

## Calcium Release and Mechanical Strength of Calcium Fluoride Doped Carbonate Apatite Cement as a Novel Pulp Capping Agent

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### Abstract

Carbonate apatite (CO<sub>3</sub>Ap) is a bone substitute material, currently developed for pulp capping agent for its ability to stimulate mineralized tissue. Calcium fluoride (CaF<sub>2</sub>) provides calcium and fluoride ions to form hydroxyapatite, fluorapatite, or fluorhydroxyapatite. Combining CO<sub>3</sub>Ap and CaF<sub>2</sub> was expected to improve the performance of CO<sub>3</sub>Ap as pulp capping material. This study aimed to assess the calcium release and mechanical strength of CaF<sub>2</sub> doped to CO<sub>3</sub>Ap in various concentration to evaluate the best ratio for this new combination. CaF<sub>2</sub> was added to CO<sub>3</sub>Ap cement in various concentration and released calcium ions were recorded by an atomic absorption spectrophotometer at day 1, 7 and 14. Mechanical strength was evaluated by Diametral Tensile Strength (DTS) using a Universal Testing Machine. The study consisted of 6 groups (n = 4); G1 (60 % DCPA, 40% vaterite), G2 (60% DCPA, 30% vaterite, 10% CaF<sub>2</sub>), G3 (60% DCPA, 20% vaterite, 20% CaF<sub>2</sub>), G4 (60% DCPA, 10% vaterite, 30% CaF<sub>2</sub>), G5 (60% DCPA, 40% CaF<sub>2</sub>) all groups were mixed with 1mL Na<sub>3</sub>PO<sub>4</sub>, and G6 (calcium hydroxide mixed with water). Data were analyzed by one way ANOVA test and Post Hoc analysis. The calcium release rate of CaF<sub>2</sub> doped CO<sub>3</sub>Ap were higher in day 7 and 14 of observation compared to CO<sub>3</sub>Ap cement. The best ratio for calcium release of the new cement combination was in G3. The addition of 20% of CaF<sub>2</sub> had the highest DTS value. In conclusion, Group 3 was the best ratio for calcium ions release and mechanical strength, however, the addition of CaF<sub>2</sub> to CO<sub>3</sub>Ap cement did not significantly improve the ability of CO<sub>3</sub>Ap to release calcium ions nor its mechanical strength. This might be due to the particle size of CaF<sub>2</sub> material used. Further study was awaited using smaller particles of CaF<sub>2</sub> based on these initial findings.

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### Introduction

Pulp capping is a vital pulp therapy performed on an exposed pulp or nearly exposed pulp to stimulate mineralized tissue barrier or reparative dentine formation and maintaining pulp vitality.<sup>1-3</sup> Besides proper case

selection, one of the important factors in the outcome of pulp capping is the choice of material. Calcium hydroxide [Ca(OH)<sub>2</sub>] has been widely used as the material of choice for pulp capping, however, insights on bioceramics leads to a better biocompatible and bioactive option such as MTA or biodentin as pulp capping material.<sup>3,4</sup>

CO<sub>3</sub>Ap is a bioceramic material that has gained much interest in orthopedics as bone substitute material. It consists of *Dicalcium Phosphate Anhydrous* (DCPA) and *vaterite* mixed with trisodium phosphate (Na<sub>3</sub>PO<sub>4</sub>) solution as the phosphate donor. CO<sub>3</sub>Ap cement will release essential inorganic substances such as carbonate, calcium, and phosphate through

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the dissolution of DCPA and vaterite with  $\text{Na}_3\text{PO}_4$ . The dissolution of these released materials supersaturates the solution, followed by precipitation of  $\text{CO}_3\text{Ap}$  formation which then forms a set and hardens the cement.  $\text{CO}_3\text{Ap}$  adapts well to hydroxyapatite, which is the major component in bone and tooth structure and will slowly be replaced by the natural structure.  $\text{CO}_3\text{Ap}$  cement can set and harden in bone defects in the presence of blood. The interface between the bone defect surface and the  $\text{CO}_3\text{Ap}$  leaves no gap, showing well adapt and good adherence of the cement to bone structure. This is imperative, because slow replacement of any bone substitute material by osteoblast and bone regeneration needs close contact between the material and the adjacent bone, otherwise osteoblasts cannot migrate to the defected area.<sup>5-8</sup>

The superior properties of  $\text{CO}_3\text{Ap}$  have gained interest not only as bone substitute material but also for dental applications. Our previous studies have been working on its potential as a bioceramic pulp capping agent, with promising outcomes.<sup>7,9-11</sup>  $\text{CO}_3\text{Ap}$  has proper mechanical properties for pulp capping material, released calcium ions and alkaline pH that contributes to antimicrobial activities and mineralization. Histologic study in exposed rat pulp showed no sign of chronic inflammation and preserve pulp vitality. The formation of reparative dentin was observed in 3 weeks of evaluation.<sup>10</sup>

Calcium ion holds an important role in the differentiation and mineralization of pulp cells to form tertiary dentin (reactive and reparative dentin) and hydroxyapatite layer. It activates stem cells, trigger the proliferation of undifferentiated pulp cells, regulates osteopontin and *bone morphogenic protein-2* (BMP-2) level in pulp calcification.<sup>12-14</sup> When in contact with carbon dioxide ( $\text{CO}_2$ ) or carbonate ions ( $\text{CO}_3^{2-}$ ) that are present in blood or tissue fluids, it will form calcium carbonate.<sup>10</sup>

Fluoride ions are also widely known for their cariostatic and remineralization effect, the later is attributed to the formation of fluorapatite or calcium fluoride -like precipitates and hydroxyl-fluorapatites.<sup>15-17</sup> Fluoride also possessed high antibacterial property that hinders adhesion and bacterial metabolism.<sup>18,19</sup>  $\text{CaF}_2$  nanoparticles are potential antibiofilm agents particularly on *Streptococcus mutans*, a commonly cariogenic bacteria. It reduces the biofilm formation up to

89% and reduces the bacteria tolerance to acid as well as its acid-producing activity.<sup>19</sup>

From the understanding of the role of calcium and fluoride ions on mineralization, we aim to dope the  $\text{CO}_3\text{Ap}$  that we developed with  $\text{CaF}_2$  to increase its bioactivity in releasing calcium ions. This is the first attempt to make such modification of the novel cement, therefore this study will evaluate whether the approach will increase the ability of  $\text{CO}_3\text{Ap}$  cement to release calcium ions and to evaluate the mechanical strength of this new combination to bear mechanical force.

## Materials and methods

The  $\text{CO}_3\text{Ap}$  cement used in this study consists of DCPA (J.T. Baker Chemical Co., NJ, USA) powder and vaterite powder that was synthesized from  $\text{Ca}(\text{OH})_2$  in a similar method as in our previous report.<sup>5</sup> The powders mixed with  $\text{Na}_3\text{PO}_4$  solution as a phosphate donor to a pasta consistency with a 0.5 L/P ratio. The average particle size of DCPA powder was approximately 0.9  $\mu\text{m}$ , vaterite powder 0.7  $\mu\text{m}$  and  $\text{CaF}_2$  powder 1.4  $\mu\text{m}$ .  $\text{Ca}(\text{OH})_2$  (Merck, Darmstadt, Germany) mixed with distilled water in a 0.5 L/P ratio was employed as a control.

Samples were divided into 6 groups (n = 4); G1 without any addition of  $\text{CaF}_2$  (60% DCPA, 40% vaterite), G2 (60% DCPA, 30% vaterite, 10%  $\text{CaF}_2$ ), G3 (60% DCPA, 20% vaterite, 20%  $\text{CaF}_2$ ), G4 (60% DCPA, 10% vaterite, 30%  $\text{CaF}_2$ ), G5 (60% DCPA, 40%  $\text{CaF}_2$ ), and G6  $\text{Ca}(\text{OH})_2$ . All groups were mixed with 1 mol/L  $\text{Na}_3\text{PO}_4$  as the cement liquid except for  $\text{Ca}(\text{OH})_2$  was mixed with distilled water, all in a 0.5 L/P ratio. Calcium released were calculated using a spectrophotometer UV/VIS (Spectronic Camspec Ltd., Leeds, UK) and Diametral Tensile Strength (DTS) method using a Universal Testing Machine (LRX Plus; Llyod Instruments, Ltd., West Sussex, UK) was used to measure the mechanical strength.

For calcium released measurement, the cement powders were mixed for 1 minute with the appropriate liquid until a homogenous paste was achieved, the pasta was packed into a stainless steel mold (6mm in diameter  $\times$  3mm in height). Both open ends of the mold were covered by glass slides and clamped by a metal clip. Furthermore, the set cement sample was

taken off the mold and was put into a tube of 10 mL of deionized water at 37 °C for 5 minutes. The stored water was added to 1 ml reagent and measurement of for Ca<sup>2+</sup> ions analysis for day(s) 1, 7 and 14 were done at 650nm.

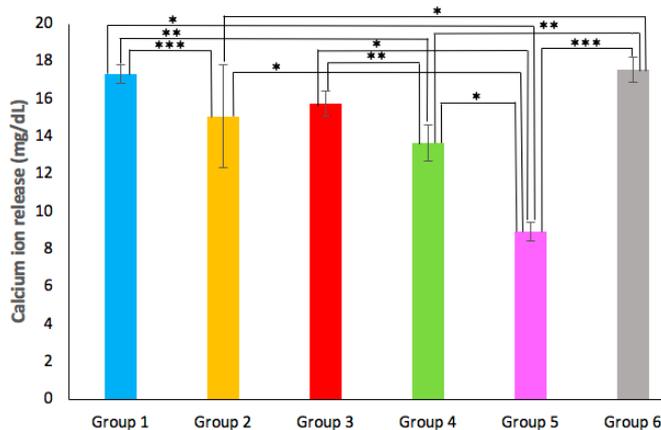
The formula to measure calcium released was below:

$$\text{Calcium} \left( \frac{\text{mmol}}{\text{L}} \right) = \frac{\text{Abs. Test}}{\text{Abs. Std}} \times \text{Standard}$$

Mechanical strength was evaluated using the DTS method. All sample cement were prepared in the same manner as before, and cement paste in the steel mold was kept in a and incubated at 37 °C, with 100% humidity for 24 hours. After 24 hours the set samples were removed from the mold, carefully immersed in the 99% ethanol for 3 min and dried at 80 °C for 3 h. For DTS evaluation, the dried samples tablets placed in the universal testing machine and crushed using a with 5.6 kN of preload stress at a crosshead speed of 1 mm/min.

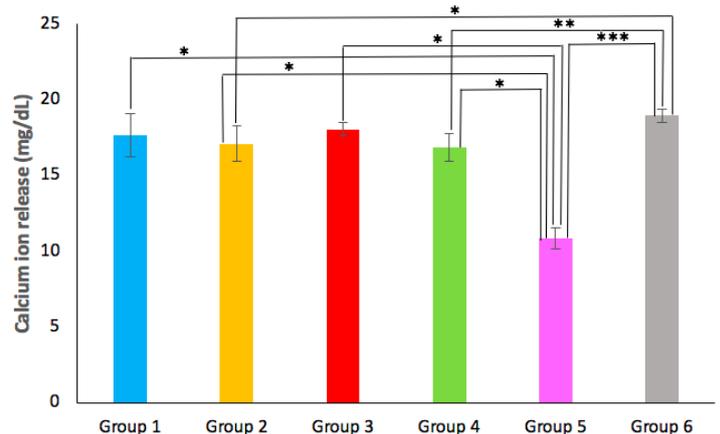
## Results

All groups released calcium ions, which gradually increased from day 1 to 7, and decreased on day 14. The mean calcium ions released on day 1 are recorded in figure 1. At day 1, the highest release was G6 which was the control group Ca(OH)<sub>2</sub>, followed with G1 and G2 with no significant differences between these groups. At day 1, the addition of CaF<sub>2</sub> to CO<sub>3</sub>Ap cement did not improved its calcium release ability among the CO<sub>3</sub>Ap groups.



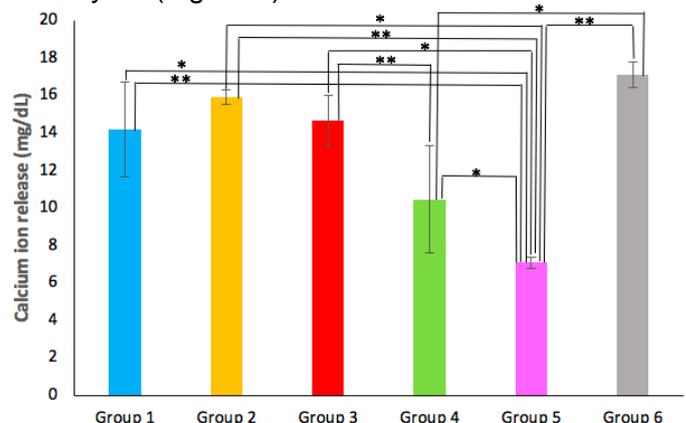
**Figure 1.** Calcium release of calcium fluoride doped carbonate apatite at day 1 of observation (\*\*\*\*\**p-value* <0.05).

On the first day, G1, where no CaF<sub>2</sub> was added to the cement, had the highest calcium release value (17.35 mmol/L), while the lowest release was in group 5 (8.92 mmol/L) where CaF<sub>2</sub> was in the highest ratio substituting the vaterite. However, this condition changed in day 7 (Figure 2), where the addition of CaF<sub>2</sub> increased the calcium release of the CO<sub>3</sub>Ap but only in the concentration of 20% (G2) and 10% (G3). Higher concentration resulted in significantly less calcium release.

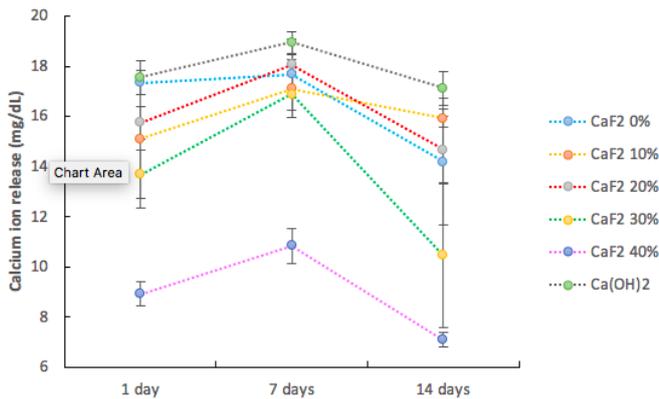


**Figure 2.** Calcium release of calcium fluoride doped carbonate apatite at day 7 of observation (\*\*\*\*\**p-value* <0.05).

On day 14, all groups had lower calcium release than day 7, G6 had the highest calcium release however the differences to G1, G2, and G3 were not significant (Figure 3). Overall in 3 days of observation, the trend of calcium release was similar to each group where it was slightly elevated from day 1 to 7 and gradually decrease on day 14 (Figure 4).

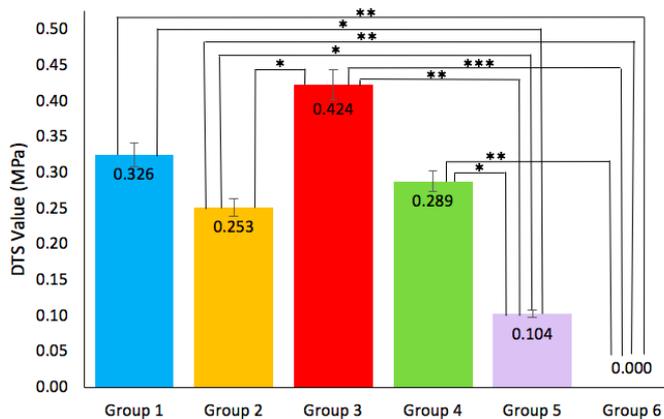


**Figure 3.** Calcium release of calcium fluoride doped carbonate apatite at day 14 of observation (\*\*\**p-value* <0.05).



**Figure 4.** Comparison of mean value of calcium release in calcium fluoride doped carbonate apatite at day 1,7 and 14 of observation.

G6 had higher calcium release in all 3 days of evaluation, followed by G3, G2 and G1 however, there was no significant difference between the G6, G3, G2, and G1. It showed that the addition of CaF<sub>2</sub> to the CO<sub>3</sub>Ap cement did elevate the calcium release as expected.



**Figure 5.** DTS value of calcium release in calcium fluoride doped carbonate apatite (\*\*\*\*\*)*p*-value <0.05).

The addition of CaF<sub>2</sub> gave an impact on the mechanical strength of the CO<sub>3</sub>Ap cement, which is dependent on its concentration. Higher CaF<sub>2</sub> concentration (30% and 40%) significantly decrease the DTS value, while 10% and 20% increased it strength. Group 3 had the highest DTS value but it did not significantly improve the mechanical strength of the original CO<sub>3</sub>Ap cement (G1). Overall, the DTS value for all groups was G3>G1>G4>G2>G5>G6 (Figure 5). The control group consists of Ca(OH)<sub>2</sub> was very fragile, it can be considered as having no DTS value at all.

## Discussion

The addition of CaF<sub>2</sub> to the CO<sub>3</sub>Ap enhances the ability of the cement to release calcium, however, this appears after the 7<sup>th</sup> day. On the first day, the CO<sub>3</sub>Ap cement without any addition of CaF<sub>2</sub> had higher calcium release. This can be explained by the ability of CO<sub>3</sub>Ap itself to releases calcium ions that is mostly from the vaterite component which dissociates calcium ions.<sup>5,7</sup> It seems that CaF<sub>2</sub> needs more time to dissociate, the macro size CaF<sub>2</sub> used in this study might be related to its low solubility. The alkaline environment as a result of the setting reaction of the carbonate apatite might also make the CaF<sub>2</sub> harder to dissolve. A study on nano CaF<sub>2</sub> displayed higher solubility and bioactivity on nano size CaF<sub>2</sub> compared to macro CaF<sub>2</sub>.<sup>20</sup> Our previous study on calcium release on bioglass doped CO<sub>3</sub>Ap also showed the same trend. The addition of bioglass that also contained calcium component did not significantly increase the number of calcium released by the material, rather it was more unstable compared to the CO<sub>3</sub>Ap itself.<sup>7</sup>

Regardless of the solubility, the bioactivity of material to leach ions will also be affected by its microstructure, particle size, environmental pH and water sorption property. Conversely, high solubility as seen in Ca(OH)<sub>2</sub> would result in the loss of material over time compromising sealing and material adaptation to the tooth structure.<sup>21-25</sup> This clarifies the high calcium release in the Ca(OH)<sub>2</sub> group, where it was easily dissolved and dissociate to calcium and hydroxyl ions. On the other hand, CO<sub>3</sub>Ap has slow solubility property, it dissolved over time and eventually be replaced by the natural cell, this was shown when implanting the CO<sub>3</sub>Ap cement in a defected bone structure.<sup>8</sup> When it comes to defect repair material as for pulp capping because it will give the natural cell the chance to regenerate and attach to the defected site and prevent an internal gap between the tooth structure and the restorative material.

The mechanical strength of a material can be determined by various factors such as the L/P ratio, material porosity, solubility, additives, manipulation, humidity, and particle size.<sup>26-28</sup> Our present study showed, that to support its mechanical strength a maximum of 20% addition of CaF<sub>2</sub> was appropriate. It seems that the more CaF<sub>2</sub> and the less vaterite in the cement

decrease its potential to precipitate to stable carbonate apatite crystals, this could be related to the less  $\text{Ca}^{2+}$ ,  $\text{CO}_3^{2-}$ , and  $\text{PO}_4^{3-}$  that are released to supersaturate. Therefore, the  $\text{CO}_3\text{Ap}$  without any  $\text{CaF}_2$  had higher strength than  $\text{CO}_3\text{Ap}$  with 30% and 40%  $\text{CaF}_2$ , because it was still able to form carbonate apatite crystals. Considering the undissolved  $\text{CaF}_2$  seen by the small amount of calcium ion released. It seems that the expectation of fluorapatite or calcium fluoride-like precipitates was not effectively obtained. The FT-IR and XRD analyses in the future study will be carried out to give more information.

Comparing to our previous study, we emphasize the importance of particle size reducing for optimal bioactivity as well as mechanical strength. Reducing its particle size can increase its surface area and enhance the hydration phase which can contribute to its bioactivity and mechanical strength.<sup>24-28</sup> Nanosized particles are generally mentioned to be more effective and have a more homogenous shape and size distribution affecting its surface area, improving the capability of releasing high levels of ions and mechanical strength.<sup>29-31</sup> This was also consistent with modified composites incorporated by nano  $\text{CaF}_2$  that increased its surface area to 20 times greater than the conventional one.<sup>31</sup> The previous study also supports the advantages of nano  $\text{CaF}_2$  by having higher solubility compared to macro  $\text{CaF}_2$ , suggesting that it could be a great candidate for reducing dentin permeability by producing massive mineral precipitates that can penetrate to the dentinal tubule and leach more F ions.<sup>21,22</sup> These features are advantageous for pulp capping, combining nano sized particle of DCPA, vaterite, and  $\text{CaF}_2$ , are expected to improve its bioactivity as well as its mechanical properties as pulp capping agent. Therefore, further study should be carried out in this matter.

## Conclusions

The addition of  $\text{CaF}_2$  to  $\text{CO}_3\text{Ap}$  slightly increased the ability to release calcium ions after 7 days and its mechanical strength compared to the original  $\text{CO}_3\text{Ap}$ . However, further studies, particularly on smaller particle size, need to be done to acquire more significant improvements.

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## Declaration of Interest

The authors declare no conflicts of interest.

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