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**The 11th Dental Faculty Consortium of Thailand**
**Academic Meeting and Research Presentation (DFCT 2013)**
**and 30th Anniversary of the Dental Faculty Consortium of Thailand**

7 - 9 May 2013
at Pullman Pattaya Hotel, Chonburi, Thailand

Prof.Dr. Sittichai Koontongkaew
Dean, Faculty of Dentistry, Thammasat University
Conference Chair

30th Anniversary DFCT

The Dental Faculty Consortium of Thailand

The Best of Thai Dental Research

# 11th Dental Faculty Consortium of Thailand Academic Meeting and Research Presentation (DFCT2013)

7-9 May, 2013

Pullman Pattaya Hotel, Chonburi, Thailand

The Dental Faculty Consortium of Thailand
Faculty of Dentistry ,Thammasat University

# Scientific Presentation Program

## 7 May 2013



## 8 May 2013

**13.00 – 14.15 Oral Presentation**

**Session 1 Group 1**
**Chair Person: Asst.Prof.Dr. Nirada Dhanesuan**



**Session 1 Group 2**
**Chair Person: Asst.Prof.Dr. Kanokporn Bhalang**

**Session 1 Group 3**
**Chair Person: Asst.Prof. Dr.Apirum Janhom**



**Session 2 Group 1**
**Chair Person: Assoc.Prof.Dr. Soisiri Thaweboon**

**Session 2 Group 2**
**Chair Person: Asst.Prof. Dr.Yosananda Chantravekin**



**Session 2 Group 3**
**Chair Person: Asst.Prof.Dr. Lertrit Sarinnaphakorn**

## Poster Presentation

### Group 1: Oral biology

## Group 2: Clinical study

**Group 3: Dental materials, Techniques**

**Group 4: Public health, education, etc.**

**9 May 2013**

09.00 - 09.30



09.30 - 10.00



10.00 - 10.30



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# Biocompatibility of monocalcium silicate – glass ionomer cement compound

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**Abstract**

*Objectives: To investigate in vitro biocompatibility of glass ionomer cement containing 30% by weight $CaSiO_3$ (30%CS-GI) compared to GIC and MTA.*

*Methods: Partial set specimens of 30%CS-GI, GIC and MTA were immersed in complete DMEM for 72 hours. Each extract was collected and serial diluted 1:1 with DMEM into 4 concentrations. All concentrations of extracts were co-cultured with human periodontal ligament fibroblast cell for 24 hours. The cytotoxic level and cellular morphology was assessed by MTT assay and phase contrast microscope. Data were compared by one-way ANOVA.*

*Results: Cells exposed to undiluted extracts from GIC was round-shaped and were significantly lesser in number than those from 30%CS-GI and MTA. Most of the cells exposed to undiluted extracts from 30%CS-GI and MTA were spindle-shaped like normal cell morphology. Cells exposed to diluted extracts (1:1, 1:2 and 1:4) showed higher level of cell viability and normal cell morphology*

*Conclusion: The addition of 30% monocalcium silicate into GIC can improve its biocompatibility.*

***Keywords :***

calcium silicate, glass ionomer cement, MTA cement, biocompatibility

## Introduction

Iatrogenic root canal perforation lowers the success rate of root canal treatment [1]. Creating a good seal between root canal space and periradicular tissue is an essential goal to achieve good prognosis. Hence, repair materials should have good sealing ability. Because the materials close contact with the tissues including periodontal ligament and alveolar bone for long period of time, they should be non-irritant and biocompatible.

Mineral trioxide aggregate (MTA) has been a material of choice for perforation repair because of its sealing ability and biocompatibility [2-5]. MTA was able to induce new cemental tissue on its surface when applied to root defects in canines [6,7] Nonetheless, MTA is relatively expensive, difficult to handle and long setting time [8].

Glass ionomer cement (GIC) has been used as restorative dental material more than 30 years [9]. Breault *et al* [10] recommended GIC as endodontic perforation repair material due to its leakage resistance properties and biocompatibility [11, 12]. Alhadainy & Himel [11] found that GIC provided a better seal than amalgam or cavit. Koulaouzidou *et al* [12] demonstrated that both GIC and MTA were biocompatible materials to fibroblastic cell lines. However, some previously published papers showed that GIC was more cytotoxic than MTA[3,13,14]. Therefore, the simplest way to improve the biological properties of GIC is adding a new material that could improve bioactivity and biocompatibility to GIC.

Recently, scientists found that adding some bioactive ceramics such as Bioglass® [15], calcium aluminate [16] and hydroxyapatite [17] into GIC could improve its biological and mechanical properties. Another bioactive glass ceramics is monocalcium silicate ($CaSiO_3$), which was found to rapidly activate the formation of hydroxyapatite layer on its surface. The formation rate was faster than that on some other bioactive glass ceramics [18,19]. Thus, adding $CaSiO_3$ to GIC might potentially improve biological properties as well.

The aim of this study is to investigate the *in vitro* cytotoxicity of glass ionomer cement containing 30% by weight $CaSiO_3$.

## Materials and Methods

### Cell culture

The protocol of the study was approved by the Ethics Committee of Faculty of Dentistry, Srinakarinwirot University. Human periodontal ligament (PDL) fibroblast cells were donated from Ms. Ratchaporn Srichan (Head of Tissue Culture section, Research Institute, Faculty of dentistry, Mahidol University, Thailand). Three cryogenic vials storing frozen human PDL fibroblast cells from 3 unidentified persons were thawed and transferred into tissue culture flasks (Nunc, Thermo Scientific, Denmark) containing culture medium. The culture medium was Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Thermo Scientific, Logan, UT, USA) and 1% antibiotic/antimycotic solution (10,000 U penicillin, 10 µg streptomycin, 25 µg amphotericin B per mL). Cells were maintained at 37°C, 5% $CO_2$ and 100% humidity in an incubator. After a confluent monolayer was obtained, cells were trypsinized and subcultured. The PDL fibroblast cells from $4^{th}$-$7^{th}$ passage were used in this study.

### Preparation of beta-monocalcium silicate (β-$CaSiO_3$) powder

Beta-monocalcium silicate powder was prepared by a coprecipitation method as described by Siriphannon et al [20]. Briefly, $Ca(NO_3)_2.4H_2O$ and $Si(OC_2H_5)_4$ (TEOS) were dissolved in 500 ml of ethanol under continuous stirring. The precipitate was obtained by the addition of 0.33 mol/l NaOH solutions into the above solution. The precipitated gel was filtered, washed twice with distilled water, dried in an oven at100°C overnight, and calcined at 500°C for 2 hours. The calcined powder was fired at 900°C for 2 hours to obtain beta-monocalcium silicate, low temperature crystalline phase. The resultant powder was ground and sieved through 230-mesh sieve before further use.

### Preparation of test materials

Ketac Molar (3M-ESPE Dental, Seefeld, Germany) was chosen as the baseline reference and white ProRoot MTA (Densply Tulsa, OK, USA) was served as control. Ketac Molar and $CaSiO_3$ powder were mixed to 7:3 by weight ratio. Ketac Molar liquid was used to wet the mixed powders; the powder/liquid ratio was 3:1for Ketac Molar powder and 2.5:1 for $CaSiO_3$ powder. Ketac Molar and white ProRoot MTA were prepared according to manufacturers' directions. The details were shown in table 1.

*Table 1 - The powder-liquid ratios of all groups*

| Groups | Materials | P:L (by weight) |
|---|---|---|
| GIC | Ketac Molar | 3:1 |
| 30%CS-GI | 30% $CaSiO_3$<br>70% Ketac Molar | 2.5:1<br>3:1 |
| MTA | White ProRoot MTA | 3.3:1 |

### Preparation of extracts

All groups of cement were mixed as previously described under aseptic condition. After mixing, a total of 0.2 g of cements was placed into a well of 24-well tissue culture plates. The GIC and 30%CS-GI specimens were kept in an incubator at 37 °C and 100% relative humidity for 30 minutes, whereas the MTA specimens were kept for 4 hours in the same condition. After incubating, the discs were exposed to ultraviolet light for 30 minutes to sterile. One milliliter of complete DMEM was poured into each well containing each specimen and incubated in the incubator for 72 hours. After incubating, each extract was transferred into a centrifugal vial for centrifugation for 5 minutes. The supernatant was collected and serially diluted 1:1 with DMEM to achieve a total of 4 concentrations of each extracts. A series of extracts of different concentrations were made to observe a possible dose-response relationship.

### Cell viability test

Human PDL fibroblast cells were seeded into 96-well plates at 20,000 cells/well and incubated for 24 hours to allow attachment. Then, 100 µL of extract was placed into the tissue culture well. Cells with 100 µL culture medium served as a control. The experiment was repeated three times of each cell line.

After an incubation period of 24 hours, cell viability was evaluated by 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Reduced MTT was measured spectrophotometrically at 570 nm in a microtiter plate reader (BIO-TEK Instrument Inc, Winooski, VT). The OD value of test wells and control wells were calculated by using the following formula:

% cell viability =

### Cell morphology

The images of PDL fibroblast cells treated with different concentration of three material groups were examined by using phase contrast microscope (Nikon TS-100-F, Nikon, Tokyo, Japan)

### Statistical analysis

The results were expressed as means ± standard deviation. Experimental data were analysed by one-way ANOVA at significant level of $p < 0.05$. Post hoc tests were done with Scheffé's test.

## Results

### MTT assays

The percentages of viable cells are shown in Figure 1. The percentage of cell viability exposed to undiluted extracts from GIC was 51.29%, which was significantly lesser than those from 30%CS-GI and MTA (90.48% and 94.4%, respectively).

The percentages of cell viability exposed to 1:1, 1:2 and 1:4 diluted extracts were not significantly different amongst all three groups. The cell viability was likely to depend on extract concentration; the lower concentration, the higher level of cell viability.

### Cell morphology

The morphology of PDL fibroblast cells exposed to various concentrations of extracts from three groups are shown in Figure 2. Most of the cells exposed to undiluted extracts from GIC were round with fewer cytoplasmic extensions, whereas those from 30%CS-GI and MTA were generally spindle-shaped like normal cell morphology.

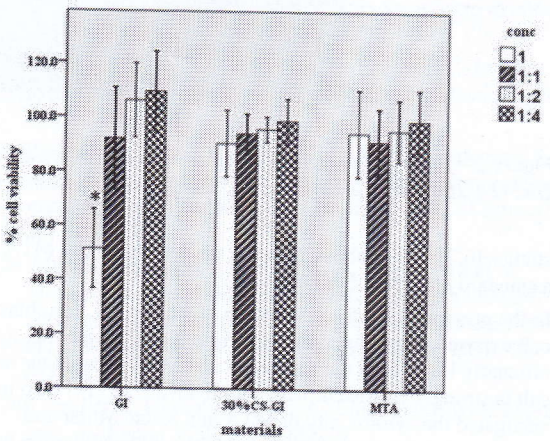


*Figure 1 - The percentage of cell viability exposed to 4 concentrations of extracts from all groups*

## Discussion

After perforation repair, the repair material can release some leachable compounds into surrounding tissue and may cause harmful effects to periradicular tissue. In the present study, we simulated this condition by using the extraction of partially set specimens and evaluated the *in vitro* cytotoxicity by using MTT assay. The specimen preparation followed ISO 10993-12:2007 standard [21], and the test protocol followed ISO 10993-5:2009 for *in vitro* cytotoxicity test [22]. However, we used human periodontal ligament fibroblast cell instead of immortalized fibroblast cells to simulate the *in vivo* situation. In addition, we also assessed cell morphology because the alteration of cellular morphology could depict the physiological state of the cells.

Ketac Molar was chosen to represent the GIC groups due to its good biological properties comparing with other commercial GICs [23]. Sengun *et al* [24], who used dentin barrier test for examining the cytotoxicity of conventional and resin-modified GIC, showed that Ketac Molar had better pulp cell viability.

MTA is universally acknowledged to be biocompatible to PDL cell cultures [3-5, 12]. Additionally, MTA could induced BMP2 expression and calcification in human PDL cell [25] and was able to form new cementum over its surface in *in vivo*

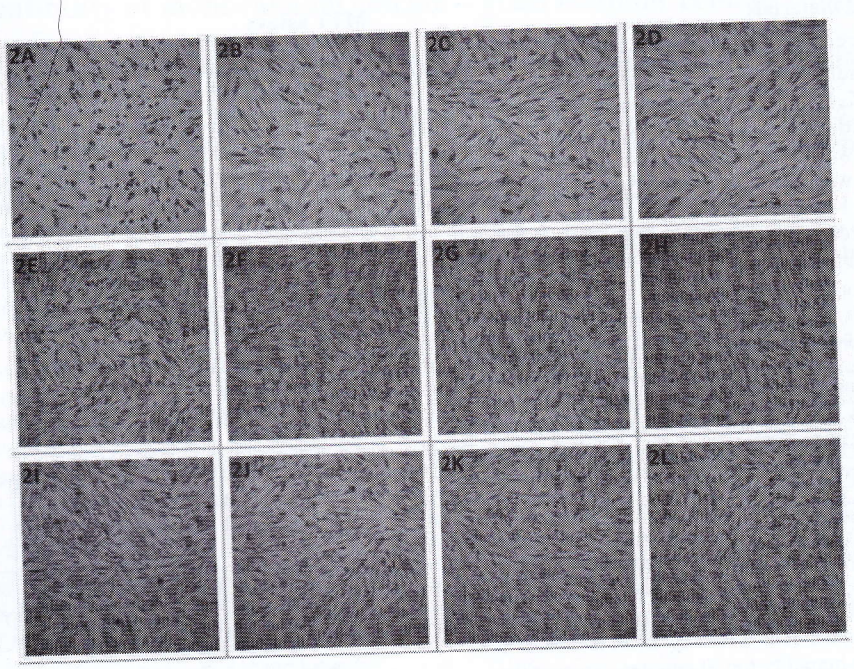


*Figure 2 – From left to right, the cell morphology treated with undiluted, 1:1, 1:2 and 1:4 diluted concentration, respectively, from GIC (2A-2D), 30%CS-GI (2E-2H) , and MTA(2I-2L) extracts*

studies [6,7]. All of above reasons, we decided to use MTA as a standard control in this study.

In the present study, the percentage of viable PDL fibroblast cell exposed to undiluted extracts from Ketac Molar was significantly lower than the test group and MTA group. Our result is strongly consistent with Vajrabhaya *et al* [14] who investigated the cytotoxicity of MTA and Ketac Molar and demonstrated that the inhibition of PDL cell proliferation by the extracts of Ketac Molar was significantly greater than that of MTA. Abdullah *et al* [13] also reported that SaOs-2 osteosarcoma cell was round morphology and not able to adhere to GIC's surface but there was an increase in cell number adhering to MTA's surface.

An explanation for our result could come from the leachable compounds of MTA and GIC. The hydration reaction of MTA formed calcium silicate hydrate gel (C-S-H) and calcium hydroxide [26]. When immersed it in solution, calcium ions from calcium hydroxide and C-S-H could release to the solution [27]. The released calcium ions stimulated the osteogenic differentiation of human PDL cells [25] and reacted to phosphate group in the solution to form hydroxyapatite [28,29]. The presence of cementum on the surface of MTA has proven this explanation [6,7].

GIC is hardened by acid-base reaction of calcium fluoroaluminosilicate powder and polyacrylic acid solution [30]. Therefore, $Al^{3+}$, $F^-$, $Si^-$ and unpolymerized acid are mainly leachable compounds that might affect the cells and tissues. Many previous studies have expressed of the toxicity of the released components. Savarino *et al* [31] reported that the release of fluoride and aluminium ions in early setting reaction combined with acidity make the GIC toxic. Consiglio *et al* [32] also found that released fluoride ions and acidic pH of GIC inhibited protein synthesis of human gingival fibroblast.

The cytotoxic level of 30%CS-GIC was similar to that of MTA. $CaSiO_3$ has been proven as one of bioactive glass ceramics because of its hydroxyapatite formation capacity. The β-$CaSiO_3$ was able to form hydroxyapatite within 1 day and could rapidly released calcium ion and silicon ions to simulated body fluid solution within 5 days [20]. Li *et al* [33], who incorporated PHBV polymer with $CaSiO_3$ bioactive glass for bioactive porous composite scaffold, reported that pH of $CaSiO_3$ powder was slightly basal pH and could neutralize the acidic by-products from PHBV polymer and stabilize the pH of the simulated body fluid solution between 7.2-7.7. For all of these reasons, adding $CaSiO_3$ to GIC could reduce the toxicity of GIC.

In the present study, the percentages of viable cell exposed to any diluted extracts from GIC, 30%CS-GIC and MTA were not significantly different. Moreover, the cytotoxic level was prone to adversely proportional to the concentration, which associated with the amount of leachable compound. The higher amount of toxic leachants, the more acidic pH of the solution, which lead to the cell damage and death.

Base on adding $CaSiO_3$ to GIC, this compound not only possesses biocompatibility, but also hypothetically possesses bioactivity. For future study, it is important to examine the bioactivity of the $CaSiO_3$-GIC compound.

## Conclusion

In this study, the adding 30% by weight $CaSiO_3$ to GIC can improve biological properties of GIC.

### Acknowledgments

This research was supported by the grants of Srinakarinwirot graduate school for graduate student research (GRAD S-1-56).

The authors would like to thank Ms. Ratchaporn Srichan, Head of Tissue Culture section, Research Institute, Faculty of dentistry, Mahidol University, Thailand, for providing human PDL fibroblast cell and valuable cell-cultured technical support.

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