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Evaluation of the Physical Properties of Cockle Shell-derived Bioceramic Pulp Capping Material: A Pilot Study

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Abstract

Objectives: To investigate the physical properties of cockle shell derived tricalcium silicate pulp capping material (C-Cap).

Methods: C-Cap was manufactured by processing cockle shell and rice husk ash under specific conditions, and various additives were added to gain the desirable physical properties. C-Cap consisted of two parts: a powder (mixture of cockle shell-derived tricalcium silicate, zirconium silicate and silicon dioxide) and a liquid (methyl salicylate and N-Butyl benzene sulfonamide). The mixing of the materials was performed via loading powder and liquid into a plastic capsule and put into an amalgamator for 8 seconds at a speed of 4,000 vibrations/minute. Life and Dycal® were tested for their physical properties and compared with C-Cap. The setting time, flowability and solubility tests were conducted in accordance with the modified ISO 6876:2012. pH was assessed at 3 hours, 1, 3, 7, 14 and 28 days. Statistical analysis was performed using Two-way repeated measures ANOVA analysis, One-way ANOVA with Tukey's test and repeated measures ANOVA analysis with Bonferroni test ($p<0.05$).

Results: C-Cap, Dycal®, and Life had setting times in the range of 1-2 minutes. C-Cap provided the highest flowability. The solubility test showed no significant difference among groups (10.6-12.4 % by weight). In comparison to Dycal® and Life, C-Cap showed the highest alkaline properties (with the pH of 9.9-10.5 over the period of 28 days).

Conclusions: C-Cap exhibited suitable physical properties for use as pulp capping material.

Keywords: biomaterials, cockle shell, pulp capping, tricalcium silicate

Introduction

Pulp capping material plays significant role on pulpal healing and regeneration for vital pulp therapy. Calcium hydroxide is commonly used for pulp capping due to its antimicrobial activity, good biocompatibility, and promoting hard tissue mineralization.[1,2] However, numerous studies have demonstrated unsatisfactory outcomes when using calcium hydroxide in vital pulp therapy, including tertiary dentin formation with tunnel defect, high solubility, and poor tooth adhesion.[3,4] Since 1993, bioceramics, especially mineral trioxide aggregate (MTA), have been used as materials with good biocompatibility, hydrophilic properties, low solubility post setting, and antimicrobial activities.[5-8] Several clinical studies have

reported greater successful outcomes of direct pulp capping using MTA as a pulp dressing material in comparison to calcium hydroxide, with the observation of higher clinical success and dentine bridge observations.[9-11]

The commercial bioceramic materials branded as ProRoot MTA (Densply Tulsa Dental, Tulsa, OK, USA) and Biodentine (Septodont, Saint-Maur-des-Fosses, France) are calcium silicate-based cements. The main composition of both materials is calcium silicate combined with various additives, such as bismuth oxide, zirconium oxide and calcium chloride to improve its physical properties.[12] Calcium silicate-based powder can be manufactured from either chemical sources; pure precipitated calcium carbonate and silica, or biological-source raw materials; cockle shells and rice husks. Cockle shell is a source of calcium carbonate and can be processed into calcium.[13] Silicon dioxide is obtained from rice husk ash via laboratory procedures.[14] Cockle shell-derived tricalcium silicate powder is manufactured by processing the powder of cockle shells and rice husk ash under specific conditions. With the addition of a radiopacifier, setting accelerator, and plasticizer to the cockle shell-derived tricalcium silicate powder, the physical properties of such material could be suitable for usage in vital pulp therapy.

Calcium carbonate rich cockle shells can be synthesized into nano-particle calcite, which in dentistry acts as a calcium source for enamel remineralization and dental hypersensitivity treatment.[15] Synthesis of hydroxyapatite paste from cockle shells significantly increase enamel surface hardness after carbonated drink immersion, as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste.[16] In medical use, nanocomposite bone scaffolds manufactured from cockle shell powder have reportedly had favorable outcomes.[17] With its biocompatibility and remineralization properties, it can possibly to be used in the manufacture of a pulp capping material.

The purpose of this study was to investigate the setting time, flowability, solubility and pH of cockle shell-derived tricalcium silicate pulp capping material (C-Cap) in comparison with commercial hard setting calcium hydroxide. The authors hypothesized that the physical properties in terms of setting time, flowability, solubility and pH of C-Cap, Dycal® and Life will be equal.

Materials and Methods

Preparation and characterization of cockle shell-derived tricalcium silicate

The raw materials for tricalcium silicate synthesis were cockle shells and rice husk ash, which were processed separately in boiling distilled water and acetic acid solution and then ground into powder. Next, the rice husk ash derived powder and the cockle shell-derived powder were mixed together in stoichiometric ratio. The mixed powder was pressed into molds having a diameter of 3 cm and a thickness of 1 cm. The molds were placed in an alumina crucible and fired at 1450°C for 2 hours and were rapidly cooled. The fired products were removed from the molds and were ball-milled using high purity ethanol as a milling medium and then dried. The chemical composition of the cockle shell-derived tricalcium silicate powder was analyzed by X-ray diffractometer (XRD). Quantitative phase analysis was conducted using Rietveld structure refinement technique. The dynamic laser scattering particle size analyzer was used to determine the particle size.

Preparation of tested materials

Cockle shell-derived tricalcium silicate pulp capping material (C-Cap) had two parts: a powder mixture of cockle shell-derived tricalcium silicate, zirconium silicate and silicon dioxide, and a liquid combination of methyl salicylate and N-Butyl benzene sulfonamide. Powder: liquid ratio is 2.5:1. Upon usage, powder and liquid are loaded in a capsule and mixed by amalgamator for 8 seconds at a speed of 4,000 vibrations/ minute.

Life (Kerr, Romulus, MI, USA) and Dycal® (Densply, Milford, DE, USA) were commercial hard setting pulp capping calcium hydroxide pastes. Following the manufacturer's instructions, base and catalyst pastes were dispensed onto the mixing paper. Using a spatula, both parts were stirred immediately until a uniform color was achieved. The tested materials' compositions are presented in Table 1.

Physical properties test

Life, Dycal® and C-Cap were tested for their physical properties–setting time, flowability and solubility following the modified ISO 6876:2012 protocol.

Table 1: Compositions of tested materials.

Materials	Compositions	Manufacturers and batch numbers
C-Cap		
Powder part:	Tricalcium silicates Zirconium silicate Silicon dioxide	Prepared at Chulalongkorn University, Thailand Mine Chem, Thailand, 070620 Ajax Finechem, Australia, 0802471
Liquid part:	Methyl salicylate N-Butyl benzene sulfonamide	Sigma-Aldrich, USA, MKCL1436 Sigma-Aldrich, USA, MKCK8702
Life		Kerr, USA, 7473277
Base paste:	Calcium dihydroxide, N-ethyl-o(or p)-toluenesulphonamide Zinc oxide, Calcium oxide	
Catalyst paste:	Methyl salicylate, 2, 2-dimethylpropane-1, 3-diol	
Dycal®		Densply Sirona, USA, 00002876
Base paste:	Disalicylate ester of 1, 3, butylene glycol, Calcium phosphate, Calcium tungstate, Zinc oxide, Iron oxide	
Catalyst paste:	Calcium hydroxide, Ethyl toluenesulfonamide Zinc sterate, Titanium dioxide, Zinc oxide, Iron oxide	

Evaluation of setting time

After the end of mixing, the samples were placed into a stainless steel ring mold with a height of 2 mm and a 10 mm diameter. Then the molds were placed into a temperature-controlled environment at 37°C and 95% relative humidity. A gilmore indenter with a diameter of 2 mm and weighing 100 g was placed onto the horizontal surface of each sample. If an indentation was visible, the needle was raised and cleaned. Then, the indenter was placed at new position until the indenter failed to produce a visible mark. This was recorded as setting time.

Evaluation of flowability

A 0.05 ml amount of material was placed at the center of a glass plate. A second glass plate was placed on top, with an additional mass on the plate, totaling 120 grams of load for 10 minutes. The diameter of the compressed sample was measured and recorded as the flow value.

Evaluation of solubility

Each sample was put into a stainless steel mold with a height of 2 mm and a diameter of 10 mm, which were then placed in temperature-controlled environment at 37°C and 95% relative humidity for 24 hours. Then, the samples were weighted by a digital balance. Two samples were placed into beaker A with 50 ml of deionized water and were stored in 37°C with 95% relative humidity for 24 hours. The samples and water from beaker A were poured through a funnel fluted filter into beaker B. Beaker B was placed in a 110°C oven until the water was completely evaporated. The difference in weight of the original and final beaker B was used to calculate the dissolved mass of the sample. The sample dissolved mass and initial sample mass were calculated as a percentage of solubility.

Evaluation of pH

Tested materials were placed in an open-ended plastic tube 1.5 mm in diameter and 10 mm in length. Each tube was stored in a sealed container with 10 ml of deionized water at 3°C. At observation intervals of 3 hours, 1, 3, 7, 14 and 28 days, pH was measured with a pH meter (Model 420A, Boston, USA).

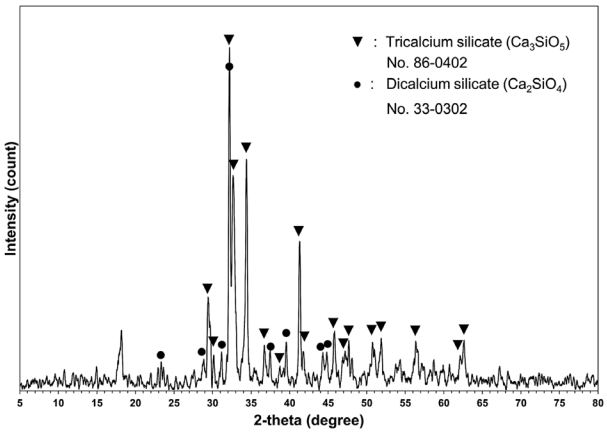
Statistical analysis

Statistical analysis was performed via IBM SPSS software version 22 (IBM Corporation, Armonk, NY, USA). All data were presented as means for each group± standard deviation (SD). Two-way repeated measures ANOVA was used to identify the effect of times, materials and interaction between times and materials on pH. One-way ANOVA with Tukey's test was used to determine the differences of pH between the material groups at each time point. The repeated measures ANOVA with Bonferroni

post-hoc analysis was performed to analyze the differences of pH among time points within each material group. The statistically significant difference was set at $p<0.05$.

Results

XRD and quantitative phase analysis of the cockle shell derived tricalcium silicate powder showed 75% tricalcium silicate as main composition and 20% dicalcium silicate (Figure 1). The dynamic laser scattering particle size analyzer revealed the average particle size was 5.48 microns.



Figurer 1: XRD analysis of the cockle shell-derived tricalcium silicate powder show the peak of tricalcium silicate (▼) and dicalcium silicate (●) formation

Physical properties of C-Cap, Life and Dycal®

The mean and standard deviation of setting time, flowability and solubility of the tested materials are shown in Table 2. The setting time of all materials was in the range 1-2 minutes. Dycal® and C-Cap set faster than Life. C-Cap showed the highest flowability (25.93±1.30 mm). There was no significant difference for solubility among groups.

pH

According to two-way repeated measures ANOVA, there is a statistically significant interaction effect between times and materials on pH, as shown in Table 3. Table 4 shows the pH value of C-Cap, Life and Dycal® after deionized water immersion at 3 hours, 1, 3, 7, 14 and 28 days. In general, all materials exhibited alkalinity at every observation interval. Among all materials, C-Cap showed the highest alkalinity with a pH of 9.9-10.5. Life and Dycal® showed the pH range of 7.8-9.5.

Discussion

Tricalcium silicate can be produced from calcium carbonate ($CaCO_3$) and silica (SiO_2) as raw materials. Instead of using Portland cement from limestone and shale as the main source of calcium silicate, this study utilized cockle shell and rice husk ash to synthesize tricalcium silicate. Both materials come from food and agricultural

Table 2: Setting time, flowability and solubility (mean and standard deviation).

Material	Setting time (n=6) (minute, second)	Flowability (n=6) (mm)	Solubility (n=6) (%)
C-Cap	1, 23 (0, 06)^a	25.93 (1.30)^a	10.64 (1.29)^a
Life	2, 01 (0, 23)^b	22.58 (0.95)^b	12.35 (1.53)^a
Dycal®	1, 10 (0, 02)^a	18.83 (0.38)^c	12.40 (1.08)^a

Different superscript letters in the same column show statistical difference between groups ($p<0.05$).

Table 3: Two-way repeated measures ANOVA test for pH.

Source	*df*	Sum of Squares	Mean Square	F	*p*
Times	5	1.284	0.257	1.328	0.262
Materials	2	75.556	37.778	122.689	<0.001
Times*Materials	10	16.884	1.688	8.729	<0.001

Table 4: pH of tested materials at different observation interval. (mean and standard deviation, *n*=6)

Material	3 hours	1 day	3 days	7 days	14 days	28 days
C-Cap	10.18 (0.09)^{a,B}	9.99 (0.33)^{a,B}	9.92 (0.61)^{a,B}	10.51 (0.38)^{a,A}	10.17 (0.88)^{a,B}	10.50 (0.59)^{a,B}
Life	8.03 (0.40)^{c,B}	8.43 (0.61)^{b,AB}	8.04 (0.33)^{c,B}	7.84 (0.46)^{c,B}	8.32 (0.54)^{b,AB}	9.07 (0.66)^{b,A}
Dycal®	9.50 (0.08)^{b,A}	8.71 (0.20)^{b,B}	8.94 (0.33)^{b,B}	8.71 (0.44)^{b,B}	8.19 (0.23)^{b,C}	7.79 (0.31)^{c,D}

Different superscript small letters in the same column show statistical difference between groups at each observation interval. Different superscript capital letters in the same row show statistical difference between observation intervals of each material (p<0.05).

waste, which are abundant, cheap and environmental-friendly. The XRD analysis of the cockle shell-derived tricalcium silicate powder demonstrated the presence of tricalcium silicate along with a small amount of dicalcium silicate. Therefore, it serves as an appropriate source material for the development of a tricalcium silicate pulp capping material.

To improve C-Cap's physical properties, materials were added to cockle shell-derived tricalcium silicate powder. Methyl salicylate was added to improve its handling properties and to accelerate the setting time of C-Cap. Methyl salicylate is a salicylic acid derivative in oily liquid form which is extracted from the leaves of *Gaultheria procumbens* and the bark of *Betula lenta.* It is widely used in pharmacology due to its analgesic and anti-inflammatory effects.[18] In dentistry, salicylate is present in Life and Dycal®. Methyl salicylate was synthesized as glycerol salicylate resin which is present in the root canal sealer for rheological properties.[19] N-Butyl benzene sulfonamide, which is used in calcium hydroxide-based cement[20], was used as plasticizer to improve flowability and handling properties. Amorphous silicon dioxide was added to improve handling properties. Camilleri *et al*. found that silicon dioxide is contained in the formulation of BioAggregate™ (Innovative Bioceramix, Vancouver, BC, Canada).[21] Zirconium silicate ($ZrSiO_4$) is used as radiopacifier. It is cheap, naturally sourced and has several desirable properties–including high atomic mass, non-toxic, low thermal expansion and mechanical strength reinforcement.[22]

Clinically, using pulp capping materials with fast setting times will enable clinicians to reduce chair-time and complete treatment in one visit. Our results showed Dycal®, Life and C-Cap set within 2 minutes. In this study, the setting time of Dycal® and Life were slightly faster than the reported values (2-3 minutes) of the manufacturers. The possible reason may partly be from different experimental settings, as this study had conditions of higher relative humidity (95% RH) and temperature (37°C).

Flowability represented the handling properties of the material when applied to the operating surface. C-Cap exhibited the highest flowability in comparison to Life and Dycal®. All materials were easy to manipulate and had a creamy consistency. The solubility of C-Cap, Life and Dycal® were not statistically different, and all were in the range of 10.7-13.4 % by weight. Our results showed higher solubility values for Life and Dycal® than occurred in other studies.[23,24] The factors affecting solubility could be surface exposure of the samples and the volume of deionized water they were immersed in, which varies among studies. C-Cap contains tricalcium silicate, which, when exposed to body fluid, will begin a hydration reaction resulting in the formation of calcium silicate hydrate with low solubility and high strength. Therefore, with a longer time period, C-Cap could be less soluble as the characteristic of bioceramic. However, the longer time observation of solubility should be investigated.

All materials exhibited alkalinity over the observation intervals. According to the result, the pH of C-Cap was statistically higher than Life and Dycal®. Other studies reported higher values of pH than this study, which might be due to a different experimental setup, with more surface exposure.[23,24]

As described earlier, the physical properties in terms of the setting time, flowability and pH of C-Cap, Dycal® and Life were not equal. Solubility of C-Cap was equal to Dycal® and Life. Therefore, the null hypothesis was rejected.

The literature reveals strong evidence of successful vital pulp therapy with the usage of calcium silicate-based materials.[9-11,25,26] When used as a material for indirect pulp capping, calcium silicate-based material, particularly MTA, has been demonstrated to provide more desirable outcomes than calcium hydroxide in terms of tertiary

dentin formation and bacterial reduction beneath restoration.[27,28] When encountering teeth with carious pulp exposure during carious-tissue excavation, direct pulp capping was indicated for teeth with clinical signs and symptoms of reversible pulpitis.[29] Various studies and systematic reviews demonstrated more satisfactory outcomes with calcium silicate-based materials in comparison to calcium hydroxide in vital pulp therapy.[11,30-34]

C-Cap is a tricalcium silicate-based material, which is manufactured from food and agricultural waste products–cockle shells and rice husk ash. The observed physical properties of C-Cap demonstrate it is easy to use and is suitable for clinical usage as pulp capping material. The observation of an alkaline pH implies the potential to enhance pulpal cell differentiation and hard tissue mineralization.[1,5,35] Further studies should evaluate the biocompatibility, regenerative potential via animal and clinical studies.

Conclusions

C-Cap exhibited desirable physical properties including short setting time, good flowability and alkaline pH. for use as a pulp capping material.

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Conflicts of interest

The authors declare no conflicts of interest.

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