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# Effect of acemannan on the dentinsialophosphoprotein and dentin matrix protein 1 mRNA expressions in primary human pulpal cells

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## Abstract

***Objective*** To investigate the effect of acemannan isolated from *Aloe vera* gel on the steady level of dentinsialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP 1) mRNAs in primary human pulpal cells.

***Materials and methods*** Cells were treated with the designated concentrations of acemannan (0.25, 0.5 and 1 mg/ml) for 24 hours. The reverse transcription-polymerase chain reaction assays were used to investigate the effects of acemannan on the steady level of DSPP and DMP 1 mRNAs.

***Results*** At 24 hours of incubation, acemannan (0.5 mg/ml) significantly enhanced the expressions of DSPP and DMP 1 mRNA levels up to 1.93 and 2.76 fold, respectively, as compared with the control group ($p < 0.05$).

***Conclusion*** Acemannan at concentration 0.5 mg/ml stimulated both DSPP and DMP 1 mRNA expressions in human dental pulp cells.

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***Key words***: *acemannan; Aloe vera; dental pulp cell; dentin matrix protein 1; dentinsialophosphoprotein*

## Introduction

Dental caries is a major dental health problem. Caries penetrates and destroys tooth structures from enamel, dentin and finally dental pulp tissue. To protect its viability from various toxic products of cariogenic microorganisms, the tooth develops a reparative dentin formation, a self-protection regenerative mechanism. The reparative dentin is synthesized by newly recruited odontoblast-like cells.[1] Recent studies have reported that dental pulp cells can differentiate into odontoblast-like cells to produce new dentin.[2,3]

Generally, hard tissue regeneration is composed of continuous processes beginning from proliferation, differentiation, extracellular matrix synthesis, and subsequently to mineralization.[4,5] For a new dentin formation, the dental pulp cells will initially enter a proliferative phase to increase the cell numbers and then differentiate into odontoblast-like cells.[1-3] Later, the odontoblast-like cells secrete dentin matrix proteins, such as collagen, dentin sialoprotein (DSP), dentin phosphoprotein (DPP), and dentin matrix protein 1 (DMP 1). The DSP, DPP and DMP 1 have been considered as dentin specific proteins and used as markers of odontoblast differentiation.[6,7] Interestingly, both DSP and DPP are transcribed and translated from the same gene, i.e. dentinsialophosphoprotein (DSPP).[8] Mutation of chromosome region containing these DSPP and DMP 1 genes results in the defect of dentin mineralization, namely dentinogenesis imperfecta.[9,10] This finding confirms the importance of these dentin specific proteins in dentin formation.

To enhance the process of dentin formation, several recombinant growth factors, such as TGF-β and BMP-2, have been successfully used to induce a new dentin formation.[11,12] However, due to limited conditions, including the short half-life of protein in the body and the method of delivery, the direct application of these recombinant growth factors into the defective sites does not always reveal good results.[13-15] Therefore, our research group has sought for molecules, especially from natural products that can stimulate the new hard tissue formation.

*Aloe vera* gel has been used in the traditional medicine of many cultures. Acemannan, a major polysaccharide fraction isolated from *Aloe vera* gel, enhances various phases of wound healing processes, such as macrophage recruitment, collagen synthesis, and wound contraction.[16] Our laboratory previously reported that acemannan induces BMP-2 expression in primary human pulpal and periodontal ligament cells.[17] However, the effect of acemannan on the differentiation of dental pulp cells into odontoblast-like cells has not been studied. The purpose of this *in vitro* study was to investigate the effect of acemannan on the mRNA expression of specific dentin matrix proteins, i.e. DSPP and DMP1 in human dental pulp cells.

## Materials and methods

### Materials

Dulbecco's modified Eagle's medium (DMEM), L-glutamine, trypsin-EDTA, penicillin/ streptomycin/ amphotericin (10,000 U/ml, 10,000 μg/ml and 2,500 μg/ml, respectively), fetal bovine serum (FBS), Superscript III RNase H Reverse transcriptase kit, oligoprimers and TRIzol reagent were obtained from Gibco-Life technologies Ltd., Paisley, UK. RedTaq DNA polymerase was purchased from Sigma-Aldrich, St.Louis, MO, USA. Tissue culture plates were obtained from Nunc, Denmark.

### Isolation and characterization of acemannan

Acemannan was isolated from *Aloe vera* Linn. (*Aloe barbadensis* Miller) leaf gel and characterized as previously described.[17] Briefly, *Aloe vera* Linn. was obtained from local plant market in Bangkok, Thailand, which was authenticated by Assoc.Prof.Dr.Chaiyo Chaichantipyuth, Department of Pharmacology, Faculty

of Pharmaceutical Science, Chulalongkorn University. The full size, fresh mature leaves of *Aloe vera* were collected. After peeling off the rind, the remaining clear gel was soaked in running tap-water for 30 minutes to 1 hour, and then in distilled water for another 30 minutes to remove the remaining yellow exudates. The colorless parenchyma gel was blended with homogenizer in the ice-cold condition and centrifuged at 10,000 x g for 20 minutes at 4° C. The supernatant was collected and the polysaccharide was precipitated with absolute alcohol. The white opaque particles were collected after centrifugation at 10,000 x g for 20 minutes at 4° C. After lyophilization, these pellets were ground and further characterized by molecular weight size exclusion HPLC, gas chromatography and 1H NMR spectroscopy. The data confirmed that polysaccharide obtained from *Aloe vera* gel was acemannan.

## Cell cultures

Human dental pulp cells were isolated as previously reported.[18,19] Cells were obtained from healthy patients of the Department of Oral Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. All teeth used for the study were freshly extracted caries-free and periodontitis-free permanent third molars. The informed consent of all the human subjects who participated in the experimental investigation described in this manuscript was obtained after the nature of the procedure and its possible discomforts and risks had been fully explained. The teeth were rinsed with 2% chlorhexidine solution before being cracked open with chisel. The pulpal tissue was removed aseptically and rinsed several times with phosphate-buffer saline (PBS) solution. Then, the pulp tissue was minced into small fragments of 1x1x1 $mm^3$ and placed in a 35 mm culture dish. Cells were grown in DMEM supplemented with 100 µg/ml streptomycin, 100 IU/ml penicillin, 25 µg/ml amphotericin B, 2 mM L-glutamine and 10% fetal bovine serum. Cultures were maintained in humidified 95% air, 5% $CO_2$ atmosphere at 37° C. When cells reached confluence, they were subcultured and then transferred to a 60 mm plate. These cells were considered the first-passage cells. Cell cultures between the third and fifth passages were used in the study.

## Total RNA preparation and reverse transcription-polymerase chain reaction

The procedures were done as previously described with some modifications.[18] For the experiments, cells were plated in a 100 mm culture dish. As the density of cells reached 80% confluence, the culture medium was changed to serum free DMEM for 6 hours, then treated with the designated concentrations of acemannan (0.25, 0.5 and 1 mg/ml) in serum free medium for 24 hours at 37° C. Total cellular RNA was extracted from treated cells using TRIzol reagent following the manufacturer's instructions. Changes in the steady-state concentration of mRNA for DMP1 and DSPP were assessed by RT-PCR. Briefly, total RNA (5 µg) was converted to single stranded cDNA using Superscript III RNase H Reverse transcriptase kit. The target cDNA was amplified using the sense and antisense primers for DSPP, DMP 1 and GAPDH (internal control). The amplification cycles were 94° C for 1 minute, 58° C for GAPDH and 53° C for DSPP and DMP 1 for 1 minute and 72° C for 1 minute. After 30 cycles, the PCR products were separated by electrophoresis on 1.5% agarose, stained with ethidium bromide, illuminated with ultraviolet light, photographed, and analyzed with an Imaging Densitometer/Gel Doc (BioRAD, USA) Program Molecular Analysis. The mRNA levels of DSPP and DMP 1 were expressed as a relative ratio of the intensity of each band to the intensity of GAPDH band. Each densitometric value was plotted on a bar graph in Figure 1 as a ratio between the experimental group and the control group. The sequence of primers is listed in Table 1.

**Table 1** Sequence of PCR primers used in this study

| Name | Primer Sequence (5 → 3) | Size (bp) |
|---|---|---|
| GAPDH | TGAAGGTCGGAGTCAACGGAT | 510 |
| | TCACACCCATGACGAACATGG | |
| DSPP | CCATTCCAGTTCCTCAAAGCA | 716 |
| | CCTCATCTGCTCCATTCCCACT | |
| DMP 1 | GATGACACCATACAAGCCAGT | 666 |
| | CTCACTCACCACCTCTTCCT | |

GAPDH; Glyceraldehyde-3-phosphate dehydrogenase

DSPP; Dentinsialophosphoprotein

DMP 1; Dentin matrix protein 1

### Statistical analysis

All experiments were repeated at least three times using cells from different cell donors. Data were presented as mean ± standard deviation. Group comparison was performed using one way ANOVA followed by post-hoc analysis with Duncan's multiple range test. Significance was chosen at an $\alpha$ level of 0.05.

## Results

### Acemannan upregulated the expression of DSPP and DMP 1 mRNAs

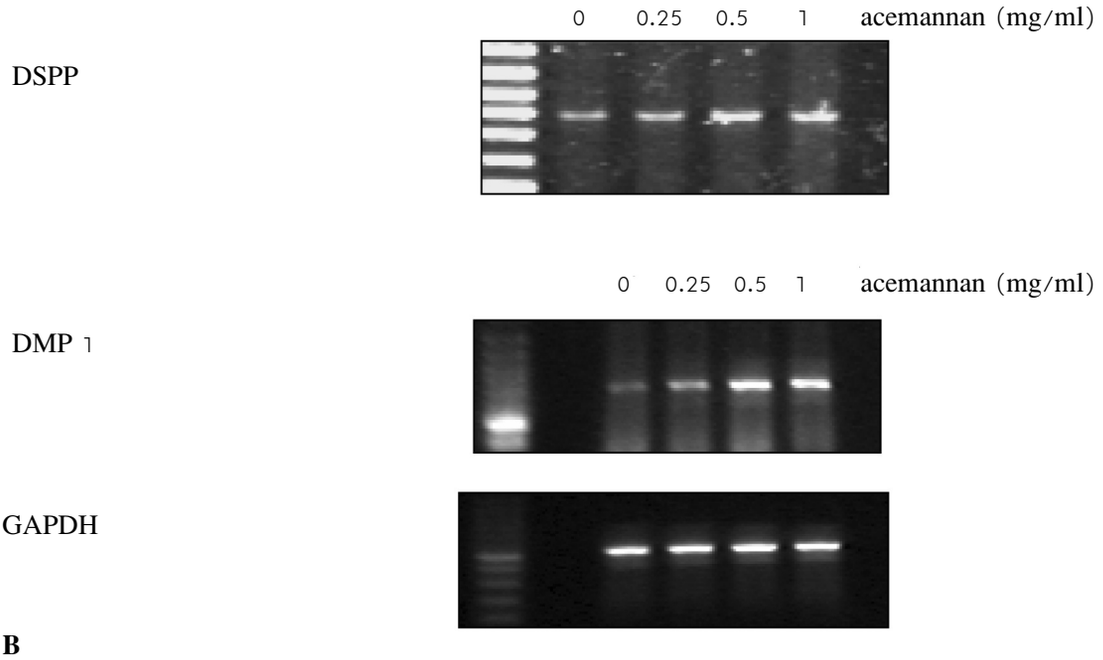
In a serum free condition, acemannan induced the steady levels of DSPP and DMP 1 mRNA in human dental pulp cells. At the concentration of 0.5 mg/ml, acemannan significantly enhanced the expression of both DSPP and DMP 1 mRNAs up to 1.93 ± 0.36 and 2.76 ± 0.99 fold, respectively, as compared with the control group ($p < 0.05$, Figure 1).

## Discussion

Dental pulp cells isolated from the dental pulp have been considered as progenitor cells for odontoblasts. Under the appropriate stimulation, these cells can proliferate and differentiate into odontoblast-like cells to produce a new dentin.[20] Therefore, dental pulp cells have been used as a model for studying the effect and mechanism of many substances on reparative dentin formation.[12,13]

From our previous report, *Aloe vera* gel extract

**A**




**B**

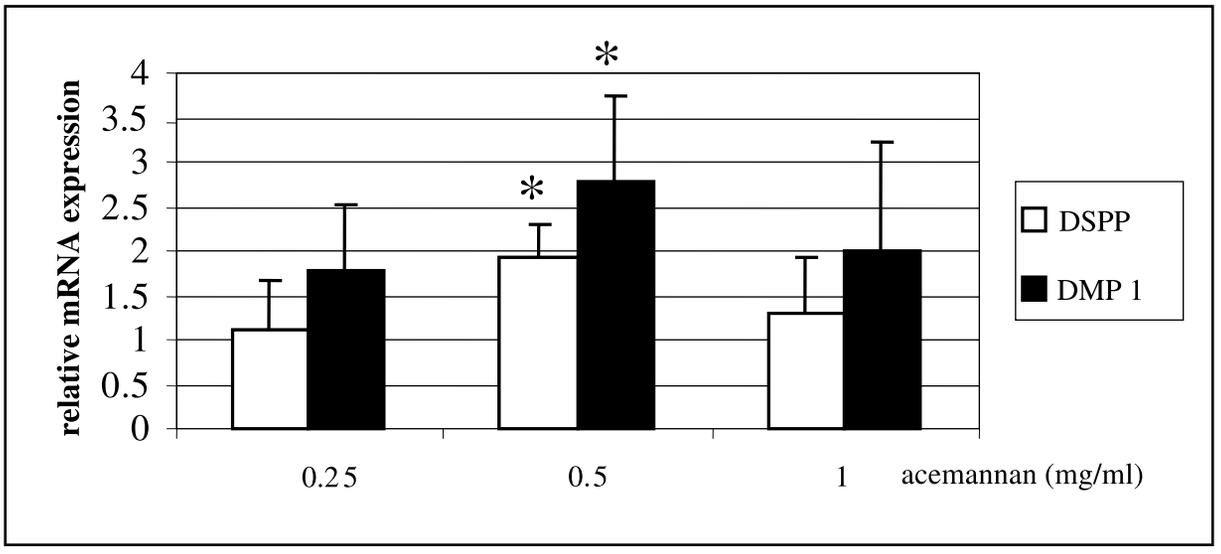



**Fig. 1** A. Expression of DSPP and DMP 1 mRNA stimulated with acemannan extracted from *Aloe vera* gel in human dental pulp cells by RT-PCR assay. Lane 1-4 represents control group, acemannan treated groups at the concentrations of 0.25, 0.5 and 1 mg/ml, respectively. B. The ratio of DSPP and DMP 1 mRNA expression of treated group compared with control. Data were shown in mean ± SD, n = 3.

* Demonstrated significant difference from the control group at $p < 0.05$

stimulated the proliferation of primary human pulpal cells, periodontal cells, and gingival fibroblasts.[19] However, many ingredients, such as polysaccharide, protein, mineral and sterol could be identified in *Aloe vera* gel. Therefore, using the *Aloe vera* gel extract in dental and medical applications may prove difficult to conclude which ingredients in the crude extract is considered active. Acemannan, isolated from *Aloe vera* gel, has been considered as active ingredient polysaccharide.[16,17,21] This polysaccharide induced the proliferation and functional maturation of dendritic cells.[21] To investigate the effect of acemannan on differentiation of dental pulp cells, the steady mRNA levels of differentiation marker genes, i.e. DSPP and DMP 1, were evaluated. As compared with that of control group, acemannan at 0.5 mg/ml significantly increased the gene expression of both DSPP and DMP 1 mRNAs to 1.93 and 2.76 fold, respectively. However, despite lacking of a dose-response induction of DSPP and DMP 1 mRNA expressions by acemannan, acemannan at the concentrations of 0.25 and 1 mg/ml still showed the slight increase of DSPP and DMP 1 mRNA levels. Therefore, acemannan may possibly induce differentiation of dental pulp cells via upregulation of DSPP and DMP 1 mRNAs.

Based on our limited data, the precise underlying mechanism how acemannan induces DSPP and DMP 1 mRNA expressions in dental pulp cells cannot be well explained. From our previous report, it was demonstrated that acemannan induced BMP-2 expression in human dental pulp and periodontal ligament cells.[17] Treated dental pulp cells with recombinant BMP-2 resulted in the upregulation of DSPP and the differentiation of dental pulp cells into odontoblasts.[22,23] Therefore, acemannan may induce the dental pulp cells to secrete BMP-2, and BMP-2 may in turn enhance differentiation of dental pulp cells into odontoblast-like cells which leads to DSPP and DMP 1 mRNA up-regulations. To verify this possibility, it is therefore interesting to use the anti-BMP-2 antibody to neutralize the inductive effect of acemannnan on the steady mRNA levels of DSPP and DMP 1 in dental pulp cells. In addition, further *in vivo* studies about the effect of acemannan isolated from *Aloe vera* gel on dentin formation must be conducted.

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# การศึกษาผลของสารอะซีแมนแนนต่อการแสดง-ออกของเดนทีนไซอาโลฟอสฟอโปรตีนและเดนทีนเมทริกซ์โปรตีน 1 ในระดับอาร์เอ็นเอนำรหัสของเซลล์เนื้อเยื่อในของมนุษย์

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## บทคัดย่อ

***วัตถุประสงค์*** เพื่อศึกษาผลของสารอะซีแมนแนนที่สกัดจากส่วนวุ้นของว่านหางจระเข้ต่อระดับอาร์เอ็นเอนำรหัสของจีนเดนทีนไซอาโลฟอสฟอโปรตีน (ดีเอสพีพี) และเดนทีนเมทริกซ์โปรตีน 1 (ดีเอ็มพี 1) ในเซลล์เนื้อเยื่อในของมนุษย์

***วัสดุและวิธีการ*** นำเซลล์เนื้อเยื่อในทดสอบด้วยสารอะซีแมนแนนที่ความเข้มข้น 0.25 0.5 และ 1 มิลลิกรัมต่อมิลลิลิตร เป็นเวลา 24 ชั่วโมง จากนั้นทำการแยกอาร์เอ็นเอและขยายสัญญาณของระดับอาร์เอ็นเอนำรหัสของจีนดีเอสพีพีและดีเอ็มพี 1 ด้วยปฏิกิริยารีเวิร์สทรานสคริปชั่นพอลิเมอร์เรสเชน

***ผลการทดลอง*** เมื่อทดสอบเป็นเวลา 24 ชั่วโมง พบสารอะซีแมนแนนที่ระดับความเข้มข้น 0.5 มิลลิกรัมต่อมิลลิลิตร มีผลกระตุ้นระดับการแสดงออกของอาร์เอ็นเอนำรหัสของจีน ดีเอสพีพีและดีเอ็มพี 1 อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ประมาณ 1.93 และ 2.76 เท่า เมื่อเปรียบเทียบกับกลุ่มควบคุม

***สรุป*** สารอะซีแมนแนนที่ความเข้มข้น 0.5 มิลลิกรัมต่อมิลลิลิตร มีผลกระตุ้นการแสดงออกของจีน ดีเอสพีพีและดีเอ็มพี 1 ในระดับอาร์เอ็นเอนำรหัสของเซลล์เนื้อเยื่อในของมนุษย์

(ว ทันต จุฬาฯ 2551;31:407-14)

***คำสำคัญ:*** *เซลล์เนื้อเยื่อใน; เดนทีนไซอาโลฟอสฟอโปรตีน; เดนทีนเมทริกซ์โปรตีน 1; ว่านหางจระเข้; อะซีแมนแนน*