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Study on Biocompatibility and Mineralization Potential of Capseal

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Purpose: Capseal I and Capseal II are calcium silicate and calcium phosphate based experimental root canal sealers. This study sought to evaluate the biocompatibility and mineralization potential of Capseal I and Capseal II.
Materials and Methods: The biocompatibility and mineralization related gene expression (alkaline phosphatase [ALP], bone sialoprotein [BSP], and osteocalcin) of Capseal I and Capseal II were compared using methylthiazol tetrazolium assay and reverse transcription-polymerization chain reaction analysis, respectively. The results were analyzed by Kruskal-Wallis test. A P-value of <0.05 was considered significant.
Result: Both Capseal I and Capseal II were favorable in terms of biocompatibility, influencing the messenger RNA expression of ALP and BSP.
Conclusion: Within the limitation of this study, Capseal is biocompatible, with mineralization promoting potential; thus, it could be a promising root canal sealer.

Key Words: Biocompatibility; Capseal I; Capseal II; Mineralization related gene expression

Introduction

Mineral trioxide aggregate has been introduced in the early 1990s in the endodontic field as root end filling material[1,2] and perforation repair material[3]. Since then, it has long been successfully used in clinical endodontics, with its use expanded to fields such as one-visit apexification[4], pulp capping[5], and pulpotomy. Mineral trioxide aggregate is known to be based on calcium silicate cement, and its main constituents are tricalcium silicate and dicalcium silicate[6]. Recently, many calcium silicate-based endodontic cements have been developed by various companies[7-9]. Capseal is one of the ex-

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perimental root canal sealers that are based on calcium silicate and calcium phosphate. An ideal root canal sealer should provide hermetic sealing of the root canal; it should be bacteriostatic, and it should neither shrink upon setting nor irritate the periradicular tissue[10]. Capseal was reported to have good root canal sealing ability[11] and biocompatibility[12-14] in previous studies.

Based on previous research demonstrating that calcium silicate based endodontic cement is biocompatible, and that it has potential to promote the formation of mineralized tissues[15,16], it is worth investigating the biocompatibility and its ability to promote mineralized tissue formation. This study sought to investigate the biocompatibility and osteogenic potential of the experimental root canal sealer, Capseal.

Materials and Methods

In this study, experimental root canal sealers Capseal I and Capseal II were used. The constituents of Capseal I and Capseal II are listed in Table 1. The main components of Capseal are calcium silicate and calcium phosphate. The liquid contains hydroxyprophyl methylcellulose.

1. Cell Culture and Cytotoxicity Test Using Methylthiazol Tetrozolium Assay

In this experiment, MG-63 human osteosarcoma cells were used. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% (wt/vol) antibiotics/antimycotics (100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B; Gibco) at 37°C in a humidified 5% (vol/vol) CO_2 atmosphere. One gram of Capseal I and II was mixed with liquid in a 1 : 1 water to powder ratio. After mixing, the materials were inserted into sterilized molding rings with inner diameter of 5 mm and thickness of 1 mm. Thereafter, the materials were set at room temperature for 3 hours with humidity of 97%. One millimeter of medium containing 5×10^4 of MG-63 cells was then seeded into each well in the 24-well plates. TheMG-63 cells were cultured with a culture plate insert with set Capseal I and II specimen on top of the culture plate insert. Empty tubes were used as the control group. After 6 and 18 hours of incubation, cell viability was evaluated using a methylthiazol tetrazolium (MTT; 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl-2H-tetrazolium bromide) assay kit. The cells were incubated with 5.7 mol/L of MTT solution in a tissue culture incubator for 4 hours. Thereafter, 200 μl of dimethyl sulfoxide solution was added to the cell culture wells. The plates were then shaken for 10 minutes at room temperature to dissolve the precipitated formazan crystals. The solutions were centrifuged for 10 minutes, and the optical density of the supernatant was measured using an enzyme-linked immunosorbent assay plate reader (PowerWave; BioTek Instruments, Winooski, VT, USA) at 540 nm. Experiments were carried out in duplicate.

Table 1. Materials used in this study

Name	Composition	
	Powder	Liquid
Capseal I	Tetracalcium phosphate and dicalcium phosphate dehydrate, Portland cement, zirconium oxide, others	Hydroxypropyl methyl cellulose in sodium phosphate solution
Capseal Ⅱ	Tetracalcium phosphate and dicalcium phosphate dehydrate, white Portland cement, zirconium oxide, others	Hydroxypropyl methyl cellulose in sodium phosphate solution

2. RNA Isolation and Reverse Transcription-Polymerase Chain Reaction

After 3 and 7 days of culture, the total RNA of the incubated cells was extracted using Trizol reagent (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's instructions. Reverse transcription (RT) of RNA was performed using an AccuPower RT Premix (Bioneer, Daejeon, Korea), which was also utilized for amplifying the RT-generated DNA. The polymerase chain reaction (PCR) products were resolved on a 1.5% agarose gel and stained with ethidium bromide.

3. Statistical Analysis

Statistical analyses of the MTT assay and RT-PCR data were performed using the Kruskal-Wallis test (SPSS Statistics version 17.0; SPSS Inc., Chicago, IL, USA). The confidence interval was 95% and a P-value less than 0.05 was considered significant.

Result

1. Cytotoxicity Test Using Methylthiazol Tetrazolium Assay

Capseal I and II showed favorable cell viability values at 6 and 18 hours' incubation times. Compared to the control, the percentage of viable cells was 110% for Capseal I and 100% for Capseal II after 6 hours of incubation. After 18 hours of incubation, the percentage of viable cells was 96% for Capseal I and 100% for Capseal II (Fig. 1).

2. RNA Isolation and Reverse Transcription-Polymerase Chain Reaction

Although there were no statistically significant differences, the messenger RNA (mRNA) expression of ALP showed increase in the Capseal II group after 7 days of incubation compared to the control group. Likewise, BSP gene expression increased within 7 days of incubation in the Capseal I group. Osteocalcin (OCN) gene expression significantly increased within 7 days of incubation in Capseal II compared to the control group ($P<0.05$; Fig. 2).

Discussion

Capseal contains both calcium silicate and calcium phosphate as its component. Based on previous research, calcium silicate produces calcium hydroxide when mixed with water; when calcium hydroxide comes in contact with phosphate ion, it could form hydroxyapatite[6], which is a major constituent of human dental tissues. Since Capseal contains calcium silicate and calcium phosphate, the formation of hydroxyapatite is expected to be enhanced by the calcium phosphate in Capseal. This would be beneficial in increasing Capseal's

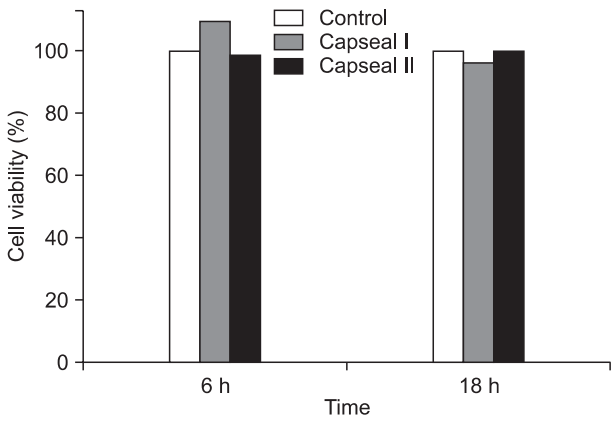

Fig. 1. The results of cytotoxicity test.

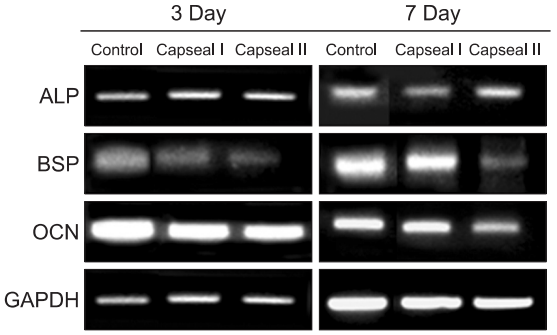

Fig. 2. The results of mineralization related gene expression test. ALP: alkaline phosphatase, BSP: bone sialoprotein, OCN: osteocalcin, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

root canal sealing ability, which was reported to be greater than that of Sealapex. Furthermore, a recent study reported that calcium ions released from calcium silicate cement play an important role in the osteoblastic differentiation of pluripotent cells[17]. Alkaline phosphatase (ALP) is one of the early markers of osteoblastic differentiation, playing an important role in mineralized tissue formation[18]. OCN is a more specific, relatively late-stage marker of osteoblastic differentiation[19]. Bone sialoprotein (BSP) is a major extracellular matrix component of bone produced mainly by osteoblast and is considered to be a marker of osteoblastic phenotypes[20]. For this reason, the upregulation of ALP, OCN, and BSP could suggest the osteogenic potential of certain dental materials. The upregulation of ALP by mineral trioxide aggregate was reported in many previous studies using rat dental papilla[21], rat dental pulp[22], and MDPC-23 cells[23]. BSP upregulation by mineral trioxide aggregate was also cited in previous studies using cementoblast[24] and dental pulp cells[25]. Since Capseal is based on calcium silicate as mineral trioxide aggregate, the results are in agreement with this study.

This study has limitations such as small sample sizes and absence of control groups using commercial root canal sealers. Thus, this study should be regarded as a preliminary pilot study. Nonetheless, this study showed the possibility of Capseal as biocompatible and bioactive root canal sealer. Further studies including *in vivo* and clinical trials could provide evidence for use of Capseal as root canal sealer.

Conclusion

Within the scope of this study, Capseal is biocompatible, with potential for promoting mineralized tissue formation.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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