hydroxide direct pulp capping of human primary molars: a randomized clinical trial. J Dent Res Dent Clin Dent Prospects. 2013;7:8-14.
13. Chithra P. Histological evaluation of pulpal response to direct pulp capping using statins with α tricalcium phosphate and mineral trioxide aggregate on human teeth pulp tissue: An In Vivo study. PhD. Thesis. Tamilnadu Government Dental College and Hospital, Chennai, India. 2018.
14. Likitpongpipat N, Sangmaneedet S, Klanrit P, Noisombut R, Krisanaprakornkit S, Chailertvanitkul P. Promotion of Dental Pulp Wound Healing in New Zealand White Rabbits' Teeth by Thai Propolis Product. J Vet Dent. 2019;36:17-24.
15. Salah M, Kataia MM, Kataia EM, El Din EA, Essa ME. Evaluation of eggshell powder as an experimental direct pulp capping material. Futur Dent J. 2018;4:160-4.
16. Dey P. Decalcification of bony and hard tissue for histopathology processing. In: Basic and Advanced Laboratory Techniques in Histopathology and Cytology. Switzerland: Springer; 2018. pp. 35-9.
17. Téclès O, Laurent P, Zygouritsas S, Burger A-S, Camps J, Dejou J, et al. Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. Arch Oral Biol. 2005;50:103-8.
18. Islam R, Toida Y, Chen F, Tanaka T, Inoue S, Kitamura T, et al. Histological evaluation of a novel phosphorylated pullulan-based pulp capping material: An in vivo study on rat molars. Int Endod J. 2021;54:1902-14.
19. Yang IS, Lee DS, Park JT, Kim HJ, Son HH, Park JC. Tertiary dentin formation after direct pulp capping with odontogenic ameloblast-associated protein in rat teeth. J Endod. 2010;36:1956-62.
20. Likitpongpipat N, Sangmaneedet S, Klanrit P, Noisombut R, Krisanaprakornkit S, Chailertvanitkul P. Promotion of dental pulp wound healing in New Zealand white rabbits' teeth by Thai propolis product. J Vet Dent. 2019;36:17-24.
21. Trongkij P, Sutimuntanakul S, Lapthanasupkul P, Chaimanakarn C, Wong RH, Banomyong D. Pulpal responses after direct pulp capping with two calcium-silicate cements in a rat model. Dent Mater J. 2019;38:584-90.
22. Nair P, Duncan H, Pitt Ford T, Luder H. Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial. Int Endod J. 2008;41:128-50.
23. Mapara M, Thomas BS, Bhat K. Rabbit as an animal model for experimental research. Dent Res J. 2012;9:111-8.
24. Ali ZH, Mubarak R, Hassan HA, Rizk NM, Hefnawy M, Awad AM, et al. 226 Histomorphological Study of Dentine Pulp Complex of Continuously Growing Teeth in the Rabbits. Culture 2012;1590:1592.
25. Davidović L, Ćuk M, Živković-Sandić M, Grga Đ, Živković S. The influence of liners on the pulp inflammation. Srp Arh Celok Lek. 2015;143:261-6.
26. Meredith A. Rabbit dentistry. EJCAP. 2007;17:55-62
27. Capello V, Gracis M. Rabbit and rodent dentistry. Lake Worth: Zoological Education Network; 2005.
28. Muruganandhan J, Sujatha G, Poorni S, Srinivasan MR, Boreak N, Al-Kahtani A, et al. Comparison of Four Dental Pulp-Capping Agents by Cone-Beam Computed Tomography and Histological Techniques—A Split-Mouth Design Ex Vivo Study. Appl Sci. 2021;11:3045.
29. Borges R, Sousa-Neto M, Versiani M, Rached-Júnior F, De-Deus G, Miranda C, et al. Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test. Int Endod J. 2012;45:419-28.
30. Accorinte M, Loguercio AD, Reis A, Carneiro E, Grande R, Murata S, et al. Response of human dental pulp capped with MTA and calcium hydroxide powder. Oper Dent. 2008;33:488-95.
31. Hsiao CT, Cheng HW, Huang CM, Li HR, Ou MH, Huang JR, et al. Fibronectin in cell adhesion and migration via N-glycosylation. Oncotarget. 2017;8:70653.
32. Tomson PL, Grover LM, Lumley PJ, Sloan AJ, Smith AJ, Cooper PR. Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. J Dent. 2007;35:636-42.
33. Laurent P, Camps J, About I. BiodentineTM induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012;45:439-48.
34. Howard C, Murray PE, Namerow KN. Dental pulp stem cell migration. J Endod. 2010;36:1963-6.
35. Qureshi A, Soujanya E, Nandakumar P. Recent advances in pulp capping materials: an overview. J Clin Diagn Res. 2014;8:316-21.
36. Estrela C, Estrela CRdA, Hollanda ACB, Decurcio DdA, Pécora JD. Influence of iodoform on antimicrobial potential of calcium hydroxide. J Appl Oral Sci. 2006;14:33-7.

37. Hilton TJ. Keys to clinical success with pulp capping: a review of the literature. Oper Dent. 2009;34:615-25.
38. Dianat O, Mashhadiabbas F, Ahangari Z, Saedi S, Motamedian SR. Histologic comparison of direct pulp capping of rat molars with MTA and different concentrations of simvastatin gel. J Oral Sci. 2018;60:57-63.
39. Pettiette MT, Zhong S, Moretti AJ, Khan AA. Potential correlation between statins and pulp chamber calcification. J Endod. 2013;39:1119-23.
40. Shayegan A, Petein M, Vanden Abbeele A. The use of beta-tricalcium phosphate, white MTA, white Portland cement and calcium hydroxide for direct pulp capping of primary pig teeth. Dent Traumatol. 2009;25:413-9.
41. Okamoto Y, Sonoyama W, Ono M, Akiyama K, Fujisawa T, Oshima M, et al. Simvastatin induces the odontogenic differentiation of human dental pulp stem cells in vitro and in vivo. J Endod. 2009;35:367-72.
42. Jia W, Zhao Y, Yang J, Wang W, Wang X, Ling L, et al. Simvastatin promotes dental pulp stem cell–induced coronal pulp regeneration in pulpotomized teeth. J Endod. 2016;42:1049-54.
43. Li Z, Cao L, Fan M, Xu Q. Direct Pulp Capping with Calcium Hydroxide or Mineral Trioxide Aggregate: A Meta-analysis. J Endod. 2015;41:1412-7.
44. Paula AB, Laranjo M, Marto C-M, Paulo S, Abrantes AM, Casalta-Lopes J, et al. Direct pulp capping: What is the most effective therapy?—Systematic review and meta-analysis. J Evid Based Dent Pract. 2018;18:298-314.
45 Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS. Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. Int Endod J. 2003;36:225-31.
46. Shah SR, Werlang CA, Kasper FK, Mikos AG. Novel applications of statins for bone regeneration. Natl Sci Rev. 2015;2:85-99.
47. Stancu C, Sima A. Statins: mechanism of action and effects. J Cell Mol Med. 2001;5:378-87.
48. Ting M, Whitaker EJ, Albandar JM. Systematic review of the in vitro effects of statins on oral and perioral microorganisms. Eur J Oral Sci. 2016;124:4-10.
49. Estanislau IMG, Terceiro IRC, Lisboa MRP, de Barros Teles P, de Sousa Carvalho R, Martins RS, et al. Pleiotropic effects of statins on the treatment of chronic periodontitis–a systematic review. Br J Clin Pharmacol. 2015;79:877-85.