2007년 2월
박사학위 논문

Mineral Trioxide Aggregate (MTA)에 의한 치수세포의 유전자 발현 변화

Gene Expression of Exposure to Mineral Trioxide Aggregate(MTA) on Dental Pulp Cells

조선대학교 대학원
치 의 학 과
최 유 석

Mineral Trioxide Aggregate (MTA)에 의한 치수세포의 유전자 발현 변화

Gene Expression of Exposure to Mineral Trioxide Aggregate(MTA) on Dental Pulp Cells

2007년 2월 25일

조선대학교 대학원

치 의 학 과

최 유 석

Mineral Trioxide Aggregate (MTA)에 의한 치수세포의 유전자 발현 변화

Gene Expression of Exposure to Mineral Trioxide Aggregate(MTA) on Dental Pulp Cells

지도교수 이 상 호

이 논문을 치의학 박사학위 신청 논문으로 제출함

2006년 10월

조선대학교 대학원

치 의 학 과

최 유 석

최유석의 박사학위 논문을 인준함

위원장	관동대학교	교수	이 종 갑
위원	조선대학교	교수	이 상 호
위원	조선대학교	교수	윤 정 훈
위원	조선대학교	교수	김 수 관
위원	조선대학교	교수	이 난 영

2006년 12월

조선대학교 대학원

Contents

국문초록 V

I. Introduction 1

II. Materials and methods

III. Results

IV. Discussion

V. Concussion

VI. Reference

List of Tables and Figures

Table 1. Primers used for RT-PCR

Fig. 1. Photograph and schematic drawing of transwell insert used in the six-well plates to prevent the direct physical contact between the pulp cells and the MTA.

Fig. 2. Survival and proliferation of dental pulp cells on MTA.

Fig. 3. Effects of MTA on alkaline phosphatase activity.

Fig. 4. A. Transcript alteration by MTA.
B. Intensities of bands mRNA shown in A were normalized with respect to those for GAPDH.

국문초록

Mineral Trioxide Aggregate (MTA)에 의한 치수세포의 유전자 발현 변화

최 유 석
지도교수 : 이상호
조선대학교 대학원 치의학과

치아 치수의 치유과 상아질 형성을 유도하는 여러 가지 약제가 사용되어 오고 있으나 각각 장단점이 있어 완벽한 치유를 기대할 수 있는 약제는 거의 없는 실정이다. 근래에 치수의 치유를 촉진하고 상아질 형성을 유도할 수 있는 mineral trioxide aggregate (MTA)가 개발, 시판되고 있는데, MTA는 치질과 조직 접합성이 좋아 변연봉쇄효과가 좋고 osteoblasts의 생물학적 반응을 자극하여 세포부착 능력을 증진시키므로써, 치수복조와 치근단형성술시 calcium hydroxide와 유사한 결과를 보인다고 알려져 있다. 이 연구의 목적은 MTA의 치수 세포에서 치유를 촉진하고 상아질형성을 유도하는지 여부와 그 기전을 규명하는데 있다.

White ProRoot MTA를 사용하여 사람치수세포에서 primary explant culture를 시행하였다. 세포의 증식능을 확인하기 위해 MTA assay를 시행하였고 석회화 여부를 확인하기 위해 Von Kossa염색을 시행하였다. 이후 석회화에 중요한 효소중 하나인 alkaaline phosphatase acitivity를 측정하였다. 뼈나 상아질 형성에 관여하는 유전자 발현을 관찰하기 위해 치수세포에서 RNA를 추출하고 RT-PCR을 수행하였다. 결과는 다음과 같다.

MTA는 치수세포에 non-cytotoxic 하였다. Calcium deposit는 관찰되지 않았다. MTA는 alkaline phosphatase activity를 증가시켰다. RT-PCR결과 alkaline phosphatase, DSPP 그리고 collagen type I 발현이 관찰되었으나 유의성은 없었다. 그러나 osteonectin (SPARC)발현을 증가시켰다.

이상의 결과를 종합하여 보면, 이 연구에 사용한 dental pulp culture system은 MTA를 포함한 치과재료의 처리 후 치수세포의 성장과 분화 그리고 상아질 형성 유도 기전을 연구하는 데 유용한 모델로 사용할 수 있다. MTA는 치수세포에 세포독성을 유도하지 않으며, alkaline phosphatase의 활성도와 유전자 발현 그리고 osteonectin (SPARC) 유전자 발현을 증가시켜 수복상아질을 형성하는 것으로 사료된다.

I. Introduction

When there is a pulp exposure, the tissue responds by laying down a tertiary dentin matrix which may be reactionary or reparative, beneath the site of injury (1). Surviving odontoblasts in response to environmental stimuli will secrete reactionary dentin. The ability to induce dentinogenesis in a controlled and timely manner through a remaining wall of intact dentin would be therapeutically useful(1-3). This concept of using biologic agents to control pulpal response across an existing layer of dentin or a transdentinal approach is a relatively new approach to a long recognized clinical problem(4,5). Stimulation of a specific cellular response in the dentin pulp complex at the site of injury would allow a biologically directed approach to tissue repair rather than a simple mechanical approach. New strategies based on these approaches will have to address the problems of delivery and control of the bioactive molecules(2,3,5)

Calcium hydroxide has long been the "gold standard" for inducing pulpal repair(6). Its effectiveness at promoting dentinal bridge formation over small pulpal exposure sites is believed to be related to a combination of antimicrobial activity (attributed to high pH) and its ability to stimulate tertiary dentin formation (attributed to the release of calcium ions). Recently, mineral trioxide aggregate (MTA) has been proposed as an alternative to calcium hydroxide for pulp regeneration. *In vitro* and *in vivo* studies suggest that MTA may be more effective at inducing dental hard tissue formation than calcium hydroxide, possibly via a physicochemical reaction in which released calcium ions react with tissue phosphates to form hydroxyapatite(7,8).

Mineral trioxide aggregate (MTA) is being widely used for root-end fillings, pulp capping, perforation repairs, and other endodontic procedures. Because of its high pH value during its fresh and setting stage, MTA caused adjacent cell lyse and medium protein denature. After setting, MTA showed favorable biocompatibility, with no effect on cell morphology and limited impact on cell growth. MTA had a similar effect to calcium hydroxide when implanted in rat subcutaneous connective tissue (9). When MTA was used as a root-end filling material, fibrous connective tissue and thin layers of hard tissue formed in direct contact to it (10,11). Formation of cementum and periodontal ligament fibers was also observed on its surface (12,13). MTA stimulates a biologic response in osteoblasts (14,15) and also provides a biocompatible surface for cell adhesion (14,16). MTA produces the same results as calcium hydroxide on pulp capping (3,17) and apexification (13). MTA has also

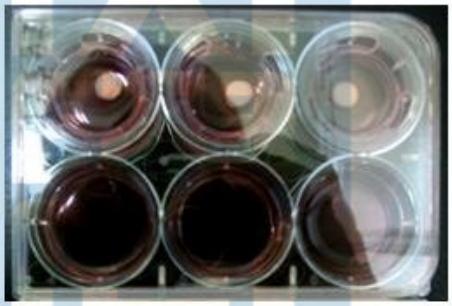
been successfully used for repairing external root resorption (18) and furcation and root perforations (6,19).

Although clinically, MTA has been shown to induce pulp tissue regeneration, the mechanisms by which MTA influences cell function are not known. The purpose of the present study is to assess the effects of MTA materials on the survival of and osteogenic or dentinogenic gene expression in human dental pulp cells. We also examined the effects of these materials on cellular alkaline phosphatase activity as a potential indicator of dentinogenesis.

II. Materials and methods

1. MTA specimen

A commercial brands of white ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland) was used. The cement was prepared according to the manufacturer's specifications, and their manipulation was carried out in a laminar flow hood under aseptic conditions. The pellet of cement was allowed to set for 24 h at 37^0C in a humidified CO_2 incubator for final setting. Subsequently, the pelletes were sterilized using EO gas. The size of each resultant pellet was approximately 1 cm x 1 cm. In one set of the specimens, the fresh pellet was immediately placed over a transwell insert measuring 24 mm in diameter (Corning, New York, NY, USA) that fits one well of a six-well cell culture plate, as depicted (Fig. 1). The transwell insert contains a permeable membrane (0.4 um-pore size) and was used to prevent direct physical interaction between the cells and the specimens while allowing for soluble compounds from the specimens to reach the cells (Fig. 1). Untreated controls were cells cultured in a six-well plate, with transwell inserts, but without the MTA specimen.



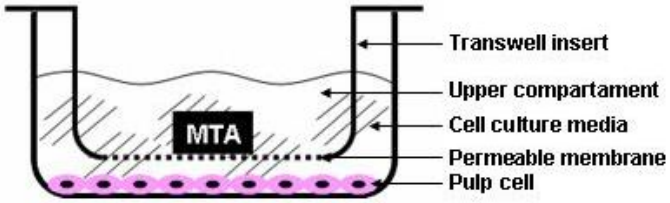


Fig. 1. Photograph and schematic drawing of transwell insert used in the six-well plates to prevent the direct physical contact between the pulp cells and the MTA.

2. Culture of dental pulp cells

All teeth used in this study were immature third molars extracted during normal treatment of 16-year-old patients. The teeth were all normal, freshly extracted, and used with the patients' informed consent. Immediately after the extraction, the teeth were swabbed with 70% (v/v) alcohol and then washed with sterile phosphate-buffered saline (PBS, 0.01 M, pH 7.4). The teeth were then transferred into a laminar flow tissue culture hood in order to perform the rest of the procedures under sterile conditions. The apical part of the teeth was removed with sterile scalpels and the dental pulps were gently removed with forceps. Dental pulps were minced with scalpels and rinsed with PBS. The explants were cultured in 100 mm diameter culture dishes (Becton Dickinson Labware, Lincoln Park, NJ) in modified Eagle's medium (MEM) (GIBCO) containing 10% heat inactivated fetal bovine serum (FBS), 100 ug/ml of streptomycin, and 100 U/ml of penicillin. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2, 95% air. The culture medium was changed every other day. Confluent cultures were collected by trypsinization (0.2% trypsin and 0.02% EDTA) and subcultured.

3. Cell Viability Assay (MTT assay)

Cells were cultured in 24-well plates at a density of $3x10^4$ cells/well. Twenty-four hours later, cells were treated with MTA for 12 h or 24 h. Cell viability was determined by using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) cell proliferation assay (measured at 570 nm).

4. Von Kossa staining

After dental pulp cells were fixed with 70% ethanol for 1 h, the culture dishes were washed with distilled water and then they were treated with 1% AgNO3 for 20 min. After they were washed with distilled water they were treated with 2.5% sodium thiosulfate for 5 min. The samples were then examined without counterstaining or after Mayer's hematoxylin counterstaining.

5. Alkaline phosphatase activity

Cells were seeded in 6-well plates at a density of 1 x 10^5cells per well and cultured in complete medium containing MTA or transwell only. After 2 or 4 days in culture, the cell layers were rinsed with PBS, scraped into 1 ml buffer (10 mM TRIS. HCl, 5 mM MgSO4, 0.1% Triton X-100, 0.1% NaNO3), frozen (-20°C) and thawed three times, sonicated for 5 min to disrupt cell membranes, and centrifuged (4,000g) at 4 C for 15 min. ALP activity was determined by the hydrolysis of p-nitrophenyl phosphate in 2-amino-2-methyl-1-propanol buffer (pH 10.4) at 37°C for 30 min. Absorbance at 405 nm was measured with a spectrophotometer (Bio-Rad 3550 microplate Reader). ALP activity was corrected for the total protein content (determined by the Bradford method with bovine serum albumin as a standard) and was expressed as nano moles of para nitrophenol per milligram of total protein.

6. Isolation of total RNA and RT-PCR analysis

The total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The integrity of RNA was verified following electrophoresis on denaturing agarose gel.

Semi-quantitative RT-PCR analysis was used to confirm the expression of several genes. Total RNA was isolated using TRIzol reagent(Invitrogen, Carlsbad, CA) and RT-PCR was performed using SuperScript One-Step RT-PCR Systems

(Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Specific primers used for RT-PCR are shown in Table 1. RT-PCR was performed by using the following conditions: One cycle of 30 min at 50°C, 1 cycle of 2 min at 94°C, and 30 cycles of 30 sec at 94°C , 30 sec at 60°C, 30 sec at 72°C, with a final extension of 10 min at 72°C. For caspase 7 and GAPDH, PCR reactions were performed for 25 cycles. The PCR products were then run on 1.5% agarose gels and visualized by ethidium bromide staining.

Table 1. Primers used for RT-PCR

Gene	Primers	Sequence (5' - 3')	Size (bp)
Alkaline phosphatase	Forward	GCACCTGCCTTACTAACTCC	626
	Reverse	CATGATCACGTCAATGTCC	
Osteonectin	Forward	ACTGAGAGCCCTCACACTGG	254
	Reverse	CAGCCAACTCGTCACAGTCC	
Osteocalcin	Forward	TGTGTGACCCAGGACTACC	617
	Reverse	CACCACTCATTGTTAGAAAGC	
Collagen type I	Forward	GATTGACCCCAACCAAGG	409
	Reverse	AGTGACGCTGTAGGTGAAGC	
DSPP	Forward	GCAGTGATGAATCTAATGGC	488
	Reverse	CTGATTTGCTGCTGCTGTCTGAC	
GAPDH	Forward	CCAACCTCATCCATGACAACTTTG	464
	Reverse	GTCATACCAGGAAATGAGCTTGACA	

III. Results

1. Cell Viability Assay (MTT assay)

The results of cell viability are shown in Fig. 2. The incubation time did not influence the cell growth after 5 days of culture. The proportion of MTA treated pulp cells exposed for 5 days to MTA was higher than the control cells. However, there was no difference in the percentages of live cells between the two groups. According to inn this result, exposure to MTA material would not induce cytotoxic response in the dental pulp cells.

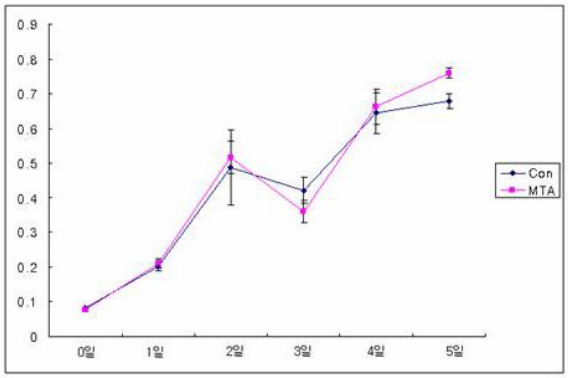


Fig. 2. Survival and proliferation of dental pulp cells on MTA.

2. Von Kossa staining

Von Kossa staining did not reveal formation of calcium-positive mineralization nodule on both groups (data not shown).

3. Alkaline phosphatase activity

Alkaline phosphatase activity of each group gradually increased, but MTA induced slight levels of alkaline phosphatase activity, and did so for control pulp cells (Fig. 3). Maximal enzyme activity was detected after 4 days in culture for both cell populations, while this expression was delayed for 2 days with MTA.

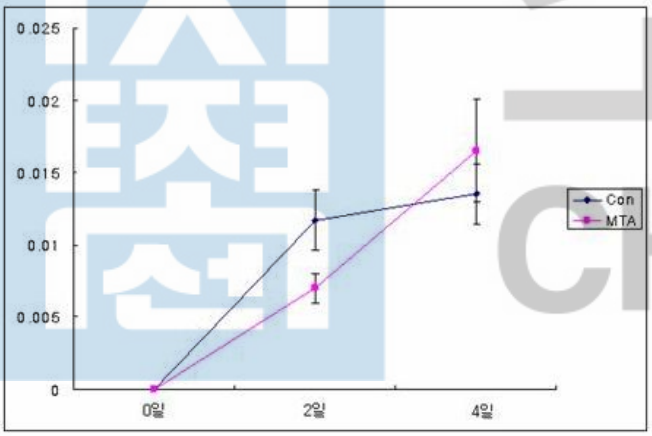
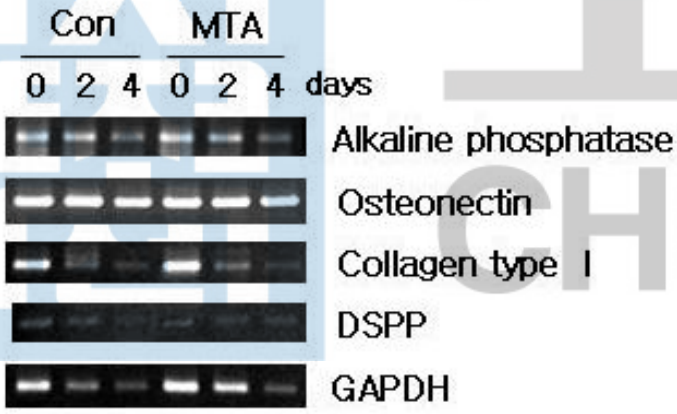
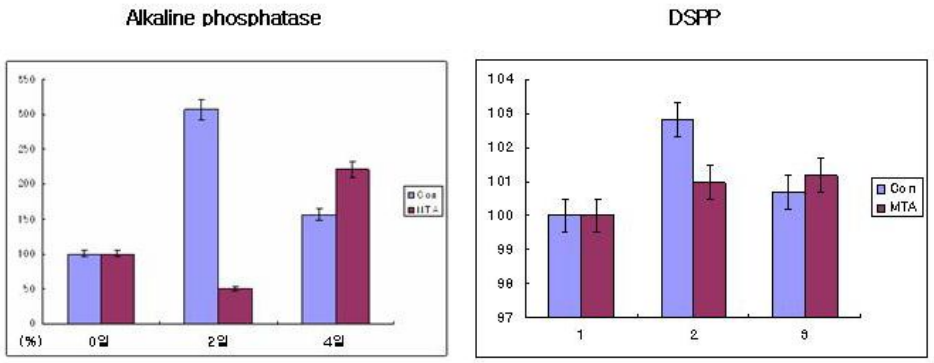


Fig. 3. Effects of MTA on alkaline phosphatase activity.

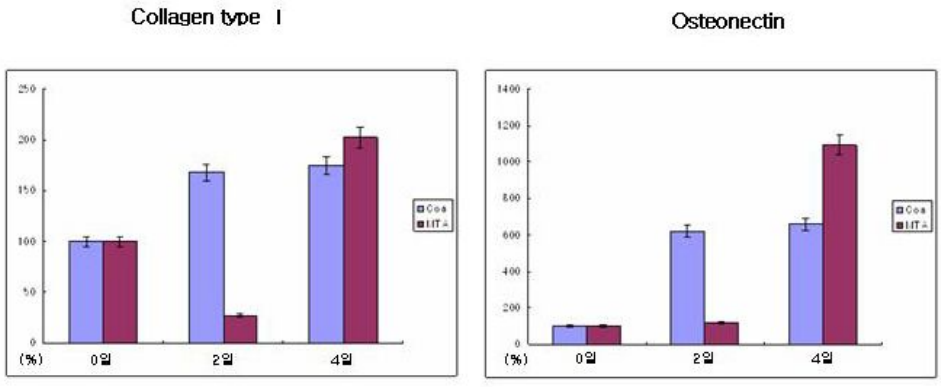
4. RT-PCR analysis

MTA had no effect on alkaline phosphatase expression after 2-day incubation. However, the expression of alkaline phosphatase was enhanced after 4-day incubation (Fig 4). This result corresponds to the alkaline phosphatase activity assay. Like the effect on expression of alkaline phosphatase, MTA also increased DSPP and collagen type I expression in all the tested period. However, there was no difference in the expression levels between the two groups ($P < 0.05$). Interestingly, the expression of osteonectin of MTA treated pulp cells exposed for 4 days to MTA was statistically higher than the control cells ($p<0.05$).





A



B

Fig. 4. A. Transcript alteration by MTA. B. Intensities of bands mRNA shown in A were normalized with respect to those for GAPDH.

IV. Discussion

Since its introduction to clinical dental practice in 1993, mineral trioxide aggregate (MTA) has gained acceptance as the material of choice for several clinical procedures. It has been shown to be an effective pulp capping material (6,17). In addition, MTA has been used to seal and repair root perforations (18,19), and to create an apical barrier in teeth with open apices (10,11). Success as a root-end filling material has also been reported (12,13). MTA seems to be an adequate material for these procedures because of its sealing ability when compared with other materials such as amalgam, IRM or SuperEBA (12,20). Several investigators have demonstraed that MTA is biocompatible with the surrounding tissues (13,14). It was shown to promote osteoblast activity (15) and was less cytotoxic than amalgam, IRM or SuperEBA (12, 20). Furthermore, MTA has been found to possess antibacterial activity (21).

The author hypothesized that MTA would induce dental pulp cells to express genes associated with dentin formation. This is based on studies in both human and dogs in which dentin deposition was histologically observed on MTA(12,17). Our results indicated that dental pulp cells are capable of expressing genes indicative of mineralization, specifically alkaline phosphatase and osteonectin. Alkaline phosphatase (ALP) is a phosphate-releasing protein, and its activity is considered an important indicator of bone formation and a phenotypic marker of osteoblasts (22). The observation that alkaline phosphatase transcript levels peak prior to any detectable increase in alkaline phosphatase activity following exposure to MTA was not unexpected; however, the finding that enzyme activity levels increased after a decline in transcript levels was unexpected. Further studies will need to be performed for a more careful examination of the correlation of transcript and protein stability with respect to the regulation of functional levels of phosphatase activity (23).

A major collagenous protein found in dentin, DSP and DPP, plays a regulatory role in the mineralization of dental hard tissue. The production of DSP and DPP is restricted to cells that function in a mineralizing capacity, including osteoblasts, odontoblasts, and cementoblasts (23). The strong showing of DSPP gene expression by dental pulp cells on both MTA and control is suggestive that the dentin formation process can proceed under experimental conditions. These results support MTA as being dentin-conductive by allowing the expression of genes and proteins consistent with the dentinogenesis process. The results of this investigation compare positively with the results of other studies that indicate a favorable biologic response by MTA

compared with other endodontic materials (9,13,17).

Changes in expression patterns of several transcripts associated with hard-tissue formation were also detected. Of the genes investigated in this study, ALP and osteonectin are most strongly associated with mineralization. ALP activity is essential for normal osteoblast function (15), and it is generally considered an early marker of mineralization. In this study ALP expression by dental pulp cells at days 2 and 4 appeared on MTA and control substrates. These results again suggest that MTA is permissive for dentinogenesis gene expression.

Osteonectin (SPARC) is a calcium binding matricellular glycoprotein expressed in odontoblasts but not by the pulp cells (24,25). Furthermore, an increasing level of SPARC was detected during the healing process of pulp tissue (26). ALP is an enzyme usually detected in mineralized tissue forming cells (22,27). Thus, an increase in SPARC and ALP synthesis might be considered as a marker of pulp cell differentiation.

SPARC is a phosphorylated glycoprotein, which is associated with development, tissue remodelling, and repair (28). It has been recently shown that odontoblasts, the only dental pulp cells expressing SPARC, demonstrated increased expression of SPARC in the initial stage of tertiary dentin formation (24-26). Although the function of SPARC in dentin formation is still unknown, it is suggested that odontoblasts may release SPARC to stimulate proliferation of some fraction of pulp cells to replace the injured due to dental caries or cavity preparation (24). SPARC may also regulate the production of extracellular matrix and matrix metalloproteinases (29,30) which might be involved in the modulation of matrix for dentinogenesis. Moreover, SPARC has been shown to play a role in differentiation of some cell types (31). Thus, upregulation of SPARC and ALP activity in dental pulp cells by the treatment of MTA, as demonstrated in this study, might also reflect the changes in their function or differentiation of these cells.

In conclusion, this dental pulp culture system may be useful in the future as a model for studying the mechanisms underlying dentin regeneration after the treatment of MTA. Exposure to MTA material would not induce cytotoxic response in the dental pulp cells. MTA could influence the behaviour of human pulp cells by increasing the ALP activity and SPARC synthesis.

References

1. Yamamura T. Differentiation of pulpal cells and inductive and influences of various matrices with reference to pulpal ound healing. J Dent Res 1985;64:530-40.
2. Tziafas D. Basic mechanisms of cytodifferentiation and dentinogenesis during dental pulp repair. Int J Dev Biol 1995;39:281-90.
3. Tziafas D, Smith AJ, Lesot H. Designing new treatment strategies in vital pulp therapy. J Dent 2000;28:77-92.
4. Smith AJ, Cassidy M, Perry H, Begue-Kirn C, Ruch JV, Lesot H. Reactionary dentinogenesis. Int J Dev Biol 1995;39:273-80.
5. Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med 2001;12:425-37.
6. Murray PE, Windsor LJ, Smyth TW, Hafez AA, Cox CF: Analysis of pulpal reactions to restorative procedures, materials, pulp capping, and future therapies. Crit Rev Oral Biol Med 2002;13:509-520.
7. Ham KA, Witherspoon DE, Gutmann JL, Ravindranath S, Gait TC, Opperman LA: Preliminary evaluation of BMP-2 expression and histologic characteristics during apexification with calcium hydroxide and mineral trioxide aggregate. J Endod 2005; 31:275-279.
8. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I: Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod 2005; 31:97-100.
9. Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabe PFE, Dezan JR E. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J Endod 1999;25:161-6.
10. Regan JD, Gutmann JL, Witherspoon DE. Comparison of Diaket and MTA when used as root-end filling materials to support regeneration of the periradicular tissues. Int Endod J 2002;35: 840-7.
11. Economides N, Pantelidou O, Kokkas A, Tziafas. Short-term periradicular tissue response to mineral trioxide aggregate (MTA) a root-end filling material. Int Endod J 2003;36:44-48.
12. Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root end filling in dogs. J Endod 1995;21:603-8.
13. Torabinejad M, Pitt Ford TR, McKendry DJ, Abedi HR, Miller DA, Kariyawasen SP. Histologic assessment of MTA as root end filling in monkeys. J Endod 1997;23:225-8.
14. Koh ET, McDonald F, Pitt Ford TR, Torabinejad M. Cellular response to mineral trioxide aggregate. J Endod 1998;24:543-7.

15. Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F. Mineral trioxide aggregate stimulates a biological response in human osteoblasts. J Biomed Mater Res 1997;37:432-9.
16. Zhu Q, Haglund R, Safavi KE, Spangberg LSW. Adhesion of human osteoblasts on root-end filling materials. J Endod 2000; 26:404-6.
17. Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS. Mineral trioxide aggregate (MTA) and calcium hydroxide a pulp-capping agents in human teeth: a preliminary report. Int Endod J 2003; 36:225-31.
18. White Jr C, Bryant N. Combined therapy of mineral trioxide aggregate and guided tissue regeneration in the treatment of external root resorption and an associated osseous defect. J Periodontol 2002;73:1517-21.
19. Holland R, Otoboni Filho JA, Souza V, Nery MJ, Bernabe PFE, Dezan JR E. Mineral trioxide aggregate repair of lateral root perforations. J Endod 2001;27:281-4
20. Keiser K, Johnson C, Tipton D. Cytotoxicity of mineral trioxide aggregated using human periodontal ligament fibroblasts. J Endod 2000; 26: 288-91.
21. Estrela C, Bammann LL, Estrela CRA, Silva RS, Pecora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal.
Braz Dent J 2000; 11: 3-9.
22. Kuru L, Griffiths GS, Petrie A, Olsen I. Alkaline phosphatase activity
is upregulated in regenerating human periodontal cells. J Periodontal Res 1999; 34:123-127.
23. Bonson S, Jeansonne BG, Lallier TE. Root-end Filling Materials Alter Fibroblast Differentiation. J Dent Res 2004; 83:408-13.
24. Reichert T, Storkel S, Becker K, Fisher LW. The role of osteonectin in human tooth development: an immunohistological study. Calcif Tissue Int 1992;50:468-72.
25. Begue-Kim C, Smith AJ, Loriot M, Kupferle C, Ruch JV, Lesot H. Comparative analysis of TGFbs, BMPs, IGF1, msxs, fibronectin, osteonectin, and bone sialoprotein gene expression during normal and in vitro-induced odontoblast differentiation. Int J Dev Biol 1994;38:405-20.
26. Itota T, Nishitani Y, Sogawa N, Sogawa C, Konishi N, Torii Y. Alteration of odontoblast osteonectin expression following dental cavity preparation. Arch Oral Biol 2001; 46:829-34.
27. Wlodarski KH, Reddi AH. Alkaline phosphatase as a marker of osteoinductive cells. Calcif Tissue Int 1986;39:382-5.
28. Yan Q, Sage EH. SPARC, a multicellular glycoprotein with important biological functions. J Histochem Cytochem 1999; 47:1495-505.
29. Tremble PM, Lane TF, Sage EH, Werb Z. SPARC, a secreted protein associated

with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. J Cell Biol 1993;121:1433-44.
30. Francki A, Bradshaw AD, Bassuk JA, Howe CC, Couser WG, Sage EH. SPARC regulates the expression of collagen type I and transforming growth factor-b1 in mesangial cells. J Biol Chem 1999;274:32145-52.
31. Bassuk JA, Birkebak T, Rothmier JD, Clark JM, Bradshaw A, Muchowski PJ, et al. Disruption of the Sparc locus in mice alters the differentiation of lenticular epithelial cells and leads to cataract formation. Exp Eye Res 1999;68:321-31.

저작물 이용 허락서

학 과	치의학	학 번	20057508	과 정	박사
성 명	한글: 최유석	한문 : 崔裕錫		영문 : Choi, You-Seok	
주 소	서울 관악구 신림 1동 1603-2				
연락처	E-MAIL :				
논문제목	한글 : Mineral Trioxide Aggregate (MTA)에 의한 치수세포의 유전자 발현 변화 영문 : Gene Expression of Exposure to Mineral Trioxide Aggregate(MTA) on Dental Pulp Cells				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(0) 조건부 동의( ) 반대( )

2007 년 2 월 일

저작자: 최 유 석 (서명 또는 인)

조선대학교 총장 귀하

감사의글

먼저 이 논문이 나오기까지 부족한 저를 지도해주고 이끌어주신 이상호 교수님께 감사의 글 올립니다. 그리고 구강병리학 교실 윤정훈 교소님과 소아치과 이난영 교수님께 감사드립니다.
항상 실험연구를 도와주고 반겨준 소아치과 의국원 여러분께 감사드립니다.
대학원 석사과정을 10년 전 치과보철학으로 마치고 다시 박사과정을 시작하고자할 때 망설이지 말고 한번해보라며 용기를 준 사랑하는 아내 홍은정씨께 고마운 마음 전합니다.
어렸을적부터 어머님은 늘 저에게 말씀하셨습니다. 너는 끝까지 공부하고 잘 살 것이라며 미래에 대한 희망과 의지를 불사르게 해주며 가르쳐주신 어머니 깊이 감사드립니다. 사랑합니다. 어머니....
항상 멀리서 지켜보며 응원해주고 울타리가 되어주신 아버님 고맙습니다. 건강하세요. 아버지. 사랑합니다.
내 인생 최고의 걸작품 고은아, 원일아 아빠는 늘 열심히 살며 공부하고 있다. 늘 건강하고 밝게 자라다오. 사랑한다.
우리 가족 형님 누나들 특히 대학원에 갈 때마다 늘 따뜻한 잠자리와 맛있는 아침을 준비 해준 큰 누나께 감사드립니다.
최유석 치과를 찾아주신 환자분들 ,늘 치과를 지키고 열심히 일하는 권소희 간호사,이선남 간호사, 고윤희 간호사께 감사드립니다.
마지막으로 정현구 박사님, 박헌동 박사님, 이창섭 교수님께 감사드립니다.

2006년 12월 8일 최 유석