Songklanakarin J. Sci. Technol.
35 (3), 293-301, May - Jun. 2013

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Comparison of polymethylmethacrylate (PMMA), native calcium sulfate, and high porous calcium sulfate beads as gentamicin carriers and osteoblast attachment

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Received 21 November 2012; Accepted 28 February 2013

Abstract

Calcium sulfate, a bioresorbable material, has been used as a bone substitute material and antibiotic vehicle. The increasing porosity of calcium sulfate beads might improve drug delivery capacity as well as enhance the antibiotic elution property. High porous calcium sulfate (HPCS) beads were fabricated using a salt leaching technique as a new bead type for antibiotic delivery system. Gentamicin-based antibiotic beads were conducted by impregnating gentamicin (3.8% w/w) with polymethylmethacrylate (PMMA), or coating of PMMA, native calcium sulfate (NCS), and HPCS with gentamicin solution. Physical properties, microstructure, and gentamicin elution from gentamicin-impregnated PMMA (GI-PMMA), gentamicin-coated PMMA (G-PMMA), gentamicin-coated NCS (G-NCS), and gentamicin-coated HPCS (G-HPCS) were compared. The osteoblast attachment revealed that PMMA, NCS, and HPCS beads were not toxic to h-OBs after co-incubation for seven days. Furthermore, more h-OBs attachment appeared in HPCS beads compared to PMMA and NCS beads after co-culture for 7 days. Eluted gentamicin from G-NCS and G-HPCS beads were greater than those from GI-PMMA and G-PMMA beads during the experimental period. All types of beads were able to elute gentamicin for 10 days except G-PMMA, which released gentamicin only for four days. The highest to lowest total concentrations of eluted gentamicin were from G-NCS, G-HPCS, G-PMMA, and GI-PMMA, respectively. These results suggested that the HPCS beads improved local antibiotic delivery and improved h-OBs attachment.

Keywords: porosity, salt leaching, elution, osteoblast

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1. Introduction

Antibiotic impregnated beads have commonly been used for standard treatment of local infected tissues especially in osteomyelitis (Roeder *et al.*, 2000; Koo *et al.*, 2001; Gondusky *et al*., 2009). Antibiotic-impregnated polymethylmethacrylate (PMMA) bone cement has been clinically used in various areas, including joint replacement surgery and osteomyelitis management (Kelsey *et al.*, 1995; Roeder *et al.*, 2000; Malizos *et al.*, 2010). However, many disadvantages of PMMA were revealed in osteomyelitis managements, including the requirement for surgical removal (Mader *et al.*, 2002; Nelson *et al.*, 2002), the enhancement of bacterial colonization (Mader *et al.*, 2002), the high cost of management, and the release of toxic substances during setting (Santschi *et al.*, 2003). Unlike PMMA, antibiotic delivery system using calcium sulfate may serve as a better antibiotic carrier material due to its biocompatible and biodegradable properties. The implanted calcium sulfate beads could be totally resorbed with minimal inflammation (Thomas *et al.*, 2009). In addition, the beneficial effects of calcium sulfate on osteoconductive activity led to use this material in both orthopedic and dental procedures (Damien *et al.*, 1991).

The porosity of bone substitute material was one of the most important features of an implanted material affecting new bone regeneration. Micropores allowed a migration of endothelial cells, promoted a differentiation of osteoblast and osteoprogenitor cells, encouraged vascularization, and contributed to osteoblast proliferation and differentiation (Kusmanto, 2008). The pore size of the material was also a significant factor for bone regeneration. The minimum pore size required for bone regeneration was approximately 100 mm (Hulbert *et al.*, 1970). The porosity of the material could be increased with various techniques, such as leaching of soluble particles (Hou *et al.*, 2003; Joël Reignier, 2006; McLaren *et al.*, 2007), mechanical and chemical techniques (Frame, 1975; Shiramizu *et al.*, 2008). The leaching of soluble particles technique was an effective method to control the amount and size of pore, which allowed the measurement of quantity and size of the particles (Hou *et al.*, 2003). Various antibiotics were used to co-operate with antibiotic beads, including aminoglycosides, β-lactam agents, and quinolones (Nandi, 2009). Gentamicin was frequently used in antibiotic-releasing beads since it has broad-spectrum antimicrobial activity and high solubility. It resisted to a high temperature during the PMMA and calcium sulfate dihydrate settings (Wahlig *et al.*, 1980). In our study, high porous calcium sulfate (HPCS) beads were developed by a salt leaching method and were studied *in vitro* for antibiotic releasing compared with native calcium sulfate (NCS) and PMMA beads. We hypothesized that HPCS beads could enhance a higher eluted gentamicin concentration than NCS and PMMA beads in an initial phase with sufficient eluted concentration in sustain phase and could be compatible with human osteoblast.

The purposes of this study were to compare the physical properties, elution characteristics of gentamicin released from gentamicin-impregnated PMMA beads (GI-PMMA), gentamicin-coated PMMA beads (G-PMMA), gentamicin-coated native calcium sulfate beads (G-NCS) and gentamicin-coated high porous calcium sulfate beads (G-HPCS), together with the human osteoblast attachment among PMMA, NCS, and HPCS beads.

2. Material and Methods

2.1 Bead preparation

The PMMA and calcium sulfate beads were categorized for elution test into four groups: gentamicin impregnated PMMA beads (GI-PMMA), gentamicin coated PMMA beads (G-PMMA), gentamicin coated native calcium sulfate beads (G-NCS), and gentamicin coated high porous calcium sulfate beads (G-HPCS).

Preparation of GI-PMMA beads: GENTAFIX3 (TEKNIMED S.A., France) consisted of 3.8% w/w gentamicin in polymethymethacrylate base. The bone cement powder was mixed with a liquid monomer according to company recommendation. The mixture was poured into the mold (diameter 5 mm, height 4 mm). For the complete polymerization, the PMMA was solidified at room temperature for 3 hours.

Preparation of G-PMMA beads: CEMFIX3 (TEKNIMED S.A., France) was a polymethymethacrylate bone cement without antibiotics. The PMMA was prepared according to company recommendation. After complete polymerization of PMMA, CEMFIX3 pellets were immersed in gentamicin solution, 40 mg ml^{-1}, (T.P.drug laboratory, Thailand) for three hours, and then were dried under an air blower.

Preparation of G-NCS beads: Calcium sulfate hemihydrate ($CaSO_4$ ½ H_2O) (Sigma, U.S.A.) was used to prepare calcium sulfate beads. Ten grams of calcium sulfate hemihydrates were mixed with 7 ml distilled water. The homogenous mixture was poured into the mold (diameter 5 mm, height 4 mm) and the beads were set overnight at room temperature. After the settlement of calcium sulfate beads were achieved, these beads were submerged in gentamicin solution, 40 mg ml^{-1}, (T.P.drug laboratory, Thailand) for three hours, and then were dried under the blower.

Preparation of G-HPCS beads: Ten grams of calcium sulfate hemihydrates (Sigma, U.S.A.) were mixed with 10 g sodium chloride (Sigma, USA) and 7 ml distilled water. The homogenous mixture was poured into the mold until the beads set as G-NCS. Sodium chloride was leached out of the calcium sulfate beads by using deionized water in an ultrasonic cleaner (JAC ultrasonic 4020P, KODO Technical Research Co. Ltd; Gyeonggi-Do, South Korea) for 30 minutes, with five times of repetition, then the calcium sulfate beads were dried overnight at 40°C. The calcium sulfate beads, subsequently, were immersed in gentamicin sulfate solution, 40 mg ml^{-1}, (T.P.drug laboratory, Thailand) for three hours and were dried overnight under the blower.

2.2 Physical properties of antibiotic beads

Physical properties of GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads were determined in aspect of weight (mg), total porosity (%), water uptake (%), and mass loss (%). The determination of total porosity of gentamicin beads was performed according to the Archimedes's principle of mass displacement (Jones, 1993; Bruckschen, 2005). Water uptake capacity of gentamicin beads was undertaken by weighing the gentamicin beads before and after the immersion in water (Baro *et al.*, 2002). In addition, mass loss level was weighed before and after the gentamicin beads were bathed in phosphate buffer saline (PBS) (Sigma, U.S.A.) for 10 days (Baro *et al.*, 2002).

2.3 Human's osteoblast attachment

Osteogenic compatibility of PMMA, NCS and HPCS beads were compared by using human's osteoblasts (h-OBs) which were subcultured from primary human osteoblasts. Bead materials were loaded with 20 mL of h-OBs 2.5×10^5 cells cm^{-3} in Dulbecco's Modified Eagle Medium (DMEM) complete medium (BioWhittaker, Lonza, U.S.A.) and placed in a 24-well plate. Then, that plate was incubated in CO_2 incubator at 37.0±1.0°C, 5.0% CO_2, and 95±5% humidity. The samples were analyzed in the first and the seventh days after the incubation for testing viability and morphology of cell-seed specimen.

The cell seed on the bead surface was observed by scanning electron microscopy (SEM). Samples were prepared according to standard method for SEM, including fixation, wash, dehydration by using critical point dryer (Bal-Tec CPD030, Bal-Tec Union Ltd., Liechtenstein), and gold coating with gold coater (JEOL JFC-1200, JEOL, Japan). The attachment of osteoblast on samples was observed by a scanning electron microscope (HITACHI S-3400N, HITACHI, Japan) on the first and the seventh day after incubation.

2.4 Microstructure investigation

The gentamicin beads in each group were sampled randomly and analyzed with scanning electron microscope after drug loading to characterize the microstructure of beads and antibiotic housing. The beads were coated with gold (IB-2, Eiko Engineering, Co. Ltd., Japan) and analyzed with scanning electron microscope (JSM-5600LV, JEOL, Japan) operated by 10 kV at ×35, ×100, ×200 and ×500 from a cross-section and a surface view.

2.5 Determination of gentamicin elution test

Five beads in each group were immersed in PBS (Sigma, U.S.A). Each bead was incubated in 1 ml PBS, pH 7.4, at 37°C for 24 hours in a tube. The dissolution PBS was collected and 1 ml fresh PBS was added every 24 hour for 10 days through the experimental period. All dissolution samples were kept at -20°C until analysis and were tested within one week. Eluted gentamicin concentrations were determined by microbiological assay using *Bacillus subtilis* (ATCC 6633) as an indicator organism (Ficker *et al.*, 1990).

2.6 Statistical analysis

All the analyses were carried out using NCSS 2007 (Kaysville, UT, USA). The concentration of gentamicin sulfate released from GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads was analyzed by repeated ANOVA followed by a Tukey's multiple comparison test. Physical properties of the beads in each group were compared with one-way ANOVA and followed by a Tukey's multiple comparison test. Data was expressed as the mean ± standard deviation. Values with $P<0.05$ were considered statistical significant.

3. Results

3.1 Antibiotic beads characteristics

Gross appearances of GI-PMMA, G-PMMA, G-NCS and G-HPCS beads are shown in Figure 1. The GI-PMMA and G-PMMA beads had no visible pores on the surface, while G-NCS beads had a small number of visible pores. Interestingly, G-HPCS beads had numerous visible pores on the bead surface. Physical properties of gentamicin beads, including weight (mg), porosity (%), water uptake (%), and mass loss (%) are shown in Table 1.

3.2 Human's osteoblast attachment

Since different concentrations of gentamicin were released from each bead and might has an effect on cellular growth and proliferation, GI-PMMA, G-PMMA, G-NCS, and G-HPCS were not used for an osteoblast attachment test. Three types of bead materials (no antibiotic) were used,

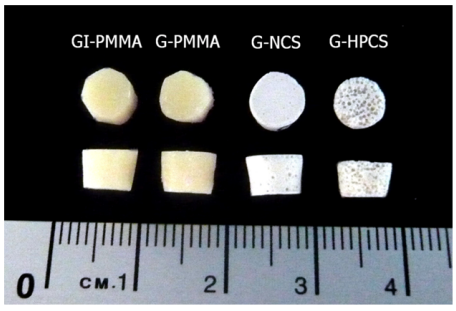

Figure 1. Beads are presented in each group in top and side views. Beads were arranged from left to right in the following order: GI-PMMA, G-PMMA, G-NCS and G-HPCS, respectively. GI-PMMA and G-PMMA beads had no visible pores on the surface, while the G-NCS bead has a few pores on the surface. Numerous visible pores were found on the surface of G-HPCS bead in both top and side views.

Table 1. Physical properties of antibiotic beads, including weight (mg), porosity (%), water uptake (%) and mass loss (%) of GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads.

Group	Weight (mg)	Porosity (%)	Water uptake (%)	Mass loss (%)
GI-PMMA	119.38±7.45[a]	2.10±2.82[a]	8.65±1.88[a]	1.50±0.25[a]
G-PMMA	117.93±8.36[a]	2.89±3.37[a]	7.56±3.16[a]	0.78±0.47[a]
G-NCS	163.08±5.73[b]	25.71±5.10[b]	27.17±2.42[b]	5.73±0.48[b]
G-HPCS	84.92±6.38[c]	42.32±4.57[c]	51.15±4.66[c]	9.19±1.52[c]

[a,b,c] Different superscripts within column indicate significant difference (p<0.05).

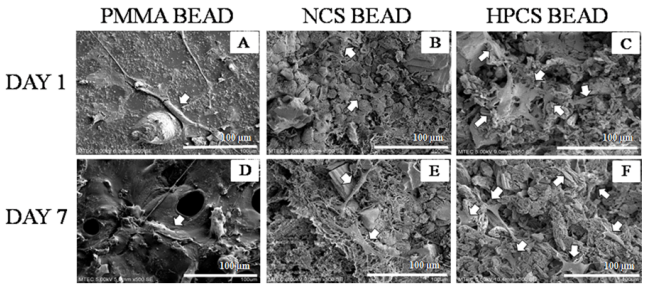

Figure 2. Human osteoblast (h-OBs) (white arrows) attached to the surface of PMMA bead (A, D), NCS bead (B, E), and HPCS (C, F) bead on day 1 and day 7 (magnification ×500).

including PMMA beads, NCS beads and HPCS beads. Scanning electron microscopic images of material surface showed the intact h-OBs cells with normal morphology and extending filopodia in which indicated the survival of h-OBs on PMMA, NCS, and HPCS bead surfaces (Figure 2). Interestingly, scanning electron microscopic pictures displayed that the h-OBs numbers were significantly increased in HPCS beads compared with that in NCS beads and in PMMA beads after cell culture for seven days.

3.3 Microstructure observation

According to observations of GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads, all had different microstructures. GI-PMMA and G-PMMA beads had no crystal structures and fewer pores, while G-NCS and G-HPCS beads had crystal structures and numerous pores (Figure 3). The surface of GI-PMMA beads was rougher than G-PMMA beads. The cross sectional view of GI-PMMA and G-PMMA beads showed the cracks on the rough surface, but gentamicin particle was not observed in both groups. Furthermore, fewer holes were noticed on both surface and the cross-section views in both GI-PMMA and G-PMMA beads.

The microstructures of G-NCS and G-HPCS beads were different in terms of porosity and crystal size on both surface and cross sectional views. The average diameters of calcium sulfate crystals of G-NCS and G-HPCS beads were 1.15±0.23 mm and 10.42±2.04 mm, respectively, whereas the average pore sizes of G-NCS and G-HPCS beads were 1.59±0.31 mm and 22.37±6.48 mm, respectively. Not only in G-HPCS beads, but also in G-NCS beads macropores were found. G-HPCS beads consisted of numerous macropores (average size 385.44±101.97 mm) in both cross sectional and surface views, while few macropores were found in G-NCS beads. In addition, both surface and cross sectional views, the crystal structure of calcium sulfate was coated with

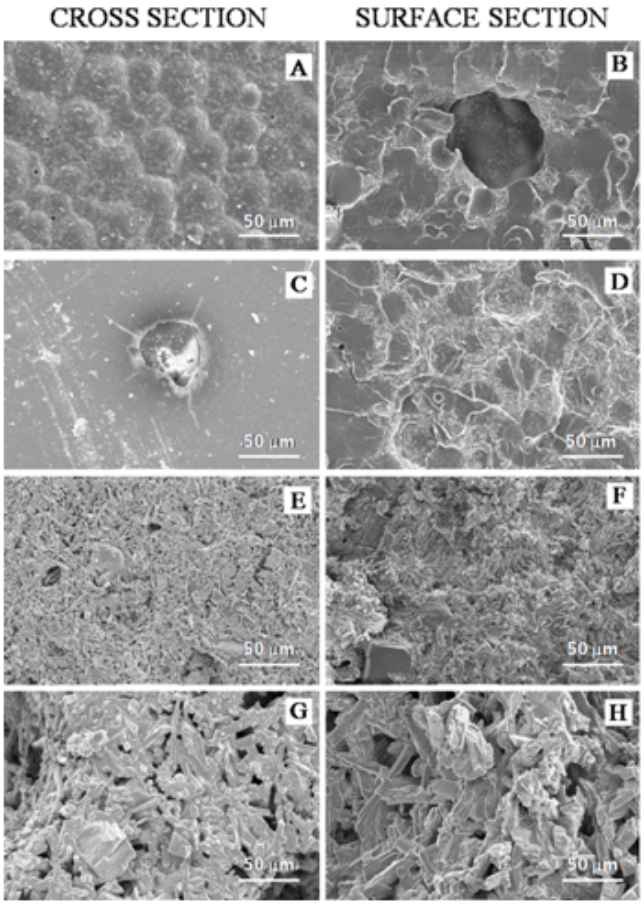

Figure 3. Scanning electron microscope micrograph showing the microstructure of a gentamicin bead including GI-PMMA (A, B), G-PMMA (C, D), G-NCS (E, F), and G-HPCS (G, H). Left column is a surface view. Right column is a cross-sectional view (magnification = ×500).

gentamicin sulfate, while it was not observed in the PMMA beads.

3.4 Antibiotic dissolution

Elution characteristics of GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads were significantly different from one another in terms of concentrations (Table 2). The total amount of gentamicin released from GI-PMMA and G-PMMA beads was 716.87±272.72 and 1381.64±486.38 μg ml^{-1}, respectively, while that in G-NCS and G-HPCS beads was 5003.45±517.27 and 4901.50±1072.69 μg ml^{-1}, respectively, during a 10-day experimental period. Gentamicin sulfate could be detected only for four days in G-PMMA beads, while GI-PMMA beads could provide gentamicin sulfate until the end of the experiment, similar to G-NCS and G-HPCS beads. Concentrations of gentamicin sulfate released from G-HPCS beads were higher than from GI-PMMA and G-PMMA beads in the first five days; after that during the next five days they decreased to the same level as of GI-PMMA beads. The highest concentrations of eluted gentamicin from all bead types were shown on the first day. As it can be seen the percentage of gentamicin released from of GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads is 13.05±7.21%, 95.44±1.07%, 52.36±6.41%, and 81.98±1.65%, respectively (Table 3). The total quantity of gentamicin sulfate in the eluted solution from GI-PMMA and G-PMMA beads was significantly lower than that released from G-NCS and G-HPCS beads (Table 2).

4. Discussion

Physical properties of commercial PMMA (GI-PMMA and G-PMMA), G-NCS and G-HPCS beads were distinct in weight, total porosity, water uptake capacity, and mass loss. PMMA beads (GI-PMMA=119.38±7.45 mg and G-PMMA=117.93±8.36 mg) were lighter than G-NCS beads (163.08±5.73 mg). However, the G-HPCS beads were the lightest one (84.92±6.38 mg) since the salt leach increased its porosity up to 42.32±4.57%. According to water uptake capacity depending on total porosity, GI-PMMA and G-PMMA beads had less such capacity since their total porosity was lower than that of calcium sulfate beads. For mass loss property, the G-HPCS beads show the highest percentage (9.19±1.52%) of mass loss after 10 days of the experiment. Since PMMA beads are a non-dissolvable material, the mass loss in this study was from getamicin release. A number of studies reported that polymers coated on the material were used for facilitating the release of growth factors (Lee *et al*., 2010; Choi *et al*., 2011). In some studies, antibiotic release was controlled by coated material with thin polymer layer containing antibiotic (Vasilev *et al*., 2011; Osaki *et al*., 2012). The mass loss of G-HPCS beads might be reduced by coating the material with polymer; it might slow down antibiotic release from G-HPCS surface.

Human osteoblasts (h-OBs) could survive in HPCS, NCS, and PMMA beads when observed with scanning electron microscope. The present study demonstrated that NCS and PMMA beads were non-toxic materials to h-OBs. This was in agreement with the previous study conducted in mouse's osteoblast (MC3T3-E1) (Lazary *et al*., 2007). That study revealed that MC3T3-E1 could proliferate two-fold in gypsum when compared with PMMA for the first 24 hours. Furthermore, they found the alkaline phosphatase activity and SMAD3 expressions in the gypsum group were higher than in PMMA group. However, our study showed that the new type of calcium sulfate beads, HPCS, has a high potential for using as an osteoconductive material similar to NCS beads, while PMMA beads did not have this property.

The microbiological assay using *Bacillus subtilis* (ATCC 6633) was an agar diffusion method for estimating the released antibiotic. The limited gentamicin concentration of

Table 2. Concentrations of gentamicin sulfate (μg ml^{-1}) released from GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads during the 10 day-experimental periods.

Day	Concentrations of gentamicin sulfate (μg ml^{-1})			
	GI-PMMA	G-PMMA	G-NCS	G-HPCS
1	571.48±279.88^{a}	1318.88±465.45^{b}	2640.54±470.28^{c}	4016.43±846.03^{c}
2	66.53±18.07^{a}	55.38±25.65^{a}	1058.85±160.67^{b}	624.56±247.94^{b}
3	26.69±4.81^{a}	6.77±1.91^{b}	852.76±204.23^{c}	159.05±25.84^{d}
4	16.28±3.51^{a}	0.61±0.33^{b}	238.21±36.87^{c}	48.03±13.96^{d}
5	9.55±3.72^{a}	UD	102.87±64.40^{c}	23.32±7.11^{b}
6	8.60±1.87^{a}	UD	52.05±15.97^{b}	12.21±3.35^{a}
7	6.55±1.82^{a}	UD	27.44±8.22^{b}	7.34±1.86^{a}
8	3.83±1.48^{a}	UD	13.99±2.32^{b}	4.93±1.15^{a}
9	4.27±1.67^{a}	UD	10.65±2.67^{b}	2.93±1.03^{a}
10	3.11±0.75^{a}	UD	6.10±1.43^{b}	2.70±0.42^{a}
Total	716.87±272.72^{a}	1381.64±486.38^{a}	5003.45±517.27^{b}	4901.50±1072.69^{b}

^{a,b,c,d} Different letters within row indicate significant difference within day (p<0.05).
UD = undetectable.

Table 3. Percentage of gentamicin release in each day from GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads.

Day	GI-PMMA	G-PMMA	G-NCS	G-HPCS
1	13.05±7.21[a]	95.44±1.07[b]	52.36±6.41[c]	81.98±1.65[d]
2	1.49±0.42[a]	3.99±1.03[a]	21.19±3.44[b]	12.27±2.91[b]
3	0.60±0.15[a]	0.52±0.15[a]	16.86±3.40[b]	3.46±1.30[a]
4	0.36±0.07[ab]	0.05±0.02[a]	4.74±0.66[c]	1.02±0.36[b]
5	0.22±0.10[a]	UD	2.08±1.42[b]	0.49±0.17[a]
6	0.19±0.05[a]	UD	1.05±0.38[b]	0.26±0.10[a]
7	0.15±0.05[a]	UD	0.55±0.19[b]	0.15±0.05[a]
8	0.09±0.04[a]	UD	0.28±0.07[b]	0.10±0.04[a]
9	0.09±0.04[a]	UD	0.21±0.06[b]	0.06±0.02[a]
10	0.07±0.02[a]	UD	0.12±0.04[b]	0.06±0.02[a]
% total release	16.32±7.22[a]	100±0.00[b]	99.44±0.22[b]	99.86±0.04[b]
Total (mg/bead)	4.48±0.31[a]	1.38±0.49[b]	5.03±0.52[a]	4.91±1.07[a]

[a,b,c,d] Different letters within row indicate significant difference within day ($p<0.05$).
UD = undetectable.

detection was 0.1 μg ml^{-1} in our study. The amount of gentamicin released from the beads each day was calculated according to this assay. The elution kinetics of gentamicin from GI-PMMA, G-PMMA, G-NCS and G-HPCS beads was significantly different. The total amount of gentamicin in G-PMMA, G-NCS and G-HPCS beads could be quantified by area under the curve, while the gentamicin amount per bead in GI-PMMA was calculated by percent concentration of gentamicin per weight of the bead. This study found that only small amounts of gentamicin (16.32±7.22% of total gentamicin) could be eluted from GI-PMMA during the 10-day experiment. This corresponded with the previous report that only 5-8% of antibiotic in PMMA was released from the exposed surface during the first week (Wahlig *et al.*, 1980). In contrast, most of gentamicin (99-100%) could be eluted from G-PMMA, G-NCS, and G-HPCS beads. These results were likely due to the differences in gentamicin coating method. The release of gentamicin could be separated into two phases: initial and sustain phases. This phenomenon was reported in many previous studies (van de Belt *et al.*, 2000; Wichelhaus *et al.*, 2001; Hendriks *et al.*, 2004; Udomkusonsri *et al.*, 2010). Likewise, the present study found that such phenomenon took place with GI-PMMA, G-PMMA, G-NCS, and G-HPCS beads. Most of gentamicin was eluted from the beads within the first day, known as an initial phase and followed by a sustain phase until the tenth day in GI-PMMA, G-NCS, and G-HPCS beads. However, the previous study found that 80% of gentamicin was released from G-NCS beads within the first day (Wichelhaus *et al.*, 2001). This might be due to the amount of solution used for dissolving gentamicin from the beads. The previous experiment used 5 ml PBS, while 1 ml of PBS was used in the present study. One milliliter of PBS might be saturated with gentamicin in the first day in our experiment. For G-PMMA beads, the gentamicin could be detected in a short period since PMMA beads had fewer pores on its surface and did not have interconnecting pores. Thus, PMMA could uptake antibiotic only in a low level when it was already in hardening form, owing to the porosity of the beads that contributed to the permeability to the bead matrix. G-HPCS beads had higher porosity than G-NCS beads, resulting in the higher percentage of gentamicin released from G-HPCS beads in the initial phase than that of G-NCS beads.

Gentamicin could coat only on the surface of the matrix. The elution characteristic of G-PMMA beads was different from GI-PMMA beads. GI-PMMA could release gentamicin until the end of the experiment, because gentamicin was added to PMMA prior to be hardened and was incorporated into the matrix of the materials. Antibiotic selection was limited for producing PMMA antibiotic beads since it needed a high temperature for the polymerization (Nandi, 2009). In this study, it was found that the antibiotic was inappropriate to be added to PMMA after hardening process. In addition, gentamicin concentration eluted from GI-PMMA and G-PMMA beads were lower than that of G-NCS and G-HPCS beads in the total amount. Our results suggested that PMMA beads have a lower ability as a local drug-release agent compared with calcium sulfate beads.

The G-HPCS bead was a new type of calcium sulfate bead used in the present study. It has high porosity, high antibiotic up taking and releasing. Furthermore, the HPCS bead is non-toxic to h-OBs and provided a positive effect on h-OBs attachment. G-HPCS and G-NCS beads could provide high concentration of gentamicin during the experiment. Although G-NCS beads could provide higher amount of gentamicin than G-HPCS beads after the second day through the end of study, the concentration of gentamicin released from G-HPCS was not difference from GI-PMMA until the

end of the study. These concentrations were above minimal inhibition concentration (MIC) for pathogenic bacteria, *Staphylococcus aureus*, which normally caused osteomyelitis exhibiting an MIC_{90} value (antibiotic concentration that inhibited the growth of susceptible strain *S. aureus* by 90%) of 1 mg ml^{-1} for gentamicin in susceptible strains (Fluit *et al*., 2000). From these reasons, the G-HPCS beads could be as applied as a local antibiotic delivery vehicle. Comparing the elution characteristics between G-NCS and G-HPCS beads, it was found that the concentration of gentamicin released from G-HPCS beads in the first day was higher than from G-NCS beads and then rapidly decreased on the following day. This result was a consequence of the difference in porosity level and water uptake capacity of the beads in each group, leading to a faster dissolution of gentamicin from G-HPCS than from G-NCS.

This result was similar to a previous study, which showed the difference of gentamicin and vancomycin releases from nanocrystalline hydroxyapatite and calcium sulfate beads (Rauschmann *et al.*, 2005). The nanocrystalline hydroxyapatite in combination with calcium sulfate exhibited a higher porosity and water uptake capacity than a pure calcium sulfate. These properties led to a higher antibiotic uptake and faster release of gentamicin and vancomycin within the first day by the composite material (Rauschmann *et al*., 2005). The results of our study also correlated with a previous study regarding the different phases of surface roughness, porosity, and wettability of gentamicin-loaded bone cements (van de Belt *et al*., 2000). They concluded that the releasing kinetics of gentamicin from bone cements was controlled by a combination of surface roughness and porosity. Interestingly, many previous studies showed the disadvantage of calcium sulfate as it might cause a transient cytotoxic effect, which lead to inflammatory reactions (Coetzee, 1980; Robinson *et al.*, 1999; Lee *et al.*, 2002). Calcium sulfate may cause more acidic microenvironment followed by a local inflammation at the site of implantation in human bone (Coetzee, 1980). From this reason, the G-HPCS bead has an advantage over the G-NCS bead since the amount of calcium sulfate per bead of G-HPCS was significantly lower than for G-NCS beads. An application of G-HPCS beads could reduce the side effects of calcium sulfate when implanted in the tissue.

The present study introduced a practical technique to apply a new type of calcium sulfate bead as local antibiotic delivery vehicle. The G-HPCS beads eluted higher concentration of gentamicin than GI-PMMA and G-PMMA beads. Moreover, G-HPCS beads are cheaper than PMMA beads and are easier to prepare with gentamicin sulfate in a clinical setting. Furthermore, gentamicin sulfate could be added to calcium sulfate beads after the hardening procedure. The advantages of dipping antibiotic in post-hardening calcium sulfate beads included the facilitation of individualized antibiotic therapy and the prevention of antibiotic degradation owing to the sterilization process or thermal instability (Dacquet *et al*., 1992).

5. Conclusion

The release characteristics of gentamicin G-HPCS, in the present study, could be compared with GI-PMMA, G-PMMA and G-NCS over ten days. The release of gentamicin from G-HPCS beads was greater than that from GI-PMMA and G-PMMA beads. The present study also demonstrated that the concentration of gentamicin from G-HPCS and G-NCS beads was higher than from GI-PMMA and G-PMMA beads in each day, except for the last five days, where the gentamicin concentration from G-HPCS and GI-PMMA was equal. In addition, G-HPCS beads in this study not only improved local antibiotic delivery, but they also had positive effects on osteoblast attachment, which was essential for new bone regeneration in osteomyelitis condition.

Acknowledgments

This research was supported by grants from Kasetsart University Research and Development Institute (KURDI), and Strategic Scholarship Fellowships Frontier Research Network, Office of the Higher Education Commission, Ministry of Education, Thailand.

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