Title
Bacillus Subtilis Mineralization Properties on Human Dentin and its Effect on Portland Cement

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Bacillus Subtilis Mineralization Porperties on Human Dentin and its Effect on Portland Cement

by

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THESIS

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Bacillus Subtilis Mineralization Properties on Human Dentin and its Effect on Portland Cement

David Cheung-Fai Lau

Abstract

Introduction

The aims of this study are (1) to explore whether *Bacillus subtilis* is capable of precipitating calicite in extracted human teeth *in vitro* and (2) to observe whether *Bacillus subtilis* is capable of strengthening (increasing microhardness) and decreasing the set time of Portland cement (similar to MTA). If successful, the ultimate goals of this study are to aid in creating a predictable form of treatment for severe tooth infractions and vertical root fractures using *B. subtilis* as well as to use the bacterium to improve qualities of MTA treatment in material strength and setting time.

Materials and Methods

Precipitation of calcite by *Bacillus subtilis* on extracted human teeth *in vitro*

Dentin defect was simulated by placing indents using a microhardness tester on mounted dentin blocks. Subsequently, the dentin blocks were exposed to the following treatment conditions: the experimental group - *B. subtilis* culture in LB broth + 2.5% calcium chloride, control group A - *B. subtilis* with LB broth, control group B - LB broth, and control group C was - LB broth + 2.5% calcium chloride. The blocks were incubated at 37º C with medium changed twice a week and were removed after one-, two-, and three-weeks of exposure for observations under light microscopy and Micro CT (week-three samples only) at 10X magnification to evaluate the formation of precipitation.

Observation of effect of addition of *Bacillus subtilis* on microhardness and set time of Portland cement

Portland cement blocks were made by mixing the following liquids with Portland cement: experimental group - *B. Subtilis* and LB broth + 0.25% calcium chloride, control group A - *B. Subtilis* with LB broth, control group B - LB broth, control group C - LB broth + 0.25% calcium chloride, and control group D - water. Microhardness indents of 1000g were placed on samples at 1, 3, 6, 12, and 24 hours to determine the setting time of each sample. After 24 hours, Portland cement block samples were polished and subjected to new indentation. The sizes of the indents were measured under light microscopy. Statistical analysis of indent width and setting time were performed using SPSS 13.0 software (SPSS Inc, Chicago, IL). Statistically significant differences (at $\alpha = 0.05$) were compared among groups using the ANOVA test.

Results

Precipitation of calcite by *Bacillus subtilis* on extracted human teeth *in vitro*

Minimal deposition was observed in all samples (controls and experimental) after 1- and 2-weeks of exposure. However, the indents in one of the dentin blocks of the experimental group after three weeks of exposure appeared to be completely filled. Micro CT scans at 10X magnification displayed presence of the indents in all control group samples, whereas no indents were observed for the experimental group sample at 3 weeks.

Observation of effect of addition of *Bacillus subtilis* on microhardness and set time of Portland cement

Samples where Portland cement was mixed with water were set at approximately 6 hours. The experimental group samples (*B. Subtilis* with LB + 0.25% calcium chloride) were set at

12 hours and Group A (control- *B. Subtilis* with LB) samples were partially set at 12 hours. Groups B and C (LB broth and LB broth + 0.25% calcium chloride, respectively) were not set at 12 hours and all samples were completely set after 24 hours. Portland cement mixed with water showed the highest microhardness but statistically significance only observed between Group D (water) and C (LB broth) (ANOVA, $P< 0.05$).

Conclusions

We found minimal deposition of calcite. However, complete filling of the microhardness indents was observed in one of the experimental samples (*B. subtilis* cultured in LB broth + 0.25% calcium chloride) after three weeks of exposure. Further study will be needed to study the nature of deposits observed in the indents on the dentin block and verify the long-term effect of mineral deposition of *B. subtilis* on dentin. In the second part of our experiment, we found that the addition of LB medium significantly delayed the set time compared to water but the addition of *B. subtilis* and calcium in LB broth reduced the set time compared to LB broth alone. Furthermore, we did not observe a significant improvement on the hardness of Portland cement when it was mixed with *B. subtilis*. Further study will need to assess whether the addition o f *B. subtilis* alone in water could help decrease the setting time and increase the hardness of MTA.

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Introduction

Two significant obstacles encountered by endodontists on a daily basis are: 1) the long setting time of Mineral Trioxide Aggregate (restorative material) which may lead to leakage and ultimate failure of treatment and 2) the inability to treat/ restore cracked teeth. In our study, we introduce two novel ideas with the use of *Bacillius Subtilis* which may resolve the problems encountered by the endodontists.

Mineral Trioxide Aggregate was created by Mahmoud Torabinejad in 1993. According to a review by Torabinejad, the ideal orthograde or retrograde root canal filling material should seal the pathways of communication between the root canal system and its surrounding tissues (Torabinejad et al., 1996). Furthermore, the filling material should also be nontoxic, noncarcinogenic, nongenotoxic, biocompatible, insoluble in tissue fluids, and dimensionally stable. Initially, MTA was created because the existing root-end (retrograde) filling materials did not possess these ideal characteristics. Subsequently, MTA has been used for pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apexes, repair of root perforations, and as a root canal filling material. Numerous studies have shown MTA to possess characteristics as an excellent orthograde/ retrograde root canal filling material. Additionally, the filling material has been recognized as a bioactive material that is hard tissue conductive and hard tissue inductive, antibacterial, and antifungal. Nevertheless, there are a few major drawbacks such as high cost, potential of discoloration, and the formation of hydroxyapatite crystals over MTA when it comes in contact with tissue synthetic fluid. Furthermore, the two most significant drawbacks of MTA which affect the clinical setting are: long setting-time (approx. 165 minutes) and significantly lower short-term compressive strength than that of amalgam, IRM, and Super EBA after 24 hours (however, after 3 weeks,

there is no significant difference between Super EBA, IRM, and MTA). The chemical composition of MTA is similar to Portland cement (Parirokh et al., 2010).

Cracks in teeth (similar to cracks/ fractures in concrete) can severely compromise the tooth and result in the loss of the tooth—fractures are estimated to be responsible for approximately one-third of the loss of molars and premolars (Geurtsen et al., 2003). The American Association of Endodontists (AAE) has divided tooth fractures into five categories: 1) craze lines—confined to enamel; 2) cuspal fracture—fractures that do not involve the pulp directly; 3) cracked teeth—incomplete vertical fractures, often involving the pulp; 4) split tooth—complete vertical fractures (progression of cracked teeth); 5) vertical root fractures (VRFs)—longitudinal complete fractures (usually associated with endodontically treated teeth). Once a tooth infraction (craze lines, cuspal fracture, cracked teeth, split tooth) has developed, neither dentin nor enamel can be permanently reunited, thus all treatment efforts are attempts to prevent the propagation of the crack and perhaps to prevent bacteria from colonizing the leaking space caused by the infraction. Treatments of tooth infractions without pulpal involvement include the use of adhesives (to bind infracted teeth), amalgam with retention on both sides of the infractions, and full coverage crowns. Infracted teeth with irreversibly involved or necrotic pulps require root canal treatment and full coverage restorations. Possible treatments for vertical root fractures in posterior teeth are root amputation and replantation of tooth after root canal treatment and placement of adhesive in the fracture. Nevertheless, the prognosis of split tooth and vertical root fractures is poor and extraction is usually recommended and the treatments are questionable. Consequently, many teeth are lost despite heroic efforts and a more predictable treatment is needed (Ingle et al., 2008).

Bacillus subtilis is a non-obligate aerobic, endospore-forming, rod-shaped bacterium commonly found in soil and associated water sources such as rivers, coastal waters, and estuaries (Nakano et al., 1998). *B. subtilis* is also known as *Bacillus natto* because one of the earliest reported uses of *Bacillus* is the fermentation of soybeans into Natto (a traditional Japanese food). Nevertheless, the formation of Natto is not the only use of the bacteria. *B. subtilis* is extremely versatile and is capable of adapting to various environments. Under the conditions of nutritional starvation, *B. subtilis* stops growing and initiates responses to restore growth by increasing metabolic diversity. These include the induction of motility and chemotaxis, and the production of macromolecular hydrolases (proteases and carbohydrases) and antibiotics. Due to the wide range of metabolism of the *Bacillus* bacteria group, these organisms are used for many industrial processes such as the production of enzymes, antibiotics, fine biochemicals, and insecticides. Furthermore, this bacterium is used as a probiotic in healthy individuals in the treatment or prevention of intestinal disorders. With its widespread use in the food, beverage, and detergents industry, *B. subtilis* has been given the GRAS (generally regarded as safe) status by the US Food and Drug Administration (Harwood, 1992).

Aside from its use in industrial processes, the versatile *B. subtilis* has another quality. *Bacillus subtilis* (calcite-forming bacteria—CFB) is capable of producing calcium carbonate. Calcium carbonate biomineralization is a common process amongst bacteria. The process occurs by two different mechanisms. One of the mechanisms is biologically controlled mineralization and the other is biologically induced mineralization (Lewin et al., 1974, Park et al., 2010). In biologically controlled mineralization, the organisms (which are usually tissue-forming multicellular eukaryotes) control the process usually occurs independent of

environmental conditions—the formation of biogenic mineral is under specific metabolic and genetic control. The mineral particles formed are generated or deposited on or within organic matrices or vesicles in a specific location, and usually intracellularly. Biologically controlled calcium mineralization results in the production of complex and specialized structures such as shells, teeth, and skeletons (De Muynck et al., 2010). On the contrary, biologically induced mineralization normally occurs in an open environment, and no specific cell structure or molecular mechanism is thought to be involved. The process has been considered as induced, and the type of mineral produced is hugely dependent on the environmental conditions (Rivandeneyra et al., 1994).

A cluster of five genes (*lcfA, ysiA, ysiB, etfB,* and *etfA*) called the *lcfA* operon was determined to be involved in calcium carbonatate biomineralization in *Bacillus subtilis* (Barabesi et al., 2007). It was determined that specifically, the *etfA* gene is essential in the precipitation of calcite crystals. The genes in the *lcfA* operon are linked to fatty acid metabolism and thus, a link between calcium precipitation and fatty acid metabolism in *Bacillus subtilis* was suggested by the investigators.

Calcite crystals in B4 precipitation medium was first demonstrated in 1973 (Boquet et al., 1973). Induced mineralization such as microbial calcium carbonate precipitation (MCP) has been utilized in biotechnology with applications such as bioremediation, control leaching (Gollapudi et al., 1995), plugging-cementation of rocks (Stocks-Fischer et al., 2001), solid-phase capture of inorganic contaminants (Warren et al., 2001) and the consolidation of carbonate materials of monuments (Barabesi et al., 2003). Furthermore, the use of calcite-forming bacteria (CFB) was also used to improve the compressive strength of mortar

(concrete remediation)—one of the bacteria used was *Bacillus massiliensis* (Park et al., 2009).

Based on the observations presented by Park et al. (improved compressive strength of mortar), we wish to investigate the use of *B. subtilis* to improve the qualities of Portland cement, which may also improve upon the properties of MTA due to its similar composition to Portland cement. Furthermore, we wish to investigate the ability of *B. subtilis* to precipitate calcite crystals and the plugging of artificial defects (indents) introduced in human teeth dentin with such precipitants (similar to plugging-cementation of rocks as observed by Stocks-Fischer et al.).

The aims of this study are (1) to explore whether *Bacillus subtilis* is capable of precipitating calicite in extracted human teeth *in vitro* and (2) to observe whether *Bacillus subtilis* is capable of strengthening (increasing microhardness) and decreasing the set time of Portland cement (similar to MTA). If successful, the ultimate goals of this pilot study is to aid in creating a predictable form of treatment for severe tooth infractions and vertical root fractures with the use of *B. subtilis* and its mineral forming capability (by further experiments) and to determine whether the bacterium is capable of improving the qualities of MTA (strength, setting time).

Materials and Methods

Precipitation of calcite by *Bacillus subtilis* on extracted human teeth *in vitro*

Bacillus Subtilis ATCC 23857 was cultured onto brain heart infusion (BHI) agar in aerobic conditions for 48 hours. Then, *B. subtilis* colonies were subsequently inoculated into six 50 mL vials: 3 vials contained 50 mL (each) of Luria-Bertani (LB) broth only, and 3 vials contained 50 mL (each) of LB broth supplemented with 0.25% calcium chloride (Barabesi et

al., 2007). All six vials were allowed to grow in aerobic conditions for another 48 hours. Optical density (OD) readings at 650 nm were measured for all each vial. The OD readings for the three LB broth-only vials were 0.47, 0.56, and 0.54; the OD readings for the three LB broth supplemented with 0.25% calcium chloride were 0.51, 0.52, and 0.57. Samples from vials with the highest OD readings (0.56 and 0.57 for LB broth-only and LB broth supplemented w/ calcium chloride, respectively) were used for the subsequent experiment. Preparation of dentin blocks were conducted following a standard protocol described in a previous paper (Dela Rosa et al., 2004). Briefly, 2mL cylindrical vials were sectioned axially with a height of approximately 5mm were used as a mold for the embedding of dentin blocks in resin. Dentin blocks of (9mm X 6mm) were prepared from extracted human teeth and polished with 240 grit sand paper. The dentin blocks were embedded into Allied Resin (composed of LX112, NMA, and DMP-30). The dentin/ resin blocks were allowed to solidify and molds were subsequently removed. Dentin/ resin blocks were polished with 600 grit sand paper, then with 6, 3, and finally, 1 micron diamond at 200 rpm for 4-8 minutes each. All samples were examined under light microscopy at 10X for evaluation of smoothness (lack of scratches) on the dentin blocks. Dentin defect (fracture/ crack) was consistently simulated by placing 1000g, 500g, 10g, and 5g indents (five indents each) were placed (using the microhardness tester) onto the polished dentin surfaces of the dentin/ resin blocks. A total of 36 dentin/resin blocks with indents were prepared. Photographs of all samples were taken at 10X magnification under light microscopy. The dentin/ resin blocks were mounted onto the lids of three twelve-five mL-well plates with the indented surfaces facing the lid of the plate. The three plates with the mounted dentin/ resin blocks were gamma irradiated.

After gamma irradiation, the dentin/ resin blocks were exposed to the different medium combinations (4 mL each well) at 37ºC. One experimental and three control groups were used in the experiment: the experimental group included *B. subtilis* with LB broth supplemented with 2.5% calcium chloride, control group A included *B. subtilis* with LB broth only, control group B included LB broth without bacteria, and control group C included LB broth supplemented with 2.5% calcium chloride without bacteria. 4 mL of medium was placed into each well and the medium was replaced every three days. Bacteria in the experimental group and control group A were only placed in the medium on day zero. Four twelve-five-mL-wells plates were used—one for each week. In each plate, one row (three wells, resulting in triplicates of each group) was used for each group (one experimental and three controls). Dentin block samples were removed after one-, two-, and three-weeks (21 days) of exposure to the different media/ media-bacteria combinations for evaluation. All samples were photographed at 10X magnification under light microscopy after exposure to the different media and media with bacteria for their respective time intervals. Micro CT scans at 10X magnification were conducted to evaluate the formation of precipitation of calcite of the week-three samples from control group B (LB broth without bacteria) and the experimental group.

Observation of effect of addition of *Bacillus subtilis* on microhardness and set time of Portland cement

Cuvets used for OD readings were modified and used as molds for Portland cement blocks. Setting times and microhardness of Portland cement mixed with *B. Subtilis* with LB broth and *B. Subtilis* with LB broth supplemented with 0.25% calcium chloride were determined. One experimental group and four control groups were used in this part of the experiment.

Portland cement block samples were made by mixing 9 mL of each of the following media/ water with 27 grams of Portland cement: the experimental group consisted of *B. Subtilis* with LB broth supplemented with 0.25% calcium chloride, control group A included *B. Subtilis* with LB broth only, control group B included LB broth only, control group C included LB broth supplemented with 0.25% calcium chloride, and in control group D, water was used. The remaining vials bacteria/ media (as described before) were used in the experimental group and control group A. Triplicates of each group were made. All samples were allowed to set at room temperature. 1000g microhardness indents were placed on samples at 1, 3, 6, 12, and 24 hours to determine the setting time of each sample—size of indents and softness of each sample were evaluated and photographs of the samples were taken.

After 24 hours, Portland cement block samples were polished with 240 grit sand paper to remove the microhardness indents placed previously and new indents (five 1000g) were placed onto each sample to aid in microhardness determination. All samples were observed at 10X magnification under light microscopy and pictures were taken. Horizontal measurements of each indent were made. Microhardness was determined by comparing the measurements of each group. Statistical analysis was performed by using SPSS 13.0 software (SPSS Inc, Chicago, IL). Statistically significant differences ($p \leq 0.05$) among groups were determined by using the ANOVA test.

Results

Precipitation of calcite by *Bacillus subtilis* on extracted human teeth *in vitro*

All samples were evaluated under light microscopy at 10X after 1, 2, and 3 weeks of exposure to various combinations of *B. Subtilis* and medium or medium alone. After one week of exposure, minor differences were observed in all samples when compared to pre-

exposure. After two weeks of exposure, the indents in all of the samples appeared to have reduced in width (Figure 1 and Figure 2). After three weeks of exposure, little difference was observed for Control groups A, B, and C when compared to week two's samples. However, the indents in one of the triplicates of the experimental group after three weeks of exposure appeared to be completely filled—the original indents were difficult to observe (Figure 1). Micro CT scans at 10X magnification of one week-three control group B sample and one experimental group sample were acquired. Indents were observed in the sagittal plane slices of the 1000g indents at the widest section in the control group B sample, whereas no indents were observed for the experimental group sample (Figures 3a, 3b and 4a, 4b).

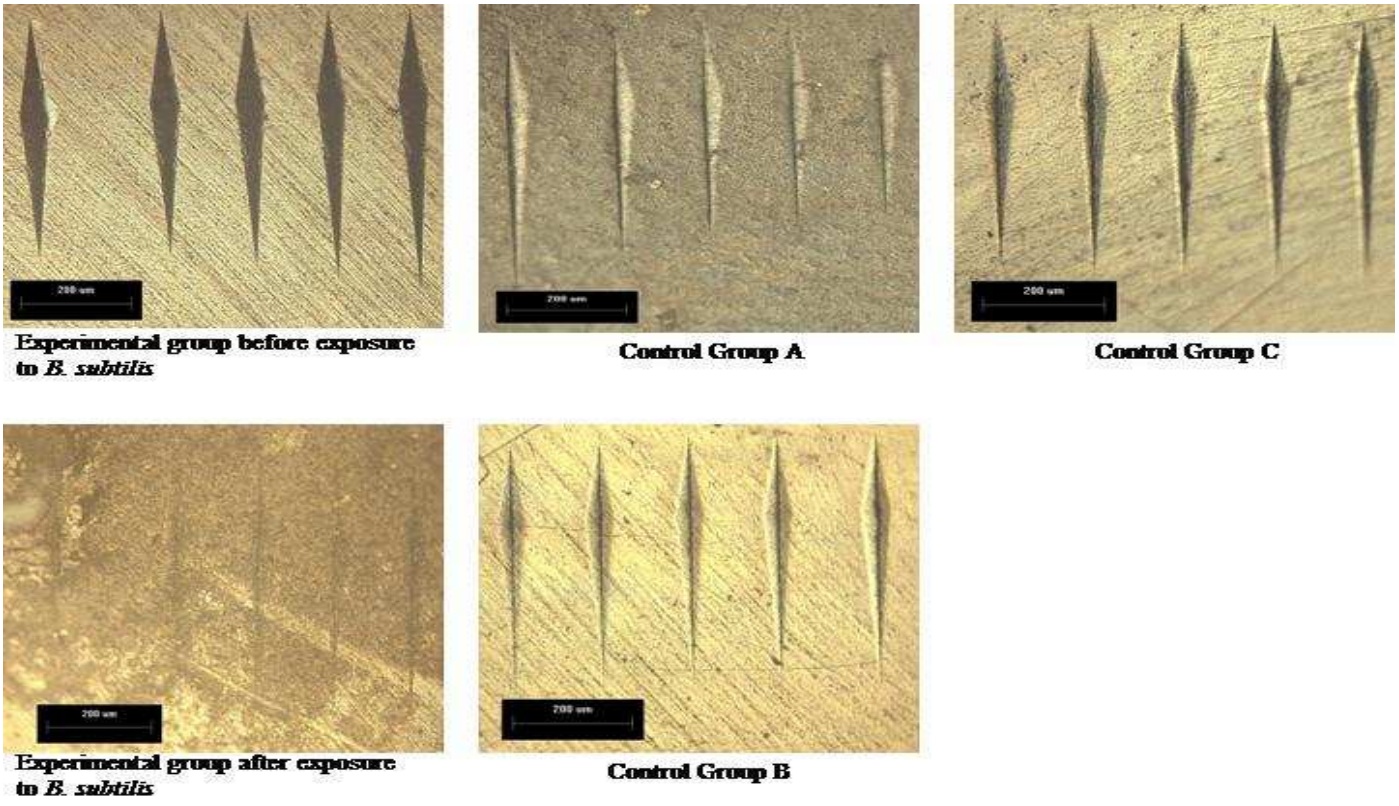

Figure 1: Precipitation by *B. subtilis* on dentin blocks with 1000g microhardness indents in different conditions. Experimental group before exposure to *B. subtilis*: no exposure; Experimental group after exposure to *B. subtilis*: three weeks exposure to *B. subtilis* + LB broth supplemented with 0.25% calcium chloride; Control Group A: three weeks exposure to *B. subtilis* + LB broth only; Control Group B: three weeks exposure to LB broth only; Control Group C: three weeks exposure to LB broth supplemented with 0.25% calcium chloride.

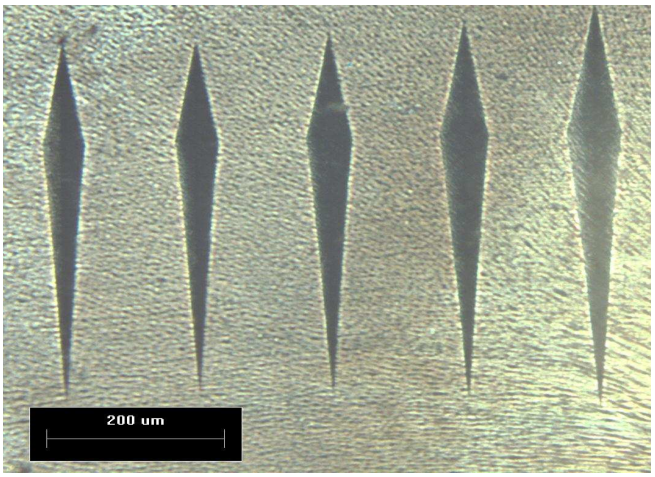

Figure 2a: Experimental group after one week of exposure, 1000g indents.

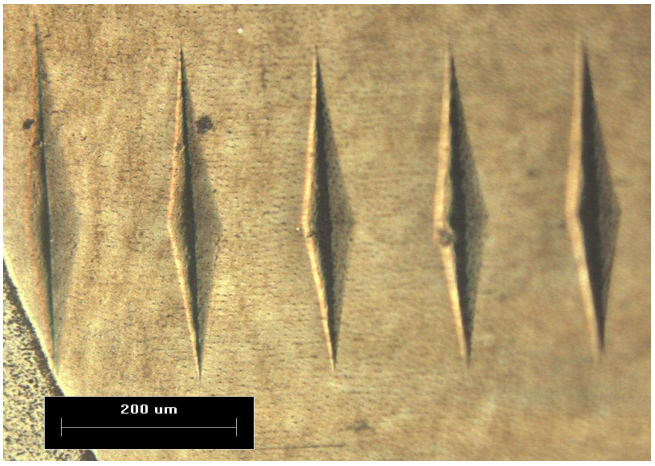

Figure 2b: Experimental group after two weeks of exposure, 1000g indents. *Note- Decreased width of indents (compare with week one sample- Fig 4a).*

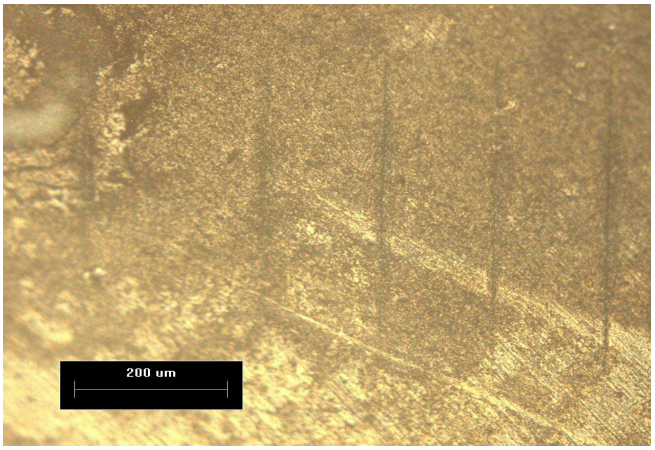

Figure 2c: Experimental group after three weeks of exposure, 1000g indents. *Note- Indents barely visible*.

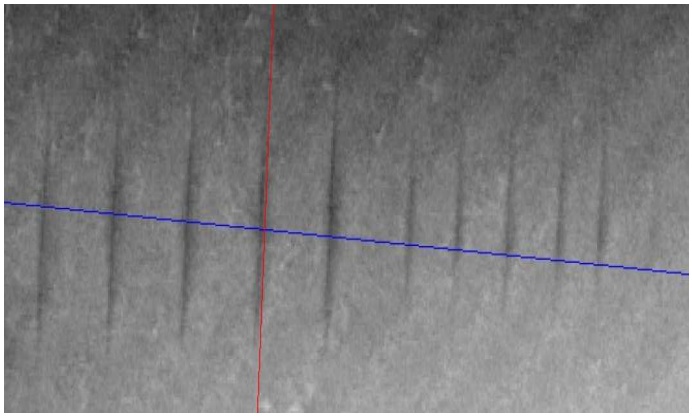

Figure 3a: Micro CT scan 10X axial view of Week 3 Control group B. Blue line placed on the widest section of indents (for sagittal section view- Figure 2B).

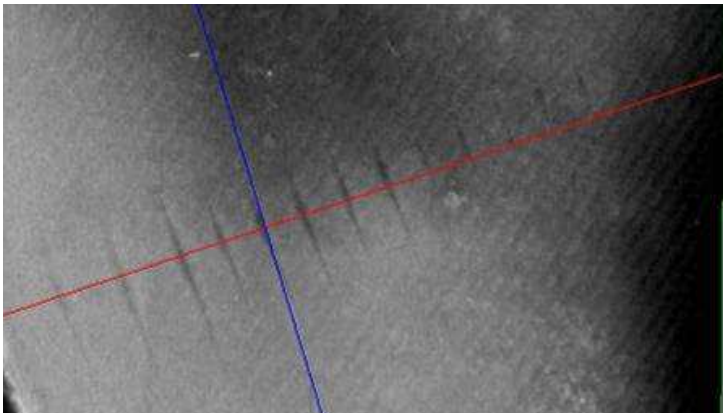

Figure 4a: Micro CT scan 10X axial view of Week 3 Experimental group. Red line placed on the widest section of indents (for sagittal section view- Figure 3B)

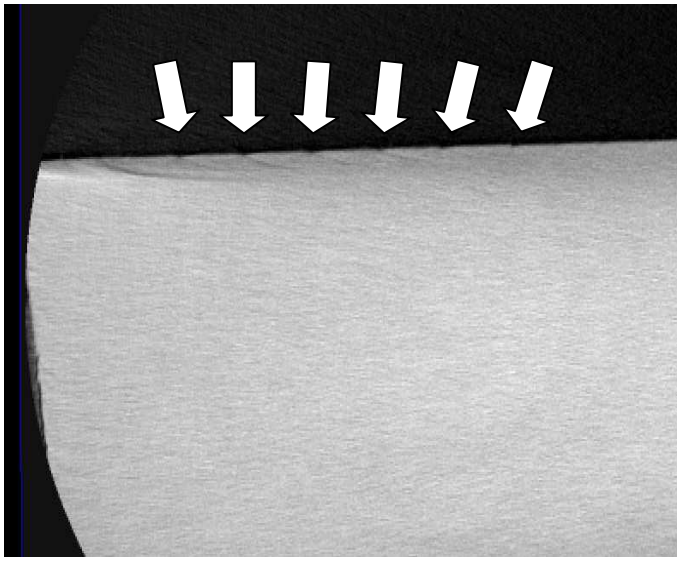

Figure 3b: Micro CT scan 10X sagittal view of Week 3 Control Group B.
-Arrows indicate indents

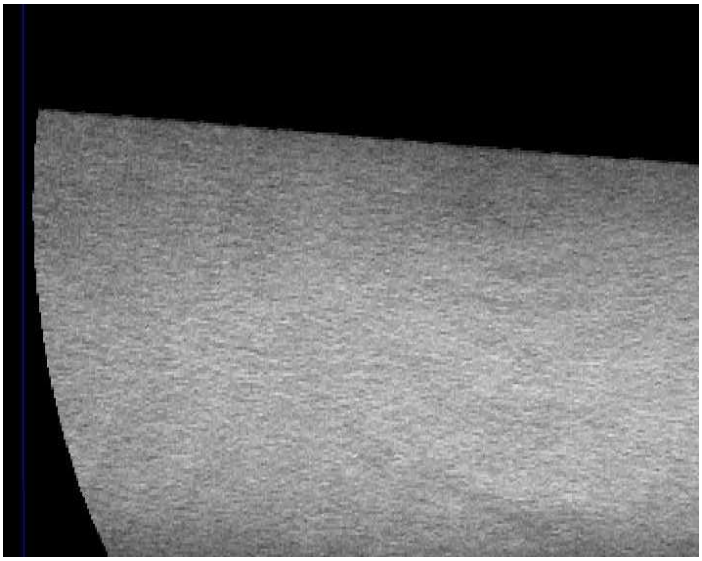

Figure 4b: Micro CT scan 10X sagittal view of Week 3 Experimental group.
-No indents observed.

Observation of effect of addition of *Bacillus subtilis* on microhardness and set time of Portland cement

Group D (blank control group, Portland cement mixed with water) samples were set at approximately 6 hours. After 12 hours, the experimental group (*B. Subtilis* with LB supplemented with 0.25% calcium chloride) and Group A (negative control- *B. Subtilis* with LB) were partially set at 12 hours. Groups B and C (negative controls- LB broth only and LB broth supplemented with 0.25% calcium chloride, respectively) were not set at 12 hours and all samples were completely set after 24 hours (Figure 5). Five 1000g indents were placed on all samples after 24 hours and the width of each indent was measured. The five measurements of each sample (triplicates made for each group) were averaged (see Table 1) for the microhardness measurements. Portland cement mixed with water (Group D) showed the strongest hardness (least width in indentation). Nevertheless, Group D was only significantly different from Control Group C (LB broth supplemented with 0.25% calcium chloride) (ANOVA, P□ 0.05).

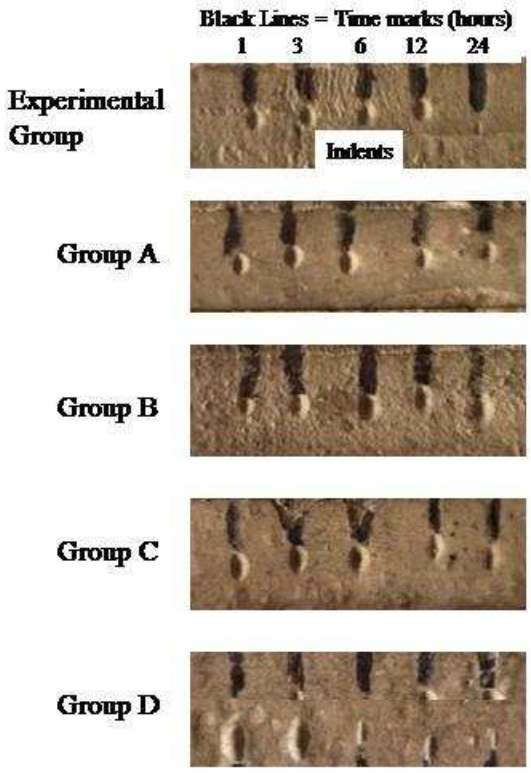

Figure 5: Setting time of Portland cement samples in different conditions. The black lines on top of the samples indicate the position of indents for each sample. The indents could be observed below the black lines. Timelines for indents from left to right: 1, 3, 6, 12, and 24 hours after the cements were mixed with the corresponding liquids in each group. Experimental group: *B. subtilis* + LB broth supplemented with 0.25% calcium chloride; Group A: *B. subtilis* + LB broth only; Group B: LB broth only; Group C: LB broth supplemented with 0.25% calcium chloride; and Group D: water.

Table 1: Measurements (width) of microhardness indents placed on Portland cement samples after 24 hours

Groups	N	Mean (μm)	Standard Deviation (μm)
Experimental	3	180.90	36.02
Control A	3	182.07	45.46
Control B	3	257.00	10.22
Control C	3	375.77*	163.47
Control D	3	121.30*	1.47

*Significant difference between these two groups

Discussion

In our investigation, we observed filling of all microhardness indents placed on a dentin block exposed to *B. subtilis* with LB broth supplemented with 0.25% calcium chloride after three weeks of exposure at 37°C. We were able to observe this by evaluating the sample at 10X magnification under light microscopy and 10X micro CT scan. A layer containing possibly organic-inorganic composites was observed on the surfaces of all the samples when observed at 10X magnification under light microscopy. A trend of decreasing width of the microhardness indents was observed from week one to week three for the experimental group (*B. subtilis* with LB broth supplemented with calcium chloride) (Figures 4a, b, c). As for the control samples, no differences were observed during the four weeks. With this observation, we can assume the results observed on the experimental samples are the effects of the presence of *B. subtilis* and a calcium source. Nevertheless, within the limitations of our study, we do not know the exact composition of the material deposited into the microhardness indents and the composition of the organic-inorganic composite layer observed on the surface of the samples. Atomic absorption spectroscopy (AAS) of the experimental samples should be done in the future to quantitatively assess the composition of the materials deposited in the indents and on the surface of the samples. Furthermore, to confirm our current findings, longer exposure times and repeated assays are needed.

Park et al. observed an increase of approximately 19.5% in compressive strength of Portland cement samples when exposed to a B4 medium inoculated with *B. subtilis*. In our experiment, we mixed Portland cement with LB broth supplemented with calcium chloride inoculated with *B. subtilis* to determine whether this has an effect on the setting time and microhardness of Portland cement. In our experiment, the shortest set time was observed when Portland cement was mixed

with water. In all groups with addition of LB broth, the setting time was longer with the longest as Portland cement mixed with LB broth. This indicates the contents in LB broth may increase the set-time of Portland cement. However, when Portland cement was mixed with LB broth supplemented with calcium chloride, the setting time decreased compared to when Portland cement was mixed with LB broth or with LB broth inoculated with *B. subtilis* without supplementation of calcium. These results indicate the presence of *B. subtilis* with calcium decreased setting time of Portland cement when mixed in LB broth. Nevertheless, the effect does not improve upon the setting time of Portland cement when mixed with water. Future study may need to investigate whether the addition of just *B. subtilis* and calcium would decrease the set-time of MTA or Portland cement and the biosafety of this procedure in dental pulpal treatments.

A similar trend was observed when we examined the samples (after complete setting) for microhardness. In a previous study performed by Park et al., the presence of *B. subtilis* increased the compressive strength of Portland cement. In Park et al.'s study, all Portland cement samples were mixed with water and after complete setting, the samples were immersed in B4 medium inoculated with *B. subtilis*. In our experiment, LB broth inoculated with *B. subtilis* was mixed with Portland cement and microhardness was determined after complete setting of the samples. The presence of *B. subtilis* did not increase the microhardness of Portland cement when compared to samples mixed only with water. Nevertheless, the presence of the bacterium appeared to have increased the microhardness of Portland cement when compared to samples mixed with LB broth or LB broth supplemented with calcium chloride only (no significant difference observed, p □ 0.05). Despite these findings, we observed a significantly lower (p □ 0.05) microhardness in Portland cement samples mixed with LB broth supplemented with 0.25%

calcium chloride when compared with Portland cement mixed with water. According to this finding, future studies should investigate the outcome of the mixture of Portland cement with water inoculated with *B. subtilis*. Additionally, immersion of set Portland cement samples (mixed with water) in LB broth (or water) inoculated with *B. subtilis* (performed by Park et al.) should also be considered.

Conclusion

In the first part of our experiment, we observed filling of all microhardness indents placed on a dentin block exposed to *B. subtilis* with LB broth supplemented with 0.25% calcium chloride after three weeks of exposure at 37°C. However, the deposits observed in the indents on the surface of the dentin block was not determined. Further studies should be performed to determine the specific composition of the deposits observed by use of AAS. If deposits are determined to be calcite, further steps should be taken to investigate the possible use of *B. subtilis* as a predictable form of treatment for severe tooth infractions and vertical root fractures. In the second part of our experiment, we investigated the effect of the addition of *B. subtilis* on microhardness and set time of Portland cement. An increased set time and no significant improvement on the microhardness of Portland cement were observed (significantly lower microhardness was observed when Portland cement was mixed with LB broth supplemented with calcium chloride only). Future studies should investigate the outcome of the mixture of Portland cement with water inoculated with *B. subtilis*. Additionally, immersion of set Portland cement samples (mixed with water) in LB broth (or water) inoculated with *B. subtilis* should also be considered. If the microhardness and setting time of Portland cement can be improved with the addition/ exposure of *B. subtilis*, additional studies should be performed to determine if the improvements can also be observed in MTA (due to its similar composition to Portland cement).

With the improved properties of MTA, greater prognosis of teeth restored with MTA may be possible.

References

Barabesi C, Galizzi A, Mastromei G, Rossi M, Tamburini E, Perito B. 2007. *Bacillus subtilis* gene cluster involved in calcium carbonate biomineralization. J Bacteriol. 91(1): 228-235

Bouquet E, Boronat A, Ramos-Cormenzana A. 1973. Produciton of calcite (calcium carbonate) crystals by soil bacteria is a general phenomenon. Nature. 246: 527-529

De Muynck W, De Beliea N, Verstraeteb W. 2010. Microbial carbonate precipitation in construction materials: A review. Ecol. Eng. 36: 118-136

Dela Rosa A, Anupama V, Sarma V, Le CQ, Jones RS, Fried D. 2004. Peripheral thermal and mechanical damage to dentin with microsecond and sub-microsecond 9.6um, 2.79um, and 0.355um Laser Pulses. Lasers in Surg and Med. 35: 214-228

Ford TR, Torbinejad M, Abedi HR, Bakland LK, Kariyawasam SP. 1996. Using Mineral Trioxide Aggregate as a pulp-capping material. J Am Dent Assoc. 127(10): 1491-1494

Geurtsen W, Schwarze T, Gunay H. 2003. Diagnosis, therapy, and prevention of the cracked tooth syndrome. Quintessence Int. 34(6): 409-417

Gollapudi, U.K., Knutson CL, Bang SS, Islam MR. 1985. A new method for controlling leaching through permeable channels. Chemosphere 30:695–705

Harwood CR. 1992. *Bacillus subtilis* and its relatives: molecular biological and industrial workhorses. Trends Biotech 10: 247-256

Ingle JI, Bakland LK, Baumgartner JC. 2008. Ingle's Endodontics 6 (Ch. 19, 20). Connecticut: People's Medical Publishing House

Lewin SZ, Baer NS. 1974. Rationale of the barium hydroxide-urea treatment of decayed stone. Studies Conserv. 19: 24-35

Nakano MM, Zuber P. 1992. Anaerobic Growth of a “Strict Aerobe” (*Bacillus Subtilis*). Trends Biotechnol. 10(7): 247-256

Parirokh M, Torabinejad M. 2010. Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part III: Clinical Applications, Drawbacks, and Mechanism of Action. J Endod 36(3): 400-413

Park SJ, Park YM, Chun WY, Kim WJ, Ghim SY. 2010. Calcite-forming bacteria for compressive strength improvement in mortar. J. Microbiol. Biotechnol. 20: 782-788.

Rivadeneyra MA, Delgado R, Moral AD, Ferrer MR, Ramos-Cormenzana A. 1994. Precipitation of calcium carbonate by Vibrio spp. from an inland saltern. FEMS Microbiol. Ecol. 13: 197-204.

Stocks-Fischer S, Galinat JK, Bang SS. 1999. Microbiological precipitation of CaCO3. Soil Biol. Biochem. **31:**1563–1571.

Warren LA, Maurice PA, Parmar N, Ferris FG. 2001. Microbially mediated calcium carbonate precipitation: implications for interpreting calcite precipitation and for solid-phase capture of inorganic contaminants. Geomicrobiol. J. **18:**93–115.

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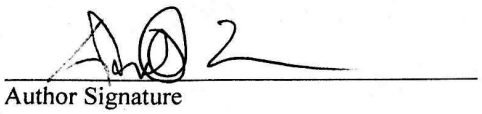

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