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骨形成性複合材的理化性質、細胞分子機制及動物試驗研究(第3年)

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中 文 摘 要 ： 鈣基骨水泥廣泛使用在牙科及骨科手術上。當中磷酸鈣(CPC)及矽酸鈣(CSC)具備許多優異性質，臨床使用在骨修補。本計畫第三年的目的在於比較商品BoneSource CPC與自行開發的CSC之體外骨形成性與抗菌效果，尤其是細胞分化能力。骨形成性評估使用人類骨髓幹細胞(hMSCs)，抗菌活性則是利用格蘭氏陽性菌(金黃色葡萄球菌，S. aureus)及陰性菌(綠膿桿菌，P. aeruginosa)，分析抑菌比與抑菌圈。相較於CPC，CSC更能促進hMSC細胞增生與分化(鹼性磷酸酶與骨鈣素)，以及礦化節點形成。值得注意的是CSC有效誘導hMSCs分化，即使培養液沒有含骨分化劑。此外，CSC亦顯著具有較高抑菌活性。這些發現可建議本計畫自行開發的CSC為有用的骨修補生醫材料，可比擬於商用的CPC。另一方面，為提高CSC不透光性以作為牙科根管治療使用，添加20 wt% 氧化鉍。使用兔子股骨模型比較此不透光性CSC與商用白色三氧礦聚合物 (white-colored mineral trioxide aggregate, WMTA)，結果指出經1個月植入後WMTA呈現灰黑色，而CSC仍是白色(即使是6個月後)，Masson’s Trichrome與Von Kossa染色皆顯示二者材料皆有新生骨生成。不透光性CSC有潛力做為WMTA替代品。

中 文 關 鍵 詞 ： 矽酸鈣骨泥、磷酸鈣骨泥、三氧礦聚合物、骨形成性、抗菌性、體內試驗

英 文 摘 要 ： Calcium-based bone cements are widely used in dental and orthopaedic surgery. Those based on calcium phosphate (CPCs) and calcium silicate (CSCs) have a number of favourable properties that encourage their clinical use in bone defect repair. The purpose of the 3-year study in this project was to compare the in vitro osteogenesis and bacteriostatic activity of BoneSource CPCs with home-made CSCs, particularly in regard to their facility for cell differentiation. Cement in vitro osteogenic activity was evaluated by incubating the cement specimens with human mesenchymal stem cells (hMSCs). Bacteriostatic activity of the two cements against Gram-positive (S. aureus) and Gram-negative (P. aeruginosa) bacteria strains was assessed using a bacteriostasis ratio assay and by inhibition zone examination. Compared with CPC, CSC was shown to promote greater proliferation and osteogenic differentiation (alkaline phosphatase and osteocalcin), and the formation of mineralization nodules of hMSCs. It is worth noting that CSC could effectively induce hMSCs differentiation, even when the culture medium did not contain osteogentic differentiation agents. In addition, CSC also showed significantly greater bacteriostatic activity, as revealed by inhibition zones and the bacteriostasis ratio. The findings suggest that CSC is a useful bioactive material for bone repair in terms of inducing cell differentiation, and may be considered an alternative to CPCs. On the other hand, to enhance the radiopacity of CSC, 20 wt% bismuth

oxide (Bi2O3) were added for the endodontic use. Thus, the regenerative potential of the radiopaque CSC and white-colored mineral trioxide aggregate (WMTA) using a rabbit femur model was compared. The results indicated that after one month of implantation, WMTA was associated with a greyish color alteration within its mass, while CSC presented color stability even at six months. Histological assay with Masson's Trichrome and Von Kossa stains showed the presence of newly formed bone surrounding the implanted sites in the rabbit femur. The conclusion drawn is that the encouraging results support the potential applications of radiopaque CSC as an improved alternative to WMTA for endodontic uses.

英文關鍵詞：Calcium silicate cement, calcium phosphate cement, mineral trioxide aggregate, osteogenesis, antibacterial activity, in vivo

1. 中文摘要

鈣基骨水泥廣泛使用在牙科及骨科手術上。當中磷酸鈣(CPC)及矽酸鈣(CSC)具備許多優異性質，臨床使用在骨修補。本計畫第三年的目的在於比較商品BoneSource CPC與自行開發的CSC之體外骨形成性與抗菌效果，尤其是細胞分化能力。骨形成性評估使用人類骨髓幹細胞(hMSCs)，抗菌活性則是利用格蘭氏陽性菌(金黃色葡萄球菌，S. aureus)及陰性菌(綠膿桿菌，P. aeruginosa)，分析抑菌比與抑菌圈。相較於CPC，CSC更能促進hMSC細胞增生與分化(鹼性磷酸酶與骨鈣素)，以及礦化節點形成。值得注意的是CSC有效誘導hMSCs分化，即使培養液沒有含骨分化劑。此外，CSC亦顯著具有較高抑菌活性。這些發現可建議本計畫自行開發的CSC為有用的骨修補生醫材料，可比擬於商用的CPC。另一方面，為提高CSC不透光性以作為牙科根管治療使用，添加20 wt% 氧化鉍。使用兔子股骨模型比較此不透光性CSC與商用白色三氧礦聚合物 (white-colored mineral trioxide aggregate, WMTA)，結果指出經1個月植入後WMTA呈現灰黑色，而CSC仍是白色(即使是6個月後)，Masson's Trichrome與Von Kossa染色皆顯示二者材料皆有新生骨生成。不透光性CSC有潛力做為WMTA替代品。

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2. 英文摘要

Calcium-based bone cements are widely used in dental and orthopaedic surgery. Those based on calcium phosphate (CPCs) and calcium silicate (CSCs) have a number of favourable properties that encourage their clinical use in bone defect repair. The purpose of the 3-year study in this project was to compare the in vitro osteogenesis and bacteriostatic activity of BoneSource CPCs with home-made CSCs, particularly in regard to their facility for cell differentiation. Cement in vitro osteogenic activity was evaluated by incubating the cement specimens with human mesenchymal stem cells (hMSCs). Bacteriostatic activity of the two cements against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria strains was assessed using a bacteriostasis ratio assay and by inhibition zone examination. Compared with CPC, CSC was shown to promote greater proliferation and osteogenic differentiation (alkaline phosphatase and osteocalcin), and the formation of mineralization nodules of hMSCs. It is worth noting that CSC could effectively induce hMSCs differentiation, even when the culture medium did not contain osteogentic differentiation agents. In addition, CSC also showed significantly greater bacteriostatic activity, as revealed by inhibition zones and the bacteriostasis ratio. The

findings suggest that CSC is a useful bioactive material for bone repair in terms of inducing cell differentiation, and may be considered an alternative to CPCs. On the other hand, to enhance the radiopacity of CSC, 20 wt% bismuth oxide (Bi_2O_3) were added for the endodontic use. Thus, the regenerative potential of the radiopaque CSC and white-colored mineral trioxide aggregate (WMTA) using a rabbit femur model was compared. The results indicated that after one month of implantation, WMTA was associated with a greyish color alteration within its mass, while CSC presented color stability even at six months. Histological assay with Masson's Trichrome and Von Kossa stains showed the presence of newly formed bone surrounding the implanted sites in the rabbit femur. The conclusion drawn is that the encouraging results support the potential applications of radiopaque CSC as an improved alternative to WMTA for endodontic uses.
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3. Introduction and purpose

A variety of cementitious calcium-based bone substitute grafts are increasingly used in minimally invasive clinical applications [1–6]. The cement can be injected into bone cavities or defects and on hardening leads to the in situ formation of solid materials. CPCs have a number of properties that favor their clinical use in the repair of bone defects in orthopedic and dental surgery [7]. Despite the significant advances made in this field, a great deal of work still needs to be conducted. Another calcium-based material, calcium silicate, has also attracted significant interest, due to its excellent biocompatibility, sealing ability and regenerative capability, together with its antibacterial properties [8–14]. It has been shown that calcium silicate cements may induce the differentiation of hMSCs [13–15]. Ionic dissolution products of calcium silicate-based cements could lead to mineral deposition at the material-dentine interface [16,17] and in the interior of the dentinal tubules [16]. Exposure of calcium silicate-based material surfaces to a physiological solution leads to the precipitation of a bone-like apatite layer, which may assist the material to integrate into living tissue [18–20]. Gandolfi et al. have pointed out that calcium silicate cements exhibit high calcium release and have a basifying effect, leading to the pronounced formation of more mature amorphous apatitic precursors [20]. Additionally, a synergistic effect between Si as an effective apatite nucleator and Ca as an apatite precipitation accelerator may assist apatite precipitation [18].

The hMSCs have a number of potential advantages over terminally differentiated cells for use in bone tissue engineering applications. Under appropriate culture conditions hMSCs from adult bone marrow can be induced to differentiate along the adipogenic,

osteogenic and chondrogenic lineages [21]. Although the osteoconductive properties of CSC and CPC have been widely studied, the comparison between them remains incomplete. It is necessary to understand the ability of the cements to induce hMSCs towards an osteogenic differentiation when cultured in the growth medium with and without osteogeneic differentiation agent. On the other hand, the use of bone graft substitutes in the body is associated with the risk of bacterial colonization of the materials [7]. Bacterial infections are a serious complication that very often leads to removal of the material. Choosing a cement with a high bacteriostasis activity helps to decrease or limit the growth of any remaining bacteria, even after treatment of infected bone defects. Gram-positive and Gram-negative bacilli are the pathogens most commonly involved. *Staphylococcus aureus* (*S. aureus*) was chosen for our preliminary investigation in view of its significance in the pathogenesis of infections associated to orthopedic implants [22].

Calcium silicate-based mineral trioxide aggregate (MTA) has been successfully used in endodontic treatment because of excellent biocompatibility, sealing ability and regenerative capabilities as well as antibacterial properties [11,12]. However, one of the main disadvantages of using MTA is its extended setting time and difficult handling [9,23]. Thus, efforts to develop novel endodontic materials as an alternative to MTA have been made, on the basis of overcoming its weakness [23,24]. In a previous study [24], we found that the aluminium-free hydraulic and radiopaque calcium silicate (β-Ca_2SiO_4) cement could set within 24 min when mixed with water, which the setting time was significantly lower than that of ProRoot white-colored mineral trioxide aggregate (WMTA) (168 min). The setting time is an important factor for satisfying clinical requirements, and a long setting time could cause problems clinically because of the cement's inability to maintain its shape and support stresses within this time interval [7]. Faster setting times would allow thorough irrigation after root-end filling, minimizing debris left in the surgical site without risking material washout [25]. In addition, the dental pulp cell responses to CSC are similar to those reported for WMTA in terms of cell cycle, proliferation, immunocompatibility and osteogenic differentiation, but the CSC material has a better cell behavior [26]. More recently, it has been found that CSC has high in vitro apatite-forming ability and low degradation [27]; thus, it may be an alternative to WMTA. Exposure of bioactive material surfaces such as MTA and calcium silicate to a physiological solution elicits the precipitation of a "bone-like" apatite layer, which may support the material's ability to integrate into living tissue [28].

In vivo models are required when in vitro systems cannot provide a reproducible approximation of the real-life in vivo or clinical setting [28]. The in vivo studies showed that MTA induces mineralized tissue formation such as dentin and cementum-like tissue [29–31]. Cintra et al. [30] found an MTA implant specimen showing irregular basophilic

areas and hard tissues in close contact to the material after one-month implantation in alveolar bone of rats. MTA does not produce any adverse effect on the microcirculation of the connective tissue [32], but it could speed up the bone healing process [33].

First of all, the purpose of the present study was to compare the in vitro osteogenesis and bacteriostatic activity of BoneSource CPCs (Striker, Kalamazoo, MI, USA) consisting of di- and tetracalcium phosphate [34–36] and experimental CSCs. BoneSource Classic is an injectable CPC intended for use in the repair of neurosurgical burr holes, contiguous craniotomy cuts and other cranial defects, as well as in the augmentation and restoration of bony contour in the craniofacial skeleton [34]. The study also evaluated the potential of the two cements as a substrate for the differentiation of hMSCs with and without osteogenic medium. On other hand, although abundant evidence in the literature has indicated that WMTA is effective for clinical applications, this field is continuously expanding. There have been few studies on in vivo osteogenesis of WMTA, given that the biomaterials should support bone ingrowth from the margins of the defects. Thus, in vivo evaluation of radiopaque CSC was reported, in comparison with WMTA.

4. Materials and methods

4.1 Specimen preparation

Reagent-grade tetraethyl orthosilicate ($Si(OC_2H_5)_4$) (Sigm-Aldrich, St. Louis, MO) and calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) (Showa, Tokyo, Japan) were used as precursors for SiO_2 and CaO, respectively. Nitric acid was used as the catalyst and ethanol was used as the solvent. The normal sol-gel procedure, including hydrolysis and aging, was employed. $Si(OC_2H_5)_4$ was hydrolyzed by the sequential addition of 2 M HNO_3 and absolute ethanol (144 mL), with 1 h stirring after each addition. $Ca(NO_3)_2{\cdot}4H_2O$ was added to the $Si(OC_2H_5)_4$ solution in an equimolar ratio, and the mixture was stirred for an additional 1 h. The mixture was sealed and aged at 60°C for one day. After vaporization of the solvent at 120°C in an oven, the dried gel was heated in air at a rate of 10°C/min in a high-temperature furnace at 800°C for 2 h and then allowed to cool to room temperature in the furnace to produce a powder. The sintered powders were then ball-milled using an agate jar with agate balls (5 mm dia.) in an ethanol medium for 12 h using a Retsch S 100 centrifugal ball mill (Hann, Germany), and dried at 60°C in an oven. For preparation of the cement the powders were hand-mixed with distilled water at a liquid to powder (L/P) ratio of 0.4 mL/g. BoneSource® classic CPC (lot no. BS08193A) was purchased from Stryker (Kalamazoo, MI), and used to investigate the differences between CSC and CPC, in particular the effectiveness of CSC as an alternative to CPC. The liquid phase was 0.25 M sodium phosphate at an L/P ratio of 0.25

mL/g according to the manufacturer's instructions. The specimens were stored in an incubator at 37°C and 100% relative humidity and allowed 1 day to set, except setting time evaluation and bacteriostatic assay. For the preparation of radiopaque cement, Bi_2O_3 (Sigma-Aldrich) was added to the ground calcium silicate powder at 20 wt % using a conditioning mixer (ARE-250, Thinky, Tokyo, Japan). ProRoot WMTA (Dentsply Tulsa Dental, Tulsa, OK) was also regarded as an experimental group. The liquid phase was water, and an L/P ratio of 0.3 mL/g was used. Prior to in vivo implantation, the hardened cement discs were sterilized by soaking in a 75% ethanol solution and exposure to ultraviolet (UV) light for 2 h.

4.2 Setting time and diametral tensile strength

After mixing, the two cements were placed in a cylindrical Teflon mold to form a cylindrical specimen of 6 mm diameter and 3 mm height for the assessment of the setting time. Six specimens were examined, and each determination was carried out using a 400-G Gillmore needle of a 1 mm diameter. Diametral tensile testing was performed using an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 0.5 mm/min. The diametral tensile strength (DTS) value of each cement specimen was calculated using the relationship of $DTS = 2P/\pi bw$, where P is the peak load (N), b is the diameter (mm) and w the thickness (mm) of the specimen. The maximal compression load at failure was obtained from the recorded load-deflection curves. Eight repeat specimens were tested for each time point.

4.3 Cell culture

The osteogenic properties of all the cement specimens were evaluated by incubation with hMSCs obtained from Cell Engineering Technologies (Coralville, IA) at passage 4-6. The cement specimens were sprayed onto each well bottom of the 24-well plate to a thickness of 2 mm, and stored in an incubator at 100% relative humidity and 37°C for 1 day to set. After this the specimens were sterilized by soaking in 75% ethanol and exposure to a 15 W ultraviolet lamp (Sankyo Denki, Tokyo, Japan) for 2 h. There was no chemical interaction between ethanol and cement. hMSCs were seeded onto the set sterilized cement specimens at a density of 5×10^3 cells per well in the 24-well plate. hMSCs cultured on tissue culture plates were used as a control. The cells were grown in Dulbecco's modified Eagle's medium (Gibco, Langley, OK) with and without osteogenic differentiation agents, comprising 10^{-8} M dexamethasone, 0.01 M β-glycerophosphate, and 2.84×10^{-4} M ascorbic acid (Sigma), supplemented with supplemented with 10% fetal bovine serum (FBS; Gibco), 1% penicillin–streptomycin (Gibco) solution. The cultures were incubated at 37°C in a 5% CO_2 atmosphere for different time periods, and

the medium was changed every three days. The pH of the media was monitored by an IQ120 miniLab pH meter (IQ Scientific Instruments, San Diego, CA). Measurements were conducted in triplicate.

4.4 Cell attachment

To observe the attachment of cells on the specimen surface, the specimens were washed three times with phosphate-buffered saline (PBS) and fixed in 2% glutaraldehyde (Sigma) for 3 h after 3 and 6 h seeding. The specimens were then dehydrated using a graded ethanol series with 20 min at each concentration and dried with liquid CO_2 using a critical point dryer device (LADD 28000; LADD, Williston, VT). The dried specimens were mounted on stubs, coated with gold, and viewed using a scanning electron microscopy (SEM; JEOL JSM-6700, Tokyo, Japan) operating in the lower secondary-electron image mode at 3 kV accelerating voltage.

4.5 Cell proliferation

The reagent Alamar Blue (Invitrogen, Grand Island, NY) was used for real-time and repeated monitoring of cell proliferation. At the end of the culture period, the medium was discarded and the wells washed twice with PBS. Each well was filled with 100 μL of solution at a ratio 1:100 of Alamar Blue to fresh medium, and incubated 2 h at 37°C. The solution in each well was transferred to a fresh 96-well tissue culture plate, and the plates were read in a Sunrise Microplate reader (Tecan Austria Gesellschaft, Salzburg, Austria) at 570 nm, with a reference wavelength of 600 nm. The absorbance results were recorded for nine independent measurements.

4.6 Alkaline phosphatase assay

The alkaline phosphatase (ALP) activity of hMSCs towards the specimens was measured after 3, 7, and 14 days' incubation. ALP catalyzes the hydrolysis of the colourless organic phosphate ester substrate, p-nitrophenyl phosphate, to p-nitrophenol, a yellow product. ALP activity was measured using the TRACP & ALP assay kit (Takara, Shiga, Japan) according to the manufacturer's instructions. To perform the assay, following incubation the cells were washed with physiological saline (150 mM NaCl) and lysed in 50 μL of lysis buffer (1% NP40 in 150 mM NaCl). For measurement purposes 50 μL of the substrate solution (20 mM Tris-HCl, 1 mM $MgCl_2$, 12.5 mM p-nitrophenyl phosphate, pH 9.5) was added to each well and allowed to react at 37°C for 30 min. The reaction was terminated by the addition of 50 μL of 0.9 N NaOH solution and read at 405 nm using a Sunrise Microplate reader. The experiments were carried out in triplicate.

4.7 Osteocalcin formation

After 7, 14, and 21 days' incubation, the samples were washed three times with PBS-T (PBS containing 0.1% poly(oxyethylene) sorbitan monolaurate (Tween 20)), followed by blocking with 5% bovine serum albumin (BSA; Gibco) in PBS-T for 1h. Primary antibodies against actin (Novus Biologicals, Littleton, CO) and osteocalcin (OC; Santa Cruz Biotechnology, Santa Cruz, CA) were added to the 5% BSA-PBS and incubated for 2 h with 20 rpm rocking. Dilutions of primary antibodies were set at 1:500 with actin as control. After primary antibody incubation, the samples were washed three times with PBS-T for 5 min and incubated with shaking with HRP-conjugated secondary antibodies at a 1:1000 dilution in 5% BSA-PBS for 1h at room temperature. The samples were then washed three times with PBS-T and twice with PBS for 5 min. A 3,3′,5,5′-tetramethylbenzidene (TMB) substrate (Invitrogen, Carlsbad, CA) was added to the wells and developed for 30 min in the dark at room temperature, followed by the addition of an equal volume of 2 M H_2SO_4 to terminate and stabilize the oxidation reaction. The coloured products were transferred to new 96-well plates and analyzed using a Sunrise Microplate reader at 450 nm, with reference at 620 nm.

4.8 Alizarin Red S staining

The mineralized matrix synthesis was analyzed using Alizarin Red S staining, which identifies calcium deposition. After culturing for 14 and 21 days, respectively, the cells were washed with PBS and fixed with a 10% formalin neutral buffer solution (Wako, Osaka, Japan) for 10 min at 4°C. This was followed by staining for 10 min in 0.5% Alizarin Red S (Acros Organics, Geel, Belgium) in PBS at room temperature. The cells were completely washed with PBS and then observed under an optical microscope (BH2-UMA; Olympus, Tokyo) equipped with a digital camera (C-5050; Olympus) at 100× magnification.

4.9 Bacteriostatic activity

The bacteriostatic activity of the freshly mixed cement was evaluated using Gram-positive S. aureus (BCRC 14957; Hsinchu, Taiwan) and Gram-negative P. aeruginosa (BCRC 10944; Hsinchu, Taiwan) bacteria. After mixing 100 mg of the powder with liquid phase, the cements were placed in a cylindrical Teflon mold to form a cylindrical specimen of 6 mm dia. A bacterial cultured on a Petri dish without cement was used as a negative control, and a $Ca(OH)_2$ (Showa, Tokyo, Japan) group as a positive control. Calcium hydroxide is a well-known antibacterial of importance in many clinical conditions, such as in infected root canals, and is also one of the setting products of CSC, which is the reason $Ca(OH)_2$ was used as a positive control. The $Ca(OH)_2$ cement was

prepared in a L/P of 0.3 mL/g. CSC and CPC were referred to as the experimental groups. To quantify colony forming units (CFU), the cement specimens were immersed in 2 mL of the bacterial suspension (10^7 CFU/mL) for various lengths of time. Afterwards, the survival of bacteria in the solutions was assessed by 105-fold serial dilutions with Bacto tryptic soy broth (Becton Dickinson, Sparks, MD), 100 μL of the treated sample being cultured on 9 cm Petri dishes and sprayed with 15 mL tryptone soya agar (HiMedia, Mumbai, India). All experimental and control groups were then incubated for one day at 37°C and the bacterial growth was assessed by Alamar Blue assay as follows. At the end of the culture period, the medium was discarded and the wells were washed twice with PBS. Each well was filled with 100 μL of a 1:100 solution of Alamar Blue in fresh medium and incubated at 37°C for 2 h. The solution in each well was transferred to a new 96-well plate. Plates were read in a Sunrise Microplate reader at 570 nm, with a reference wavelength of 600 nm. The bacteriostasis ratio (%) was calculated as follows: ([absorbance of the negative control] – [absorbance of the treated sample])/(absorbance of the negative control) × 100%. The results were obtained in triplicate.

To evaluate the zone of inhibition using the agar diffusion test, Petri dishes were filled with 15 mL of tryptone soya agar (HiMedia). The bacterial suspension (100 mL, 10^7 CFU/mL) was homogeneously dispersed on each agar plate containing the cement specimens, and the plates incubated at 37°C for 6 h. The growth inhibition zone around each cement specimen (the distance between the edge of the sample and the macroscopically observable bacteria) was measured with a 0.01 mm digital micrometre (Mitutoyo, Tokyi, Japan). The results were expressed (mm) as the mean and standard deviation of three independent experiments.

4.10 Surgery

Fourteen male New Zealand white rabbits (4 months) with a weight of approximately 3 kg were used as experimental animals. The animal experiment was approved by the Animal Ethical Committee of the Chung Shan Medical University Experimental Animal Center and national guidelines for the care and use of laboratory animals were observed. Before surgery, the implant sites were shaved and sterilized with 70% ethanol 10% povidone iodine. Premedication of each animal was performed by intramuscular injecting Zoletil 50 (1 mL/kg) (Vibac Laboratories, Carros, France) and subcutaneous injecting atropine (0.5 mL/kg) (Septodont, St. Maur, France) for analgesia. For implantation of the cement samples, a 6 mm sized defect was created in the left and right distal medial femur using a low-speed trephine drill and the incision was washed with normal saline in the process of defect preparation to eliminate bone debris. Each rabbit received two samples of test cement that could be put in the surgical defect securely without fixation. The bone

defect without filling cement was used as a control. Following implantation of materials, the muscle and the skin were sutured using 4-0 polysorb (Syneture, Mansfield, MA, USA) in layers. Wounds were covered and protected with antibiotic ointment. The samples were harvested after 1, 3 and 6 months of implantation (three samples of each experimental group at each time point).

4.11 Color stability

After sacrifice, implant areas were examined to observe the color stability of the cement samples under a light microscope (BH2-UMA; Olympus, Tokyo, Japan) equipped with a digital camera (C-5050; Olympus, Tokyo, Japan) at 50× magnification.

4.12 Histological assay

The retrieved implants with their surrounding tissue were prepared for histological evaluation. The samples were fixed in 10% buffered formalin at 4℃ for 2 weeks and decalcified. Then the samples were embedded with paraffin. After which, 5 μm thick, longitudinal sections were prepared per specimen using a sawing microtome technique. The decalcified sections were prepared and stained with modified Masson's Tricrome stain kit (ScyTek Lab., West Logan, UT) and Von Kossa kit (ScyTek), according to the manufacturer's instructions. Trichrome stain in blue was used for identification of collagen. Von Kossa staining in red can be used to observe the difference between the osteoid tissue and the calcified bone. Sections were examined using an Olympus BH2-UMA light microscope equipped with a digital camera (C-5050) at 200× magnification.

4.13 Statistical Analysis

One-way analysis of variance (ANOVA) statistical analysis was used to evaluate the significance of differences between mean values. Scheffé multiple comparison testing was used to determine the significance of the deviations in the data for each specimen. In all cases, the results were considered statistically significant at a p value less than 0.05.

5. Results

5.1 Setting time and diametral tensile strength

The setting time of CSC (22 min) was significantly ($p < 0.05$) lower than that of CPC (42 min). CSC had a strength value of 2.6 ± 0.1 MPa, which was significantly ($p < 0.05$) lower than that of CPC (3.8 ± 0.2 MPa).

5.2 Cell attachment

Figure 1 shows the morphology of hMSCs attached to the two cements after 3 and 6 h

of culture in the growth medium, with and without osteogenic differentiation agents. After the third hour, stem cells attached to the CSC surfaces were spread out, but a relatively reduced degree of spreading on the CPC surfaces was found, irrespectively of osteogenic differentiation agents. When cultured for 6 h, the SEM images revealed that the cells cultured on CSC were flat, with an intact well-defined morphology and extending filopodia, presenting better cellular adhesion than CPC.

5.3. Cell proliferation

The absorbance of Alamar Blue was highest with CSC, indicating that significantly more cells ($p < 0.05$) was proliferated on the CSC surfaces than on the CPC surfaces, at all culture times apart from day 1 (Figure 2). When there was no differentiation reagent in the medium, on day 7, CSC increased approximately 39% ($p < 0.05$) in absorbance value compared with CPC (Fig. 2(A)). When cells were seeded on the two cement specimens in the presence of osteogenic differentiation medium, similar trend was found (Fig. 2(B)).

5.4 Cell differentiation

To observe the functional activity of cells, the intracellular ALP level was measured, as shown in Figure 3. It is clearly seen that substantial higher ALP activity was induced when cells were grown in differentiation medium. The ALP levels on CSC was significantly ($p < 0.05$) higher than those on the FDA-approved CPC at most of culture times. As an example, in the case of osteogenic differentiation medium after seven days of culture, a significant 46% increment ($p < 0.05$) in ALP was determined for CSC compared with CPC (Figure 3B). Similarly to these findings, the OC amount was lower with CPC than with CSC (Figure 4). On day 21 a significant ($p < 0.05$) increase of 20% and 18% was found for OC secretion by cells cultured on CSC, (B) in the presence and (A) the absence of osteogenic differentiation medium, respectively, compared with CPC.

5.5 Calcium deposit

Low-magnification views of hMSCs stained with Alizarin Red S after culture for 14 and 21 days are shown in Fig. 5. Noticeable differences were observed after cell culture on the CSC and CPC surfaces. When there was no osteogenic differentiation medium, nodule formation was not significantly in cells cultured on CPC on days 14 and 21 (Figur 5A). In contrast, clearly calcified tissue formation was observed in the cultures grown on CSC after 21 days. When culture medium was added with osteogenic differentiation agents, the mineralized matrix synthesis was more evident in the CSC group than the CPC group (Figure 5B).

5.6 pH variation

The variations of the pH in culture medium during the culture interval of hMSCs seeded on the specimens are shown in Figure 6. Regardless of osteogenic differentiation medium, it can be clearly seen that the pH values for CSC increased from initially 7.4 to 7.7 after 1 day of assessment, reaching 7.8 after 7 days. Conversely, the decreased pH value of CPC-immersed medium was within the ranging 6.8-7.1 during the culture time periods. There were significant ($p < 0.05$) differences in extracellular pH between CPC and CSC at all incubation times.

5.7 Bacteriostasis ratio

The bacteriostatic effects of the two cement specimens were investigated by a comparing the numbers of viable *S. aureus* (Figure 7A) and *P. aeruginosa* strains (Figure 7B) after contact with the cement specimens at different time points. The $Ca(OH)_2$ control exerted a greater bacteriostatic effect again the bacterial species at all culture time points than did the two cements. The values of bacteriostasis ratios in CPC were significant lower ($p < 0.05$) than those found in CSC at all culture time points. More importantly, it seems that CPC exerted no bacterial activity whatever against *S. aureus* and *P. aeruginosa*.

5.8 Inhibition zone

Figure 8 shows a tryptone soya agar plate containing three cement specimens after 6 h culture. Bacteria were inhibited from growing around the two calcium hydroxide and CSC cement specimens, but it was not possible in this test to be sure of their ability of killing bacteria colonies. The $Ca(OH)_2$ control had a higher inhibitory effect against the growth of *S. aureus* (Figure 8A) and *P. aeruginosa* (Figure 8B) strains in comparison with CSC and CPC. CSC resulted in inhibition zones of about 2-3 mm around the cement specimens, whereas the $Ca(OH)_2$ control showed larger growth inhibition zones of approximately 3-4 mm against the two strains. The zone of inhibition clearly indicated that CSCS had a stronger bacteriostatic behavior of CSCs than CPCs.

5.9 Inflammatory Response

Bone tissue samples were excised from the site of implantation and analyzed using light microscopy. The macroscopic evaluation results showed that both cement implants and the control group exhibited no obvious inflammatory response, rejection or necrosis in the adjacent host tissue and they incorporated well with the surrounding tissue.

5.10 Degradation

Radiographs of cements implanted in the defect of the distal medial femur indicated the dimension of the cements did not change with time. Even after six months of implantation the cement specimens underwent little change in diameter, independent of the type of cement specimens. More importantly, it seems that the materials were in close contact to the bone tissue without the formation of fibrous interface.

5.11 Color Stability

Figure 9 shows color changes in the implanted materials after 1, 3 and 6 months of implantation. It is worthwhile to note that WMTA showed the greyish (or dark) discoloration at month 1, while CSC implants kept the original color. The RDSC group did not exhibit discoloration even after 3-month and 6-month implantation.

5.12 Histological Observation

The surrounding tissue of the retrieved implants was analyzed after 1, 3 and 6 months of implantation. The control group, at month-1 of observation, showed collagen formation (Figure 10A). Similarly, the two cements samples stained with Masson's Trichrome at month-1 showed deposition of collagen surrounding the implanted sites which increased quickly over time. This process was characterized by a great quantity of connective tissue surrounding the bone defect area, which might elicit one of the initial phases of the bone healing process (Figure 10B). The implantation site was surrounded by connective tissue that was organized and dense with the presence of osteoblasts and osteoid matrix (Figure 10C). The staining in WMTA and CSC groups showed no significant differences at all implantation time-points.

Mineralization was localized around the implants and displayed by black mineral in tissue samples stained with Von Kossa. Black, red, and pink correspond to calcified areas, nuclei, and cytoplasm, respectively. After one month of implantation, the control was only lightly stained (Figure 11A). The images of sections stained showed that WMTA and RDSC began to deposit mineral and the degree of bone apposition was better than the control group and exhibited a superficial layer with basophilic areas. Newly grown bone tissue in direct contact with cement surfaces was clearly visible one month after surgery. Mineral deposition in large areas was also confirmed by Von Kossa assay harvested *in vivo* at three (Figure 11B) and six months (Figure 11C) after implantation. Six months after implantation, osteoid formation was appreciated in the two cements at the defect edges.

6. Discussion

CPCs as bone graft materials have given promising results in the reconstruction of the

frontal sinus and spine, augmentation of craniofacial skeletal defects and osteoporosis, and also in dental fillings. A wealth of literature has reported that CPCs are demonstrated to be effective in clinical applications, and this field is continuously expanding. As mentioned earlier, CSCs have created increasing interest for use as bone substitute materials due to their high bioactivity and good biocompatibility. To validate the clinical applications of CSCs it was necessary to investigate their *in vitro* osteogenesis and bacteriostatic activity compared with CPCs. The characteristics of these two cement systems depended on the powder composition, liquid phase and liquid/powder ratio. With regard to the difference in strength between the two cements, CSC (2.6 MPa) gave a lower diametral tensile strength than BoneSource CPC (3.8 MPa). On the other hand the setting time of CSC (22 min) was shorter than that of CPC (42 min), consistent with a previous report [37]. Poor mechanical properties were the main disadvantage of the two cements. The low strength of CPC and CSC limited their use in low- or non-load-bearing applications such as craniofacial repair or small bone defects. Setting time is an important clinical factor, and an extended setting time could cause problems clinically due to the inability of the cement to maintain its shape and to support stresses within this time interval [7]. Fernández *et al.* have suggested 10–15 min to be a suitable setting time [38], and Lewis has proposed a setting time of approximately 15 min for the injectable bone cements used in vertebroplasty and kyphoplasty [39]. The setting time may be shortened by setting reagents such as Na_2HPO_4, which can be used to accelerate the hardening of either CPC or CSC [18,40]. Gandolfi *et al.* have also reported that the inclusion of hydroxyapatite, tricalcium phosphate or calcium hydrogen phosphate can significantly reduce the setting time of CSC, indeed the final setting time may be more than halved [41].

Cell attachment, spreading and shape are important modulators of cellular function. The close proximity of stem cells to the CSC surface indicates a favorable interaction between the cells and these materials. Cell-materials interactions may influence the proliferation, differentiation and mineralization of the cells. Indeed, greater numbers of cells initially grew on the CSC surfaces than on CPC at all culture times, regardless of the presence of osteogenic differentiation medium. Supplementing the culture medium with osteogenic agents can produce stronger and more effective differentiation (ALP, OC, and mineralization) of hMSCs, but not increase their proliferation, in agreement with a previous article [42]. Cell differentiation studies (ALP and OC), in common with results of cell proliferation assay, showed a significant impact of material composition, with the emphasis on the superior biological function of CSC compared with CPC.

It is generally accepted that an increase in the specific activity of ALP in bone cells reflects a shift to a more differentiated state [43]. ALP enzyme activity is also associated

with bone formation, and this enzyme is produced at high levels during the bone formation phase [44]. OC is the most abundant noncollagenous bone matrix protein characteristic of osteoblast synthetic function. The synthesis of OC is recognized at the late stage in osteoblast differentiation, and its expression increases rapidly as mineralization increases [45]. An increase in ALP and OC in cells cultured on CSCs were observed after seven days' incubation in comparison with CPCs, when cultured in either growth or osteogenic medium.

Similar results were also obtained after Alizarin Red S staining for calcium deposits. When the culture media were supplemented with osteogenic differentiation agents (*e.g.* dexamethasone, β-glycerophosphate or ascorbic acid), the formation of bone-like nodules with a mineralized extracellular matrix was strongly encouraged, in agreement with a previous report.8 More importantly, calcium deposits were more evident with CSCs than with CPCs. Results in vitro indicated that the present CSC showed significantly higher osteogenesis than CPC. Similarly to these findings, Chang and co-workers have reported that calcium silicate ceramics ($CaSiO_3$) could promote the attachment, proliferation and differentiation of osteoblast-like cells more effective than β-tricalcium phosphate ceramic [46]. Gandolfi *et al.* have demonstrated that CSCs doped with α- tricalcium phosphate sustained the survival of human orofacial mesenchymal stem cells (OFMSCs), and maintained steady- state levels of vascular cell adhesion molecule-1, ALP, and bone sialoprotein, while up-regulating their respective gene transcripts [14]. The adipogenic and in vivo bone regenerative capacities of OFMSCs were also unaffected by calcium silicate. Ion-releasing CSCs supported a biomimetic micro-environment conducive to the survival and differentiation of OFMSCs. A combination of OFMSCs and CSCs can promote tissue regeneration in periapical bone defects.

In vitro, hMSCs can be directed along a restricted set of lineages by the addition of osteogenic differentiation agents such as dexamethasone, bone morphogenetic protein-2 (BMP-2), β-glycerophosphate, ascorbic acid, or transforming growth factor-β (TGF-β), all of which have been identified as involved in regulating osteogenesis, in commitment to the osteoblast lineage, but this is difficult to apply in a clinical setting [16]. An alternative approach would be to use a scaffold or matrix to provide cues for differentiation without the need for osteogenic differentiation medium [47]. Since osteogenic differentiation agents stimulate osteoblastic differentiation of hMSCs, it is difficult to ascribe the expression of 'osteoinduction' originating from the cements on the basis of the data [48].

Cell viability and functions associated with a biomaterial are closely related to the physical, chemical and biological characteristics of the materials used. Thus, in the present study hMSCs were also seeded onto cement surfaces in the absence of osteogenic

stimuli, to examine the ability of the two cements to induce differentiation of stem cells. Indeed, the osteogenic differentiation medium induced completely an early phase of differentiation (ALP) when cells were seeded on the two cement surfaces. The levels of ALP and OC were higher in the cells cultured in osteogenic medium compared with the control growth medium and also varied with the material (CSC > CPC), indicating that osteoblastic differentiation has occurred and that the effect was material-dependent.

It is interesting to note that an intensely mineralized extracellular matrix was seen in cells grown on CSC in comparison to cells on CPC in the control and differentiation-inducing media. This confirmed that CSC may be more effective than CPC in providing a potentially osteoinductive micro-environment without the addition of exogenous growth factor or pre-treatment with osteogenic medium. Some studies have found that bone nodules may be formed in the absence of osteogentic differentiation medium when cells are cultured in the presence of bioactive glass [49]. This is highly important with regard to the development of materials for bone repair. Many biomaterials use specific cell types to assay the cell functions, but frequently require the addition of osteogenic agents to improve the cell responses. An osteoinductive material can trigger primitive, undifferentiated and pluripotent cells into the bone-forming cell lineage. Abundant evidence in the literature indicates that the differentiation of hMSCs into osteoblasts is sensitive to the chemical and physical properties of a material upon which the cells are grown [48]. Thus, the osteoinductive potential of biomaterials can be controlled by tailoring material characteristics such as chemical composition and surface morphology, which in turn affect cell-material interactions [50]. At this point it is important to note the inductive effect of CSC on differentiation of hMSCs, possibly due to the release of Si ions into the culture medium, as has been reported in other studies [46,47]. The idea that ceramics could be formulated in such a way as to provide an osteoinductive microenvironment is a natural outgrowth of these studies [48].

It is important to understand the reactivity of CSC and CPC in the medium. The pH of the medium conditioned by CSC cells incubated with CSC was pH 7.6-7.8, considerably more alkaline than CPC or the control pH 7.4. Some alkalinisation of the extracellular medium with CSC cement rather than the normal pH (7.4) during cell culture was not unexpected. The pH changes in the CSC-immersed medium were indicative of a two-stage reaction. The formation of Si–OH groups on the silica-based cement surface in the physiological environment led to an increase in interfacial pH, to values above 7.4 due to exchanging its Ca^{2+} ions with H_3O^+ ions. At the same time, the dissolution of CaO (or $Ca(OH)_2$) component of CSC also produced a remarkable increase in the pH of culture medium.30 Subsequently, the precipitation of an apatite layer consumed PO_4^{3-} and OH^- in the medium and gave rise to a decrease in pH. The kinetics of the binding of Ca ions by

Si-O and P groups by both Si-O and Si-OH groups with the subsequent nucleation and precipitation of calcium phosphate on the surface of CSCs, when exposed to a phosphate-containing solution. The interplay between ionic exchange, dissolution and apatite precipitation may modulate the pH changes in the medium for calcium silicate materials. Such an increase in pH of the medium is likely to be a key event in the promotion of cell functions, in addition to the role of Si. CPC added to cultured cells caused a rapid and persistent acidification of the medium, which was evidenced by the rapid color change of Phenol red (culture media stain) from pink at physiological pH of 7.4 to orange at pH 7, and yellow at pH 6.6. A lower pH value than pH 7.4 in the CPC-immersed medium was not surprising, due to the fact that Ca and P release originated from the underlying cement or the release of unreacted acidic dicalcium phosphate. BoneSource CPC is a mixture of tetracalcium phosphate and dicalcium phosphate in a ratio 73%:27%. That this change in the tissue culture environment was not toxic to the cells was clear both from morphological observations and their proliferation.

Cellular mechanisms involved in bone formation and resorption may be responsive to the acid-base balance. During bone formation, the pH of the bone interstitial fluid may shift to alkaline pH and during resorption to acid pH. More importantly, the alkalinisation of the medium is relevant to the enhancement of osteoblastic function, since it is accompanied by a shift in the intracellular pH in the same direction as in the culture systems of osteoblastic clone cells and cell lines. Although the extent to which the effect of high pH effects applies to the clinical situation has yet to be determined, it is reasonable to postulate that the beneficial effect of CSC on in vitro cell growth compared with CPC might to some extent be due to alkalinisation.

Bacterial infection in clinical treatment remains a significant complication. Antibiotic-loaded bone cement is a well-accepted adjunct for the treatment of an established infection. To treat and eradicate infection, the increasing use of antibiotic-loaded bone cement has developed. The combination of bioactive carriers with a sustained antibiotic release might produce a beneficial effect, not only offering protection to bone implants against foreign-body infections, but also improving fracture healing and bone-defect filling. But it gives rise to another concern, in developing resistant strains of bacterial. The choice of the cement with high bacteriostatic activity helps to decrease or avoid growth of the remaining bacterial, even after treatment in the infected bone defects. Additionally, it can prevent the antibiotic in use from damaging the kidneys, which is a potential problem. It is therefore interesting to note bacteriostatic effectiveness of the two bioactive cements. Two different assays have been used to test the bacteriostatic activity of the cements: the agar diffusion test and the direct contact test. The agar diffusion test is the most commonly used technique in evaluating bacteriostatic activity. In the direct

contact test, bacteria are placed in direct contact with the test material, and subsequent bacterial growth is then monitored by changes in absorbance.

Bacterial inhibition potency was shown to occur in the order $Ca(OH)_2$ > CSC > CPC, and it was independent of the bacterial strains used in this study. Unsurprisingly, $Ca(OH)_2$ paste showed higher bacteriostatic activity against *S. aureus* and *P. aeruginosa* than the two calcium-based cements. Calcium hydroxide was shown to be appropriate for the elimination of bacterial depends on ionization to some degree, releasing OH ions and causing an increase in pH. This increase in the pH value may kill bacteria by damaging the cytoplasmic membrane and DNA and denaturing proteins. Gram negative bacteria such as *P. aeruginosa* attempt to maintain a constant intracellular pH through the active transport of ions, whereas Gram positive bacteria such as *S. aureus* preserve a constant pH gradient across the cellular membrane. Maintaining a steady intracellular pH becomes significantly more difficult as the environmental pH moves toward either extreme.

A number of calcium silicate-based materials have been shown to possess antimicrobial properties against a variety of bacteria strains. The current study has showed great differences in bacteriostatic activity between CSC and CPC, CSC having a higher bacteriostasis ratio than CPC. Similarly, significant differences in inhibition growth zone between the two calcium-based cements have been found. CSC possesses higher bacteriostatic activity than CPC, the mechanism of which is probably a function of pH, although additional factors may be involved. Surface chemical composition, charge, hydrophobicity, roughness, and other physical characteristics are the main factors influencing the bacterial adherence and other molecules on material surfaces. During the hydration process, calcium silicate powder reacts with water to produce calcium silicate hydrate and $Ca(OH)_2$ and the latter is released into an aqueous environment, imparting a local high pH environment, as evidenced by pH changes in the culture medium. In contrast, the hydration product of CPC was apatite. Due to the high pH value, it is reasonable to consider that CSC should possess bacteriostatic activity lower than pure $Ca(OH)_2$. On the contrary, there is little evidence of considerable bacteriostatic effect by BoneSource CPCs. Similarly, in the present study CPCs were seen to have an insignificant effect on the viability of the bacterial strains under investigation. CPCs can have antimicrobial capacity if $Ca(OH)_2$ is added to the cement matrix, due to their strongly basic pH during and after setting.

The *in vivo* assay was tested to further contribute to research in this area and evaluate the potential for use in clinical trials. The two cement groups showed normal bone development with no inflammation during the implantation time. It is important to highlight the absence of inflammatory cells or acute inflammation processes at the interfaces between tissue and implanted material, in agreement with previous studies [7].

McNamara *et al.* [7] reported that grey-colored MTA displays no inflammation at 8-week implantation into rat mandibles. In a dog's teeth model grey-colored MTA was associated with less periapical inflammation and tissue response, even when no root filling or coronal restoration was present [51].

An important requirement for any dental material used for permanent treatment is its resistance to degradation (or solubility) when exposed to a host environment for a prolonged time period. The two cement samples exhibited few variations in size even after six months of implantation. WMTA is used as root-end filling materials that are in direct contact with the periapical tissues and for this reason, the ideal material should be biocompatible, impervious to dissolution or breakdown by the tissue fluids, nonresorbable, adapting as closely as possible to the dentinal walls of the root-end preparation and possess good handling characteristics. Gandolfi *et al.* [24] also reported the increase of the weight of calcium silicate-based materials when they came in contact with simulated body fluids. As previously stated, high apatite forming activity and low degradation were the characteristics of CSC [27].

In addition to the noted shortcomings in setting time, the potential of discoloration of WMTA has been a concern. Indeed, severe greyish (or dark) discoloration of WMTA was found in the present *in vivo* results. In contrast, CSC presented color stability. The unexpected findings have also been reported in *in vitro* and *ex vivo* studies related to the color stability of WMTA [52,53]. Although the exact explanation for WMTA-induced discoloration is not yet understood, the difference in chemical composition between the two cements can be one factor. WMTA is typically comprised of a variety of oxide components such as SiO_2, CaO, and Al_2O_3 with trace FeO [54], while CSC consists of SiO_2 and CaO. Felman and Parashos suggested that the oxidation and incorporation of the remaining iron content within the WMTA powder into the calcium aluminoferrite phase of the set WMTA in the presence of blood might be the mechanism of discoloration [26].

Masson's trichrome was used to stain the harvested tissue to visualize the formation of collagen in blue. The collagen fibers may indicate matrix development for future mineralization and bone formation. Mineralization borders were also identified with Von Kossa staining. Histological evaluation revealed that the two cement implants were encapsulated by the surrounding bone tissue, and the new bone was in direct contact with the implant at 1-month after implantation. As an ideal implanted biomaterial candidate, the cement samples studied herein presented good biocompatibility and osteoconductive properties. The significant findings, from a clinical perspective, were the osteogenesis with which both cements can induce new bone formation.

The implantation of both CSC and WMTA samples into bone defects in rabbit femur resulted in enhanced mineral deposition *in vivo*. The results may be partially explained by the fact that a similarity in chemical composition and biocompatibility occurred.

According to the literature [55], calcium silicate-based cements could remineralize the partially demineralized dentin and induce calcium-phosphate deposit formation. Dreger *et al.* [16] reported that calcium silicate-based MTA or Portland cements released some of their components in the tissue capable of stimulating mineral deposition in the cement-dentin interface and in the interior of the dentinal tubules. When a biomaterial is implanted into a body, a calcium and phosphate-rich layer forms on its surface, which then bonds to the living bone through this biologically active carbonated apatite layer. On the other hand, that the presence of calcium carbonate (calcite) can be confirmed on the surface of calcium silicate cement when in contact with simulated body fluids [56]. Xu *et al.* [57] found that newly formed bone tissue grew into the porous calcium silicate-based materials in a rabbit calvarial defect model, along with the deposition of a bone-like apatite layer at the tissue/material interface. Nevertheless, it seems that the mineral deposition in the regenerated tissues was more extensive in the CSC group than in the WMTA group at three and six months after implantation.

The chemical composition of implant materials has been shown to affect cell behavior, including cell shape, attachment, proliferation, differentiation and mineralized matrix synthesis. Ion-releasing calcium silicate cements support a biomimetic microenvironment conducive to survival and differentiation of human orofacial bone mesenchymal stem cells. The previous investigation performed with cementoblast cells demonstrates the ability of WMTA to allow cell growth and to induce biomineralization [29]. The Ca/Si atomic ratio of WMTA is more than 3, which indicates higher than CSC of 1.5. It seems therefore reasonable to suspect that CSC has a higher Si content in the materials' mass than WMTA. Bioactive Si-containing substrates, which release soluble silicate species, have been shown to accelerate the formation of new bone tissue by promoting the genetic activity of bone-regulating cells [58]. An appropriate Si concentration is effective in supporting the proliferation of osteoblast-like cells and actively stimulating a biological response in MG63 cells through the production of bone-specific proteins. More importantly, the cement with a higher Si content is beneficial for collagen adsorption and secretion, in which in turn more cells remains on the cement surfaces with higher Si content than on those with lower Si content. Last but not least, the CSC provided a favorable environment for bone tissue ingrowth, resulting in a large amount of mineralized tissue formation.

6. Conclusions

The results of the current study have consistently indicated that CSC is more effective than CPC in supporting the proliferation of hMSCs and in actively stimulating a biological response in these cells through the production of bone-specific proteins. CSC without osteogenic differentiation medium showed higher cell proliferation, higher ALP

expression, OC production, and calcium deposition than CPC. CSC induced higher alkalinisation of the environment and showed a higher bacteriostatic effect and growth inhibition towards *S. aureus* and *P. aeruginosa* than did CPC. These encouraging results support the potential applications of CSC as an alternative to CPC for bone repair. CSC implants were implanted in the rabbit femur defects to evaluate the *in vivo* bone-regenerative capacity of CSC, and the results were compared to WMTA implants. Contrary to CSC having color stability, noted discoloration of WMTA was found. Histological assay with Masson's Trichrome and Von Kossa staining showed abundant osteoid and mineralized bone tissue between the cement-tissue interfaces compared with the control without implants. The encouraging results support the potential applications of CSC as an improved alternative to WMTA for endodontic uses.

7. Evaluation

This three-year project focuses on expanding the applications of calcium silicate for bone repair. The results have been accepted in SCI journal. We also do our best to push commercialization of the potential materials.

8. 參考文獻

[1] LeGeros RZ,. Chohayeb A, Shulman A, J Dent Res 1982, 61, 343.
[2] Driessens FCM, Boltong MG, Bermudez O, Ginebra MP, Fernández E, Planell JA, J Mater Sci Mater Med 1994, 5, 164–70.
[3] Bohner M, Merkle HP, Van Landuyt P, Trophardy G, Lemaitre J, J Mater Sci Mater Med, 2000, 11, 111–6.
[4] Takagi S, Chow LC, J Mater Sci Mater Med 2001, 12, 135–9.
[5] del Real RP, Wolke JGC, Vallet-RegíM, Jansen JA, Biomaterials 2002, 23, 3673–80.
[6] Barralet JE, Grover L, Gaunt T, Wright AJ, Gibson IR, Biomaterials 2002, 23, 3063–3072.
[7] Sugawara A, Asaoka K, Ding SJ, J Mater Chem B 2013, 1, 1081–9.
[8] Gough JE, Notingher I, Hench LL, J Biomed Mater Res A, 2004, 68, 640–50.
[9] Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR, Int Endod J 2005, 38, 834–42.
[10] Huffman BP, Mai S, Pinna L, Weller RN, Primus CM, Gutmann JL, Pashley DH, Tay FR, Int Endod J 2009, 42, 34–46.
[11] Parirokh M, Torabinejad M, J Endod 2010, 36, 16–27.
[12] Torabinejad M, Parirokh M, J Endod 2010, 36, 190–202.
[13] Gandolfi MG, Ciapetti G, Taddei P, Perut F, Tinti A, Cardoso M, VanMeerbek B, Prati C, Dent Mater 2010, 26, 974–92.
[14] Gandolfi MG, Shah SN, Feng R, Prati C, Akintoye SO, J Endod 2011, 37, 1102–8.
[15] Ding SJ, Shie MY, Hoshiba T, Kawazoe K, Chen G, Chang HC, Tissue Eng A 2010, 16, 2343−54.
[16] Dreger LAS, Felippe WT, Reyes-Carmona JF, Felippe GS, Bortoluzzi EA, Felippe MCS, J Endod 2012, 38, 324–9.

[17] Gandolfi MG, Taddei P, Siboni S, Modena E, de Stefano EE, Prati C, Dent Mater 2011, 27, 1055–69.
[18] Ding SJ, Shie MY, Wang CY, J Mater Chem 2009, 19, 1183−90.
[19] Gandolfi MG, Taddei P, Tinti A, Prati C, Int Endod J, 2010, 43, 917–29.
[20] Gandolfi MG, Taddei P, Modena E, Siboni F, Prati C, J Biomed Mater Res B, 2013, 101, 1107–23.
[21] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR, Science 1999, 284, 143−7.
[22] Montanaro L, Campoccia D, Arciola CR, Biomaterials 2007, 28, 5155–68.
[23] Chiang TY, Ding SJ, J Endod 2010, 36, 1683−7.
[24] Gandolfi MG, Taddei P, Siboni F, Modena E, Ciapetti G, Prati C, Dent Mater 2011, 27, e134–57.
[25] McNamara RP, Henry MA, Schindler WG, Hargreaves KM, J Endod 2010, 36, 1851–5.
[26] Chen CC, Shie MY, Ding SJ, Int Endod J 2011, 44, 836–42.
[27] Chiang TY, Ding SJ, Int Endod J 2013, 46, 234−41.
[28] Muschler GF, Raut VP, Patterson TE, Wenke JC, Hollinger JO, Tissue Eng B 2010, 16, 123−45.
[29] Hakki SS, Bozkurt SB, Hakki EE, Belli S, J Endod 2009, 35, 513–9.
[30] Cintra LTA, de Moraes IG, Estrada BPF, Gomes-Filho JE, Bramante CM, Garcia B, Bernardinelli N, J Endod 2006, 32, 556–9.
[31] Bernabé PFE, Holland R, Morandi R, de Souz AV, Nery MJ, Otoboni-Filho JA, Dezan Junior E, Gomes-Filho JE, Braz Dent J 2005, 16, 149–55.
[32] Masuda YM, Wang X, Hossain M, Unno A, Jayawardena JA, Sato K, Nakamura Y, Matsumoto K, J Oral Rehabil 2005, 32, 145–50.
[33] do Nascimento C, Issa JPM, Iyomasa MM, Regalo SCH, Siéssere S, Pitol DL, de Oliveira Wolga N, Pedrazzi V, Micron 2008, 39, 868–74.
[34] Friedman CD, Costantino PD, Takagi S, Chow LC, J Biomed Mater Res (Appl Biomater) 1998, 43, 428–32.
[35] Dias LA, Nakanishi M, Mangussi-Gomes J, Canuto M, Takano G, Oliveira CA, Clin Neurol Neurosurg 2014, 117, 116– 9.
[36] Sverzut AT, Rodrigues DC, Lauria A, Armando RS, de Oliveira PT, Moreira RWF, Clin Oral Impl Res DOI: 10.1111/clr.12346.
[37] Barone CM, Jiminez DF, Boschert MT, Beckert BW, J Craniofac Surg 2000, 11, 495–9.
[38] Fernández E, Gil FJ, Ginebra MP, Driessens FCM, Planell JA, Best SM, J Mater Sci Mater Med 1999, 10, 223–30.
[39] Lewis G, J Biomed Mater Res B 2006, 76, 456–68.
[40] Pina S, Olhero SM, Gheduzzi S, Miles AW, Ferreira JMF, Acta Biomater, 2009, 5, 1233–40.
[41] Gandolfi MG, Iacono F, Agee K, Siboni F, Tay F, Pashley DH, Prati C, Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009, 108, e39–e45.
[42] Meseguer-Olmo L, Aznar-Cervantes S, Mazón P, De Aza PN, J Mater Sci Mater Med 2012, 23, 3003–14.
[43] Malaval L, Liu F, Roche P, Aubin JE, J Cell Biochem 1999, 74, 616–27.
[44] Sabokbar A, Millett P, Myer B, Rushton N, Bone Miner 1994, 27, 57–67.

[45] Mizuno M, Kuboki Y, J Biochem 2001, 129, 133–8.
[46] Ni S, Chang J, Chou L, Zhai W, J Biomed Mater Res B 2007, 80, 174–83.
[47] Tsigkou O, Jones JR, Polak JM, Stevens MM, Biomaterials 2009, 30, 3542–50.
[48] Boyan BD, Schwartz Z, Nat Rev Rheumatol 2011, 7, 8–9.
[49] Gough JE, Jones JR, Hench LL, Biomaterials 2004, 25, 2039–46.
[50] Yuan H, Fernandes H, Habibovic P, de Boer J, Barradas AMC, de Ruiter A, Walsh WR, van Blitterswijk CA, de Bruijna JD, Proc Natl Acad Sci USA, 2010, 107, 13614–9.
[51] Bernabé PFE, Gomes-Filho JE, Rocha WC, Nery MJ, Otoboni-Filho JA, Dezan-Júnior E, Int Endod J 2007, 40, 758–65.
[52] Jacobovitz M, de Lima RK, Int Endod J 2008, 41, 905–12.
[53] Belobrov I, Parashos P, J Endod 2011, 37, 1017–20.
[54] Felman D, Parashos P, J. Endod 2013, 39, 484–7.
[55] Tay FR, Pashley DH, Biomaterials 2008, 29, 1127–37.
[56] Gandolfi MG, Taddei P, Tinti A, Dorigo De Stefano E, Rossi PL, Prati C, Clin Oral Invest 2010, 14, 659–68.
[57] Xu S, Lin K, Wang Z, Chang J, Wang L, Lu J, Ning C, Biomaterials 2008, 29, 2588–96.
[58] Shie MY, Chang HC, Ding SJ, Int Endod J 2012, 45, 337–45.

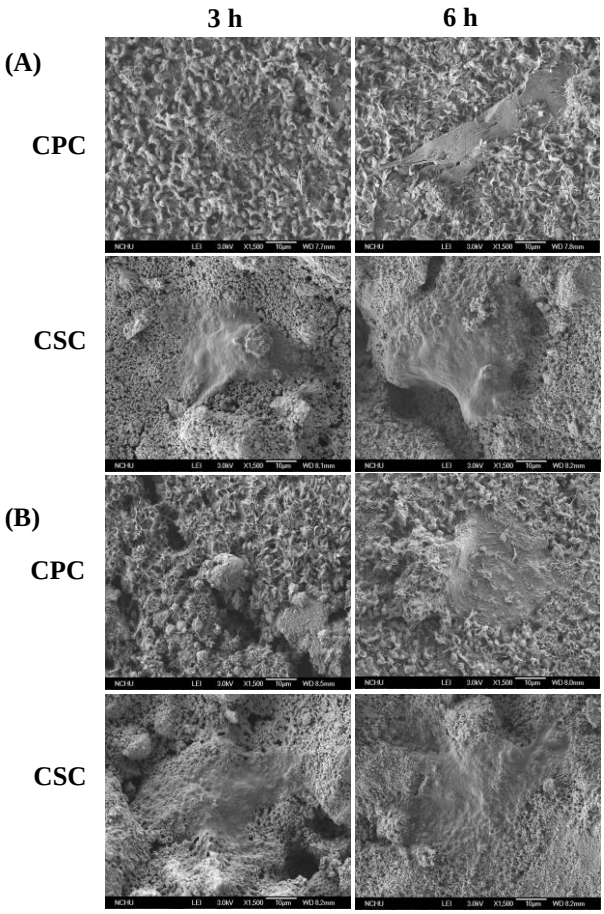

Figure 1 SEM images of hMSCs cultured on the CPC and CSC surfaces for 3 and 6 h in the culture medium (A) without and (B) with the osteogenic differentiation agents.

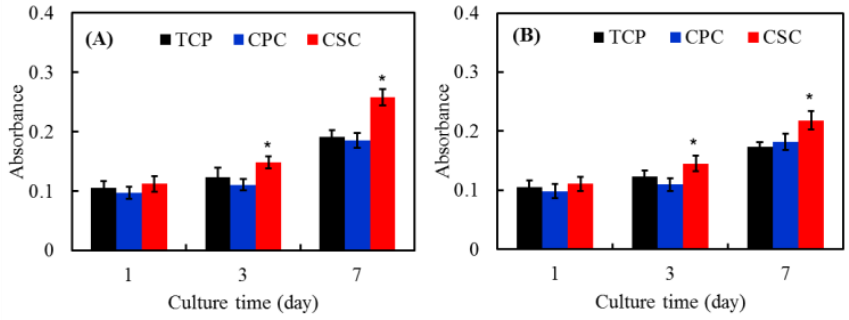

Figure 2 Alamar Blue assay for hMSC proliferation cultured on the specimens, (A) in the absence and (B) presence of the osteogenic differentiation medium, at various time points. * indicates a significant increase relative to CPC ($p < 0.05$).

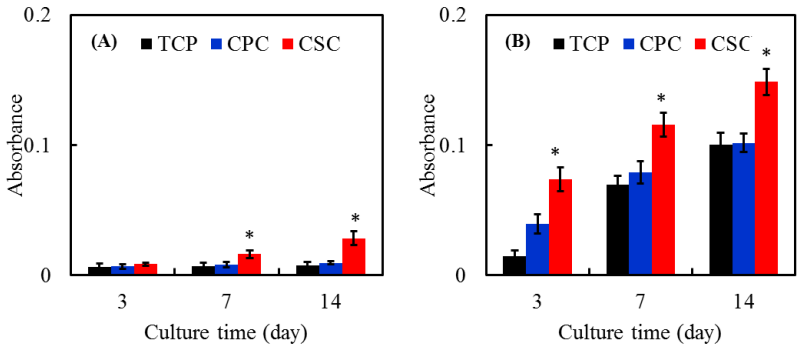

Figure 3 ALP assay on hMSCs cultured (A) in the absence and (B) the presence of the osteogenic differentiation medium. * denotes a significant increase relative to CPC ($p < 0.05$).

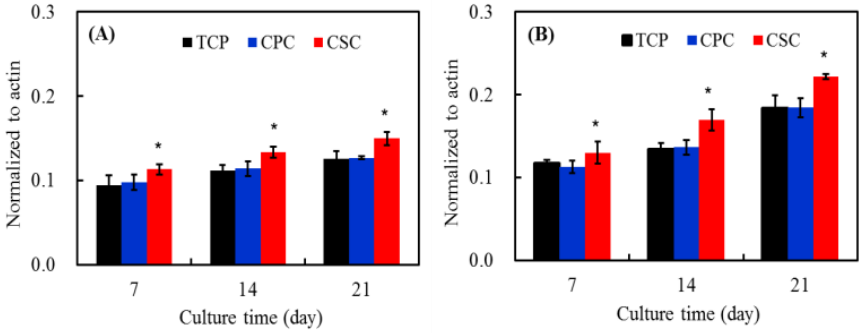

Figure 4 OC quantification for cell cultured in medium (A) without and (B) with the osteogenic differentiation medium. * indicates a significant increase relative to CPC ($p < 0.05$).

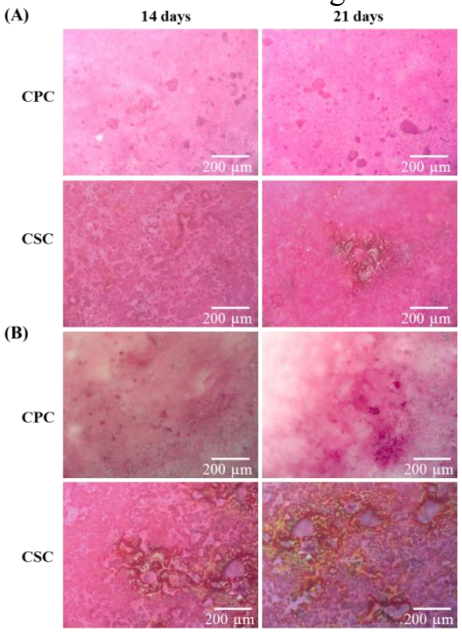

Figure 5 Photographs of hMSC cells in culture medium without (A) and with (B) osteogenic differentiation agents after staining with Alizarin red S for identification of the calcium deposit in cells on days 14 and 21.

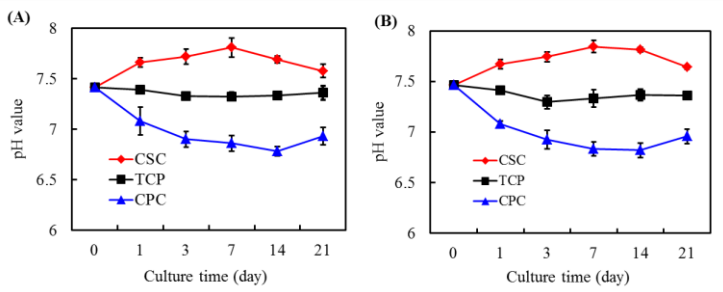

Figure 6 pH variations as a function of time in the culture medium, (A) without and (B) with the osteogenic differentiation agents.

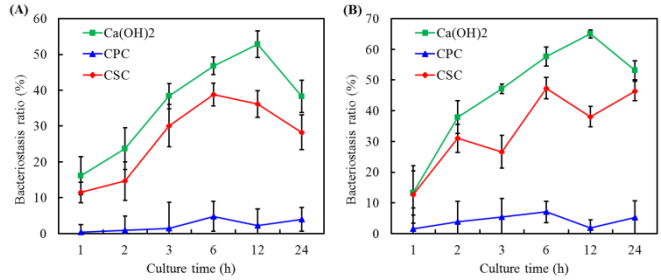

Figure 7 Bacteriostasis ratio (%) of fresh Ca(OH)2, CSC and CPC after culture in (A) *S. aureus* and (B) *P. aeruginosa*, in contact with the cements at various time points.

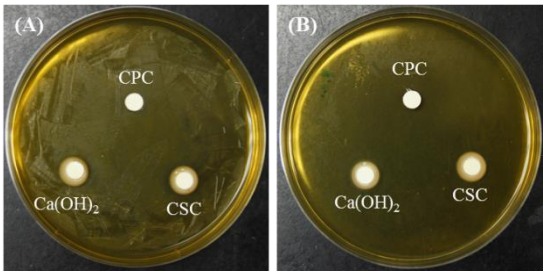

Figure 8 Growth inhibition zone for fresh $Ca(OH)_2$, CSC and CPC specimens after culture, in (A) *S. aureus* and (B) in *P. aeruginosa* for 6 h.

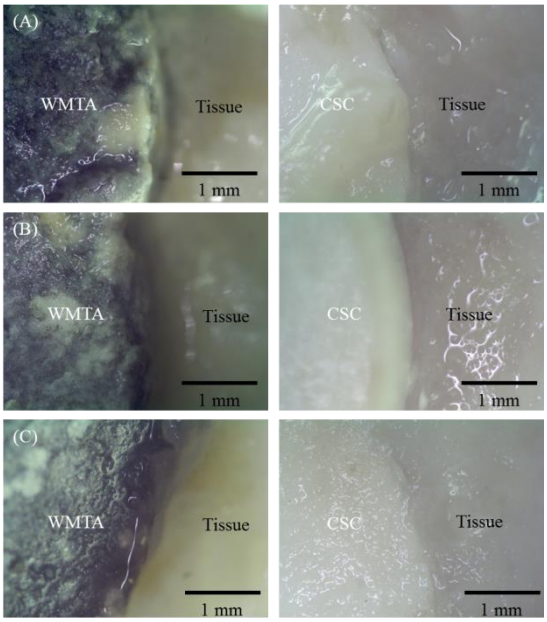

Figure 9 Photographs of WMTA and CSC implants after (**A**) 1; (**B**); 3 and (**C**) 6 months of implantation.

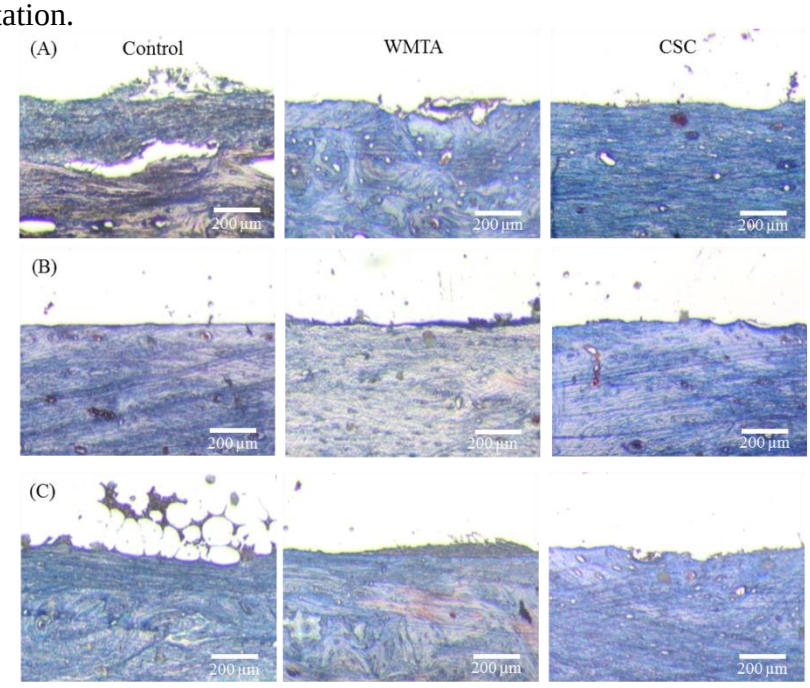

Figure 10 Modified Masson's Trichrome staining photographs of rabbit femur surrounding the control, WMTA, and RDSC for different implantation time-points. (A) The 1-month WMTA and RDSC implant samples showed the deposition of collagen surrounding the implanted sites; (B) More staining was seen at the bony tissue adjacent to implant; (C) After six months implantation sites were surrounded by a connective tissue that was organized and dense with the presence of osteoblasts and osteoid matrix for all groups.

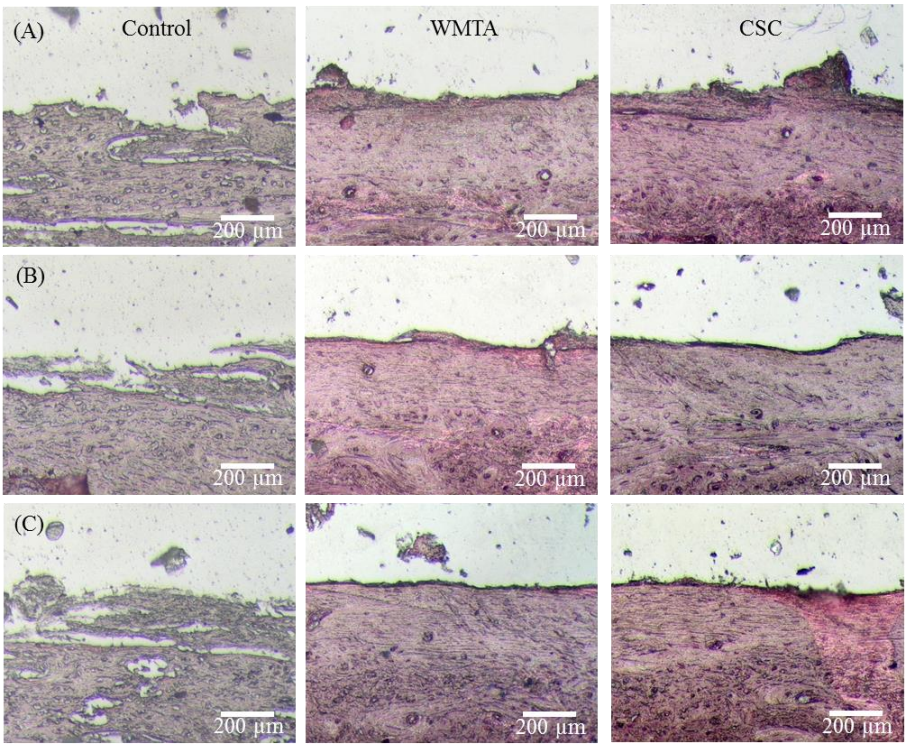

Figure 11 Von Kossa staining photographs of rabbit femur surrounding the control, WMTA, and RDSC for different implantation time-points. (A) At 1-month the control was only lightly stained. WMTA and RDSC began to deposit mineral and the degree of bone apposition; (B) Mineral deposition in large areas was also confirmed at 3-month implantation; (C) Six months after implantation, osteoid formation was appreciated in the two cements at the defect edges.

出席國際學術會議心得報告

計畫編號	NSC 102-2221-E-040-001-MY3
計畫名稱	骨形成性複合材的理化性質、細胞分子機制及動物試驗研究
出國人員姓名 服務機關及職稱	丁信智 中山醫學大學　口腔科學研究所　教授
會議時間地點	104/12/9-11　日本東京
會議名稱	2015 年第 15 屆亞洲生物陶瓷研討會(ABC2015)
發表論文題目	Porous calcium silicate-based scaffolds with high strength for bone tissue engineering

一、參加會議經過

第 15 屆亞洲生物陶瓷研討會由日本東京醫科齒科大學生醫材料暨工程研究所 K. Yamashita 教授主導，地點在該校國際會議廳舉行，此屬於小而美且領域專門的國際會議，參與的學者皆是 SCI 論文上常見的人物。除二場 Plenary lectures 之外，另有 8 次 Invited lectures。口頭及海報貼示亦是重點項目。整個會議研討涵蓋與生醫陶瓷材料相關的各種不同議題，如磷酸鈣陶瓷、氧化鋁/氧化鋯、生物活性玻璃、骨取代材、生物活性鈦金屬、組織工程、生物相容性研究等。會場討論氣氛十分熱絡。本次會議沒有廠商展示，實屬可惜。

二、與會心得

亞洲生物陶瓷研討會主要由日本陶瓷學會發起，每年在日本舉辦 1 次，隔年在亞洲其他國家。如同往年般主要是亞洲生醫材料學者參加，本人另帶本所 5 位研究生參加會議。生醫（陶瓷）材料研究為一跨領域且理論、應用並重的學門，從與會中所發表的論文可知仍有相當大的研究空間，但有待臨床醫師與生醫材料研究者雙向交流與合作，才能更加突破目前所面臨之瓶頸。從國外學者的研究趨勢及發表主題，顯示台灣生醫材料界研究方向與世界並進、並未偏離。台灣已將醫療器材產業規劃為重點科技，目前有更多的產業與學者投入生醫陶瓷研發，因此實有必要強化國際交流與曝光度，有利於醫療器材產業輸出。下一屆 2016 年 12/5-6 在澳洲，由昆士蘭科技大學 Prof. Yin Xiao 主辦。

Porous Calcium Silicate-Based Scaffolds with High Strength for Bone Tissue Engineering

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2 School of Dentistry, Chung Shan Medical University, Taichung City 402, Taiwan

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Creating functional engineered tissue for load bearing bone reconstruction using biocompatible and biodegradable scaffolds is of crucial importance. Although ceramics are still emerging as bone substitute materials because of high strength, an increasing trend is seen towards the construction of composites aiming at obtaining biodegradable and more versatile bone substitute materials taking the advantageous properties of ceramics and polymers. Calcium silicate-based materials have attracted a great deal of interest due to their osteogenesis [1]. Gelatin is widely used in the biomaterials because of biocompatibility and biodegradability. In the present study, porous calcium silicate-gelatin composites for bone tissue engineering applications were prepared. Physicochemical properties of the composites were evaluated after soaking in a simulated body fluid (SBF). Human mesenchymal stem cells were used to examine the osteogenesis and angiogenesis. Experimental results indicated that the porosity and diametral tensile strength of the samples was about 60% and 2.0 MPa, respectively, before soaking. When soaked in a SBF solution for 1 week, precipitation took place on all sample surfaces which were covered with clusters of precipitated spherulites. SBF-immersed samples with and without gelatin increased the weight loss with the increasing soaking time, while the diametral tensile strength of the samples gradually decreased. After 12 weeks, the samples with and without gelatin lost significantly about 90% in strength. The gelatin-containing specimens had a greater weight loss and porosity than the control without gelatin. For attachment and proliferation, a higher expression of human mesenchymal stem cell was found on the gelatin-containing composite than the control. The presence of gelatin in the composite was effective in stimulating the cell differentiation and actively promoted the angiopoietin level. The porous composites with high initial strength may be used for bone tissue engineering.

[1] Ding SJ, Shie MY, Hoshiba T, Kawazoe K, Chen G, Chang HC. Osteogenic differentiation and immune response of human bone marrow-derived mesenchymal stem cells on injectable calcium silicate-based bone grafts. Tissue Eng A 2010;16:2343−2354.
[2] Ding SJ, Wei CK, Lai MH. Bio-inspired calcium silicate-gelatin bone grafts for load-bearing applications. J Mater Chem 2011;21:12793−12802.

出席國際學術會議心得報告

計畫編號	NSC 102-2221-E-040-001-MY3
計畫名稱	骨形成性複合材的理化性質、細胞分子機制及動物試驗研究
出國人員姓名 服務機關及職稱	丁信智 中山醫學大學　口腔科學研究所　教授
會議時間地點	105/4/15-18　大陸深圳
會議名稱	第 26 屆可注射式骨關節生醫材料研討會(GRIBOI 2016)
發表論文題目	Injectable calcium silicate-based bone cements

一、參加會議經過

第 26 屆可注射式骨關節生醫材料研討會(GRIBOI 2016) 在大陸深圳威尼斯飯店的國際會議廳舉行，由香港大學 William Lu 教授主導，深圳科學院協助，台灣只有本人參加。會議基礎與臨床研究並重美，參與的學者皆是可注射式材料領域上的熟面孔，每天上下皆有 Invited lectures。即是海報貼示也需要 3 分鐘口頭報告及發問。整個會議研討涵蓋與注射式骨關節相關的各種不同研究，更特別地是有眾多此方面醫師參加。會場討論氣氛十分熱絡。本次會議有廠商展示。

二、與會心得

可注射式骨關節生醫材料研討會的創始人是 Acta Biomaterialia 的編輯 M. Bohner 教授，Bohner 教授研究為磷酸鈣骨泥系統，此會議以往皆在歐洲舉行。可注射式骨材研究為一跨領域且理論、應用並重的學門，從與會中所發表的論文可知仍有相當大的研究空間，但有待臨床醫師與生醫材料研究者雙向交流與合作，才能更加突破目前所面臨之瓶頸。從國外學者的研究趨勢及發表主題，顯示台灣生醫材料界研究方向與世界並進、並未偏離。台灣已將醫療器材產業規劃為重點科技，目前有更多的產業與學者投入生醫材料研發，因此實有必要強化國際交流與曝光度，有利於醫療器材產業輸出。下一屆 2017 年將在雅典舉辦。

Injectable calcium silicate-based bone cements

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Institute of Oral Science, Chung Shan Medical University, Taichung 402, Taiwan

With the increasing popularity of minimally invasive techniques, the development of injectable systems that can mould to the shape of the bone cavity and polymerize (or harden as solid implanted materials) when injected in situ has attracted a great deal of attention. Such devices should shorten the surgical operation time, minimize the damaging effects of large muscle retraction, reduce the size of scars, and lessen post-operative pain, allowing the patients to achieve rapid recovery. Thus, a great number of injectable bioceramic-based bone substitute grafts such as calcium phosphate, calcium silicate, calcium carbonate, and calcium sulphate, have been developed [1-3]. Among injectable cements, calcium silicate has attracted significant interest, due to its excellent osteogenesis, sealing ability and regenerative capability. Exposure of calcium silicate-based material surfaces to a physiological solutions leads to the precipitation of a bone-like apatite layer, which may assist the material to integrate into living tissue. Calcium silicate bone cements may not only induce the differentiation of human mesenchymal stem cells, bit also have considerable bacteriostatic activity, as revealed by inhibition zones and the bacteriostasis ratio against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacterial strains [3]. The translation of the cements from the bench to bedside has been quite successful, but there are several documented shortcomings of the cement systems, including anti-washout ability, radiopacity, osteoinduction, and angiogenesis.

In general, the unmodified ceramic cements have some difficulty maintaining the original grafted shape at defect sizes when implanted because they do not have enough washout resistance (or viscosity) to the body fluid within their hardening periods. To overcome the disadvantage of anti-washout, polymers, such as cellulose, alginate, gelatin, chitosan, and polylactic acid, can be added to ceramic cements. The use of vertebroplasty and endodontics require good visualization during injection and post-treatment, such as in situ monitoring of cement microleakage. Bismuth oxide, zirconium oxide, barium sulphate, and strontium carbonate can be introduced to improve radiopacity due to their high molecular weight. However, the addition of radiopacifiers may be detrimental to some of the physical, mechanical, and biological properties.

A bone graft that induces vascularization by supporting endothelial cell migration, adhesion, and proliferation is more conducive to bone defect healing. Therefore, a promising strategy to promote angiogenesis within the cement is the local and sustained delivery of angiogenic factors by the material itself. The use of stable and low-cost inorganic ions (e.g. Cu) or polymer (e.g. gelatin) to induce a regenerative response within the materials offers a complementary approach to the use of recombinant or concentrated protein or peptide growth factors. The kinetics of ion release from any scaffold must be tailored because high doses of metallic ions could be toxic. On the other hand, the osteoinductivity is also desired because the cement can recruit the primitive, undifferentiated and pluripotent cells from the surrounding tissues and stimulate their differentiation into osteoprogenitor cells, which develop into differentiated bone cells over time. The incorporation of an osteoinductive factor, such as bone morphogenetic proteins, could be one of the most effective ways to improve the efficacy of cement materials, but this is difficult to apply in a clinical setting. Therefore, a material that can provide a potentially osteoinductive micro-environment without the addition of exogenous growth factor would be challenges facing clinical applications.

In conclusion, although the significant advances made in the injectable calcium silicate-based bone cements for tooth and bone regeneration and repair, a great deal of work remains to be conducted. The synergistic combination of biofactors and ceramics has led to a promising class of bone grafts as the next-generation materials with unique properties for specific clinical applications.

REFERENCES: [1] M. Bohner (2010) *Eur Cell Mater* **20**:1−12. [2] A. Sugawara, K. Asaoka K, S.J. Ding SJ (2013) *J Mater Chem B* **1**:1081−1089. [3] S.C. Huang, B.C. Wu, S.J. Ding (2015) *J Mater Chem B* **3**:570−580.

科技部補助計畫衍生研發成果推廣資料表

日期:2016/08/12

科技部補助計畫	計畫名稱: 骨形成性複合材的理化性質、細胞分子機制及動物試驗研究
	計畫主持人: 丁信智
	計畫編號: 102-2221-E-040-001-MY3　　學門領域: 生醫材料

無研發成果推廣資料

102年度專題研究計畫成果彙整表

計畫主持人：丁信智	**計畫編號：**102-2221-E-040-001-MY3
計畫名稱：骨形成性複合材的理化性質、細胞分子機制及動物試驗研究	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			2		丁信智，魏忠楷，陳俊儒，“In vitro and in vivo behaviors of calcium silicate bone cement”，中華民國104年生物醫學工程科技研討會，台灣大學，台北，台灣，中華民國，11/13-14，2015，接受。 丁信智，何全城，“鈣基骨移植材轉譯研究”，2016年中山醫學大學口腔醫學院與廣西醫科大學聯合學術研討會，廣西醫科大學，南寧，大陸 ，3/18，2016，接受。
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			2	篇	Wu BC, Huang SC, Ding SJ*. Comparative osteogenesis of radiopaque dicalcium silicate cement and white-colored mineral trioxide aggregate in a rabbit femur model. Materials 2013;6(12):56755689. Huang SC, Wu BC, Ding SJ*. Stem cell differentiation-induced calcium silicate cement with

						bacteriostatic activity. Journal of Materials Chemistry B 2015;3(4):570580.
		研討會論文			4	Ding SJ, Wei CK, Chen CJ. Cell differentiation-induced bone cement with bacteriostatic activity. The 2st Global Conference on Biomedical Engineering (GCBME 2016), Taipei, Taiwan, Aug 17-19, 2016, accepted. Ding SJ. Injectable calcium silicate-based bone cements. 26th Interdisciplinary Research Conference on Injectable Osteoarticular Biomaterials and Bone Augmentation Procedures (GRIBOI 2016), Shenzhen, China, April 15-18, 2016, accepted. Ding SJ. Silica Biofactor in bone repair and regeneration. 14th Asian BioCeramics Symposium (ABC2014), Shanghai, China, Oct 28-30, 2014. Ding SJ, Huang SC. Comparative osteogenesis of calcium silicate cement and calcium phosphate cement. The 26th Annual Conference of the European Society for Biomaterials (ESB), Liverpool, UK, August 31- September 3, 2014, accepted.
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
參與	本國籍	大專生			0	人次	
		碩士生			0		

計畫人力					
		博士生	2		
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：■已發表　□未發表之文稿　□撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   本計畫乃將先前發展出的具優異生物相容性及骨形成性矽酸鈣材料，比較其與臨床商品材料之性質差異，作為商品化之依據，如生物性、抗菌活性與骨形成性。結果發現本計畫所開發的透光性矽酸鈣材料與商品磷酸鈣材料有較高誘導幹細胞分化功能，且具較高抑菌活性。此外不透光性矽酸鈣與商用白色三氧礦聚合物動物植入相較之下，矽酸鈣材料不會變色，三氧礦聚合物則呈現灰黑色，組織染色皆顯示二者材料皆有新生骨生成。與商品磷酸鈣材料或三氧礦聚合物相比，本計畫所開發出的矽酸鈣材料深具技術創新及臨床實用性，可應用於牙科、骨科與脊椎外科等骨缺損修補。藉此新材料開發可減少國內醫療器材與裝置的進口依賴，兼而提升國內醫療產品開發能力，將產品推向國際舞台。

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）
   本計畫乃開發新式矽酸鈣材料，應用於硬組織缺損修補。計畫內容是分析開發出的骨修補材料與現階段臨床所用的材料之性質差異，以凸顯本材料臨床應用的可行性，因此成果不影響公共利益。