The Application Potential of Sintered β-Dicalcium Pyrophosphate in Total Joint Arthroplasty

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Abstract: An *in vitro* bone cell culture model was used to evaluate the potential application of sintered β-dicalcium pyrophosphate (SDCP) in arthroplasty surgery. Primary osteoclasts and osteoblasts were cocultured with different sizes of SDCP particles. The changes in cell counts and the synthesis and secretion of alkaline phosphatase, acid phosphatase, and prostaglandin E_2 in response to the SDCP particles were monitored. When bone cells were cultured with SDCP particles smaller than 53 μm, both the osteoblast and osteoclast cell counts decreased significantly. When the SDCP particles were larger than 177 μm, although the osteoblast population increased significantly, the osteoclast population decreased significantly. Simultaneously, the titer of prostaglandin E_2 in the medium and the cytoplasmic prostaglandin E_2 increased significantly. We concluded that SDCP is a potentially useful bioceramic for the prevention of osteoclast-mediated periprosthetic osteolysis. **Key words:** sintered β-dicalcium pyrophosphate, particle size, osteoclasts, osteoblasts, arthroplasty.

With advances in ceramics technology, the application of calcium phosphate bioceramics as a bone substitute has recently received considerable attention. During the past decade, a large number of biomaterials have been proposed as artificial bone fillers for repairing bone defects. The material most widely used in clinical medicine is hydroxyapatite (HA) ceramics [1]. However, the use of block forms of porous HA has resulted in an unacceptably high failure rate in clinical applications [2]. As an intermediate product in the biological mineralization process [3], sintered β-dicalcium pyrophosphate (SDCP, formula β-$Ca_2P_2O_7$) has been proved to have great potential as an *in vivo* biodegradable bone substitute [4,5].

Host tissue responses to these materials are generally assessed by morphologic and histologic examinations of the implant site to evaluate their biocompatibility and cytotoxicity. The inertness of biomaterials is relative: even materials considered inert in bulk form can elicit inflammatory reactions in particulate form. Researchers have recognized that particulate debris around an orthopedic implant can have an adverse effect on the surrounding tissue [6]. The adverse effect depends on the particulate nature of the material more than on chemical biocompatibility [7]. It is difficult to examine the *in vivo* reaction of a specific cell to the substrate because numerous cell populations and chemical factors are involved in implantation.

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To determine the sequence of events and the parameters influencing the interactive process, a cell culture model has great importance for biomaterials. In previous *in vitro* biocompatibility tests, we compared the various sizes of calcium phosphate particles and the reaction of osteoblasts, and we found that SDCP was more biocompatible than HA [8]. The larger size hydroxyapatite particles seemed compatible with the bone cells, but the smaller sized hydroxyapatite particles may both activate the osteoclasts and decrease the population of osteoblasts [9]. However, the effect of SDCP particles on the osteoclasts was not clear in that study.

To quantify the effect of particle size on bone cell metabolism, an osteoclast cell culture model system was prepared in the presence of various sized SDCP particles. The goal of this study was to evaluated possible effects and identify the mechanism by which various sizes of SDCP particles and osteoclasts interacted. This study would lead to an evaluation of the possible clinical application of SDCP bioceramics in arthroplasty surgery.

Materials and Methods

Osteoclast Cell Culture

The rat spleen-calvaria coculture system was performed as described by Tanaka and Takahashi et al. [10, 11]. Newborn Wistar rats (3–5 days old) were obtained from the laboratory center of the Medical College, National Taiwan University. Primary osteoblastic cells were prepared from newborn rat calvaria as previously described [7]. Osteoblastic cells (1×10^4 cells/well) and spleen cells (7.5×10^5 cells/well) were cocultured for 7 days in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in the presence of 10 nM 1α, 25 $(OH)_2$ vitamin D_3 in 6-well plates. The culture dishes were incubated at 37°C in an atmosphere supplemented with 5% CO_2. When the cells grew to 80% confluence, various sizes of SDCP powders were added.

Preparation of Sintered β-Dicalcium Pyrophosphate Powder

The powder of SDCP was prepared as previously described [4]. In short, the β-DCP powder (β-DCP: $Ca_2P_20_{7;}$ Sigma, St Louis, MO) mixed with 5% weight $Na_4P_2O_7 \bullet 10H_2O$ in water and dried at 70°C for 3 days. The well-mixed and dried cake was ground and sieved to obtain 40–60 mesh particles. The sieved particles were placed in a platinum crucible and heated to 930°C at a heating rate of 3°C/min in a conventional Ni-Cr coiled furnace and then maintained in air for 1 hour after the sintering temperature of 930°C was reached. The sintered particles were ground and separated by mesh into various grain sizes of 0.5–3.0 μm, 37–63 μm, 177–250 μm, and 420–841 μm. The SDCP particles were spherical in shape at various grain sizes when observed using scanning electron microscopy. Before use, the particles were processed using dry heat sterilization (130°C, 40 min) as previously reported [12].

Treatment of Osteoclast Cell Cultures With Sintered β-Dicalcium Pyrophosphate

The medium surrounding a confluent rat bone cell culture was removed and the cells washed twice with phosphate buffer solution. Dulbecco's modified Eagle's medium supplemented with ITS (insulin, 10 μg/mL medium; transferrin, 5 μg/mL medium, and sodium selenite 5×10^{-3} μg/mL medium; Sigma) was mixed with 0.1% (1 mg/mL) of the various size SDCP particles and added to the cells. The day the new medium was added was considered day 0 of culture. The test medium was removed from wells at 3 hours, 1 day, 3 days, and 7 days, divided into 500 μL batches and frozen at −80°C for further analysis.

Identification and Quantification of the Osteoblast and Osteoclast Cells

At the end of the experiment, cells in the wells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) using a commercially available assay kit (procedure No. 386, acid phosphatase, leukocyte; Sigma). Cells were briefly fixed with a citrate-acetone solution for 30 seconds at room temperature. The fixed cells were incubated in the dark with a staining mixture for 1 hour at 37°C. The cells were washed with deionized water for 3 minutes, counterstained with acid hematoxylin, and then observed using inverted microscopy (Olympus, IMT-II, Hatagaya, Shibuya-ku, Tokyo, Japan).

The cell counts of the control and experimental wells were determined using an MICD image-analyzing system (MICD Software Series, Image Research, Catharine, Ontario, Canada). The mean osteoclast and nonosteoclast cell (osteoblast) populations in 4 randomly selected high-power fields (0.06 mm^2) were measured by counting the number of nuclei caught by a CCD-72 camera (Dage-MII, Michigan, IN) through the microscope. The cell population per unit area (millimeter squared) was measured as a mean of these 4 measurements.

The total cell count per well was calculated by multiplying to obtain the count for the area of each well (940 mm^2/well). The cellular responses of the treated samples were compared with control samples and expressed as a percentage of the control values.

Analysis of Alkaline Phosphatase in Culture Medium

Alkaline phosphatase (ALP) activity released from the cells into the medium was measured using a commercially available assay kit (procedure No. ALP-10, Sigma). Briefly, an aliquot (20 μl) from the media was mixed with 1 mL ALP reagent. The absorbance at 405 nm caused by p-nitrophenol production was followed for 5 minutes at 30°C. The change in rate of absorbance was directly proportional to ALP activity.

Analysis of Acid Phosphatase in Culture Medium

Acid phosphatase (ACP) activity released from the cells into the medium was measured using a commercially available assay kit (procedure No. 435, acid phosphatase, leukocyte, Sigma). In short, an aliquot (20 μL) from the media was mixed with 200 μL ACP reagent. The absorbance at 405 nm caused by p-nitrophenol production was followed for 5 minutes at 30°C. The change in rate of absorbance was directly proportional to APC activity.

Analysis of Prostaglandin E_2 (PGE_2) in Culture Medium

The production of prostaglandin E_2 (PGE_2) in culture medium was analyzed by enzyme-linked immunosorbent assay (ELISA). In short, 50 μL of standard PGE_2 (Assay Designs, Ann Arbor, MI) or sample was added per well. The test samples were incubated on the bench top for 18 hours at room temperature. Then 200 μL of Ellman's reagent were added to each well and incubated for 1.5 hours at room temperature. The reaction was stopped and read using a Microelisa reader (Molecular Device, Sunnyvale, CA) at 405 nm.

Analysis of Cytoplasmic ALP, ACP, and PGE_2

At the end of the experiment, cellular ALP, ACP, and PGE_2 activities were determined after lysis of the cells with the detergent Triton X-100 (Sigma T 8787; 1% in Hanks' balanced salt solution). Cytoplasmic ALP, ACP, and PGE_2 values were determined by the same methods as for the measurement of these activities in the culture media.

Statistical Analysis

The differences between the effects of the various sizes of SDCP particles were evaluated using an analysis of variant statistic method. The post hoc test performed was Bonferroni's *t*-test. The level of statistical significance was defined as $P<.05$.

Results

Cell Count

The osteoblast cell populations changed significantly after SDCP particles were added to the culture. The osteoblast cell population decreased significantly when cultured with the SDCP particles smaller than 53 μm ($P<.05$), and it increased when culturing with the SDCP particles larger than 177 μm ($P<.05$) (Fig. 1). When measured as a percentage of the control cell counts, the osteoblast counts decreased to 41.7% – 54.3% of the control counts for SDCP particles smaller than 53 μm and increased to 140.1%–153.8 % of the control counts for SDCP particles larger than 177 μm after being cultured for 7 days (Fig. 2). The changes in the osteoblast cell population, which were detectable in both the 3- and 7-day cultures were both statistically significant.

The osteoclast cell populations also changed significantly after adding SDCP particles into the culture. The osteoclast cell population decreased significantly when cultured with the SDCP particles smaller than 250 μm ($P<.05$). The smallest size SDCP particles gave the lowest cell population (Fig. 1). When measured as a percentage of the control cell counts, the osteoclast counts decreased to 1.8%–24.8% of the control counts for the SDCP particles smaller than 53 μm after 7 days of culture (Fig. 2). These changes were statistically significant at all the time intervals tested (3 hours, 1 day, 3 days, and 7days). When bone cells were cultured with SDCP particles smaller than 53 μm, both the osteoblast cell population and the osteoclast cell population were significantly lower than the control groups.

Alkaline Phosphatase

The release of ALP into culture medium and its production by bone cells under various conditions were measured. Except for the ALP titer in the 3-hour culture, the changes in ALP secretions in the

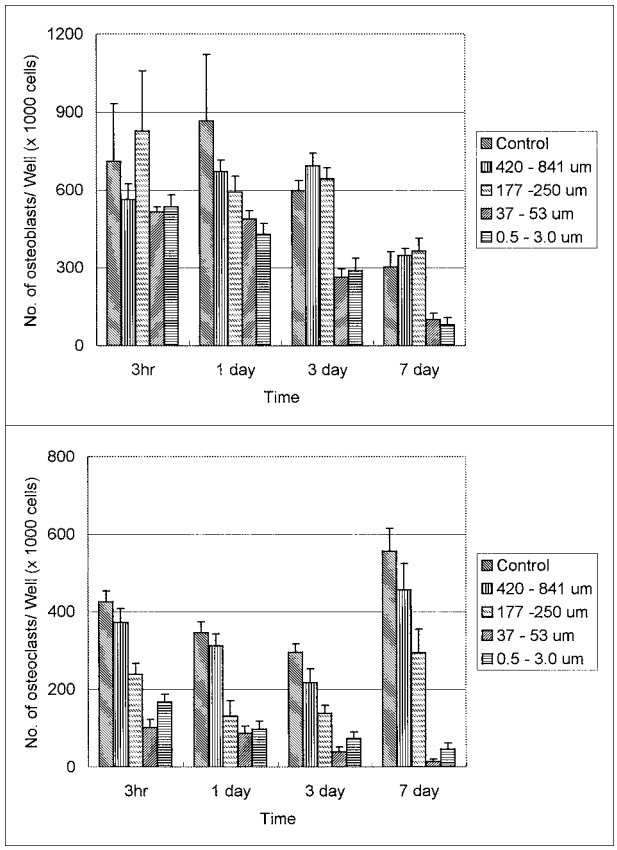

Fig. 1. Changes in osteoblast and osteoclast cell populations after 7 days' culture with various sizes of sintered dicalcium pyrophosphate (SDCP) particles. (Error bar shows standard error). (A) Statistically significant changes were seen in the osteoblast cell population at 3 and 7days. The osteoblast cell population increased significantly when cultured with SDCP particles larger than 177 μm ($P<.05$) and decreased significantly when cultured with SDCP particles smaller than 53 μm ($P<.05$). (B) Statistically significant changes were seen in the osteoclast cell population at all the time intervals tested (3 hours, 1 day, 3 days, and 7days). The osteoclast cell population decreased significantly when cultured with SDCP particles smaller than 250 μm ($P<.05$).

presence of the various sizes of SDCP particles were all statistically insignificant ($P>.05$). The changes in cytoplasmic ALP titer for the various sizes of SDCP particles were all statistically significant ($P<.05$; Table 1). A decrease in cytoplasmic ALP titer was more evident when the bone cells were cultured with the smaller SDCP particles, and this result correlates with the changes in the cell counts for the osteoblast cells.

Acid Phosphatase

The release of ACP into culture media and production by the osteoclast cells under various conditions were also measured. The changes in ACP secretions by bone cells were all statistically insignificant ($P>.05$), except for the 3-hour culture. The changes in cytoplasmic ACP titer of bone cells for

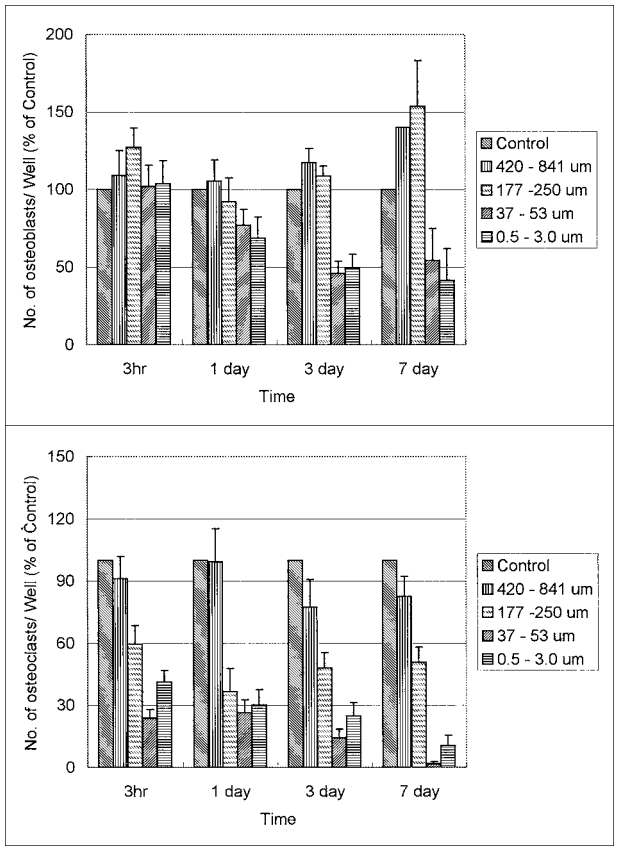

Fig. 2. Changes in osteoblast and osteoclast cell populations (as a percentage of the control) after 7 days' culture with various sized SDCP particles are seen. (Error bar shows standard error.) (A) A statistically significant increase in the osteoblast cell population was seen when bone cells were cultured with SDCP particles sized larger than 177 μm. When bone cells were cultured with SDCP particles smaller than 53 μm, the osteoclast cell population was significantly lower than that of the control groups. At the end of 7 days' culture, the osteoblast counts had increased by 140.1% to 153.8% of the control counts for the SDCP particles sized larger than 177 μm and had decreased by 41.7% to 54.3% of the control counts for the SDCP particles sized smaller than 53 μm. (B) The decrease in osteoclast cell population attained statistical significance when bone cells were cultured with SDCP particles sized larger than 420 μm for 7 days. When bone cells were cultured with SDCP particles smaller than 53 μm, the osteoclast cell population was significantly lower than that of the control groups at all time intervals tested. At the end of 7 days' culture, the osteoclast counts had decreased by 1.8% to 10.4% of the control counts.

Table 1. Changes in Intracellular and Extracellular Alkaline Phosphatase Titer After Culture with Various Sized Sintered Dicalcium Pyrophosphate Particles at a Concentration of 1 mg/mL

Size	3 h (units)	1 d (units)	3 d (units)	7 d (units)
Medium Control	117912.2	100499.0	123882.5	141792.5
	SE: 1442.6	SE: 4489.6	SE: 4965.7	SE: 4255.1
420–841 μM	106966.8	96021.4	104976.7	117912.2
	SE: 1314.0	SE: 3782.1	SE: 3032.3	SE: 5621.5
177–250 μM	94031.3*	91543.7	101494.1	106966.8
	SE: 1807.4	SE: 2699.1	SE: 2874.6	SE: 5031.7
37–53 μM	91543.7†	84080.9	98011.4	121394.5
	SE: 2143.8	SE: 2866.6	SE: 2915.2	SE: 4927.0
0.5–3.0 μM	100996.6*	92041.2	108459.4	103484.2
	SE: 2024.3	SE: 3392.0	SE: 4172.1	SE: 3563.7
P value	.0076	.85	.52	.36
Cell Control	13488.3	69044.7	49530.9	196921.5
	SE: 1049.5	SE: 16454.4	SE: 4122.1	SE: 142336.0
420–841 μM	13156.6	40465.0*	37977.4*	149161.0*
	SE: 754.4	SE: 6278.5	SE: 3361.6	SE: 147800.7
177–250 μM	10116.24†	33389.12*	31233.2†	111610.3*
	SE: 755.5	SE: 4236.7	SE: 3365.8	SE: 91798.6*
37–53 μM	11387.7*	33333.8*	21669.8‡	46874.6
	SE: 705.0	SE: 4639.0	SE: 1749.7	SE: 36265.4
0.5–3.0 μM	12769.7	37645.*	22830.6‡	29299.8*
	SE: 635.4	SE: 5198.4	SE: 2003.4	SE: 21905.6
P value	.023	.029	1.37×10^{-7}	.0031

NOTE. The differences between various sized sintered β-dicalcium pyrophosphate particles were evaluated by an analysis of variance statistical method. The post hoc test performed was Bonferroni's *t*-test.

Abbreviation: SE, standard error.

*$P < .05$.

†$P < .001$.

‡$P < 0.00001$.

All *P* values are when compared with the control group.

the various sizes of SDCP particles were all statistically significant ($P<.05$; Table 2). The decrease in cytoplasmic ACP titer was more evident when the bone cells were cultured with the smaller SDCP particles, and this also correlates with the changes in the cell counts of osteoclast cells.

Prostaglandin E_2

The synthesis and secretions of PGE_2 by the bone cells cultured with the various preparations of SDCP are shown in Table 3. Changes in both the cytoplasmic PGE_2 and the secreted PGE_2 were all statistically significant ($P<.0001$ by analysis of variance [ANOVA] test; Table 3). When bone cells were cultured with SDCP particles smaller than 53 μm, the PGE_2 titers in the test culture medium reached a peak on the third day after the addition of the SDCP particles, then decreased a little by the seventh day. After 3 days of culture, the PGE_2 titers in the media cultured with 37–53 μm SDCP particles were 2.59 times that of the control, and PGE_2 titers in the media cultured with 0.5–3 μm sized SDCP were 2.80 times that of the control (Table 3). The cytoplasmic PGE_2 titers reached a peak on the first day after implantation, then decreased gradually to the seventh day. At the end of 7 days, the cytoplasmic PGE_2 titers in the bone cells cultured with 37–53 μm and 0.5–3 μm size SDCP were 2.39 and 3.13 times that of the control, respectively (Table 3).

Discussion

Continuous advances in the field of implantable calcium phosphate bioceramics have produced impressive progress with respect to biocompatibility and have shown an ability to promote tissue formation. Biodegradable biomaterials have a wide variety of applications in clinical medicine. Certain biodegradable calcium phosphates have been used as bone substitutes to avoid complications associated with bone grafts, such as supply shortage, immunogenicity, and transfer of disease with the graft [13–16]. One of the main advantages of biodegradable substances is that they obviate eventual surgical removal. Sintered β-dicalcium pyrophos-

Table 2. Changes in Intracellular and Extracellular Acid Phosphatase (ACP) Titer After Culture With Various Sized Sintered Dicalcium Pyrophosphate (SDCP) Particles at a Concentration of 1 mg/mL

Size	3 h (units)	1 d (units)	3 d (units)	7 d (units)
Medium Control	30.8	46.2	53.9	92.3
	SE: 17.0	SE: 12.6	SE: 11.8	SE: 34.08
420–841 μM	23.1	23.1	46.2	38.5
	SE: 11.8	SE: 2.8	SE: 12.6	SE: 17.2
177–250 μM	30.8	46.2	38.5	46.2
	SE: 12.6	SE: 12.6	SE: 12.8	SE: 12.6
37–53 μM	30.8	53.9	46.2	38.5
	SE: 12.6	SE: 11.8	SE: 12.6	SE: 12.8
0.5–3.0 μM	46.2	53.9	61.6	61.6
	SE: 17.0	SE: 11.8	SE: 10.3	SE: 15.4
P value	.85	.37	.71	.29
Cell Control	63.3	138.5	137.5	163.3
	SE: 9.7	SE: 29.5	SE: 17.1	SE: 25.2
420–841 μM	57.3	92.3*	126.3	150.5
	SE: 10.8	SE: 13.0	SE: 12.9	SE: 22.4
177–250 μM	47.0	100.9*	110.9*	155.6
	SE: 8.0	SE: 17.7	SE: 10.9	SE: 17.7
37–53 μM	38.5	57.3†	67.9†	88.1*
	SE: 7.2	SE: 10.7	SE: 7.8	SE: 14.4
0.5–3.0 μM	39.3	52.2†	69.7†	88.1*
	SE: 8.0	SE: 11.1	SE: 9.5	SE: 18.6
P value	.21	.0085	.00017	.013

NOTE. The differences between various sized sintered β-dicalcium pyrophosphate particles were evaluated by an analysis of variance statistical method. The post hoc test performed was Bonferroni's *t*-test.

Abbreviation: SE, standard error.

*$P < .05$.

†$P < .001$.

All *P* values are when compared with the control group.

phate has been proved to have great potential as an *in vivo* biodegradable bone substitute [4,5]. In this study, we further demonstrated that larger sized SDCP particles are potentially useful for preventing osteoclast-mediated periprosthetic osteolysis by an *in vitro* bone cell culture model.

The events at the interface between the implanted material and the adjacent tissue are the direct results of the cellular, chemical, physiologic, and mechanical reactions evoked by the presence of the biomaterial [17]. Host tissue responses to these materials are generally assessed by morphologic and histologic examinations of the implant site to evaluate their biocompatibility and cytotoxicity [18]. In our institution, SDCP has been shown to be an effective biodegradable bone filler for repairing bone defects [4,5]. The *in vivo* experiments do not allow the examination of specific cell to substrate relationships. A cell culture model in the presence of biomaterials is of great usefulness for determining the events and the parameters influencing the interactive process [19]. Although cell culture studies cannot directly duplicate the conditions that exist *in vivo*, the effects of specific types of particles on cellular metabolism can be directly quantified using this technique [20].

Working with primary bone cells populations has several advantages. For example, because these cells are not transformed, any observed response can be attributed to the phenotype of the bone cells and are not a possible by-product of the transforming process [21]. In previous experiments, we isolated and cultured subpopulations of osteoblast cells to characterize their response to SDCP particles. It revealed that the addition of smaller size SDCP particles ([0.5–3.0 μm and 37–53 μm) into culture could significantly affect the cell counts of osteoblasts [22]. In this study, the main concern was to study the interactions of SDCP bioceramic particles originating from on the coexisting and cocultured osteoblast and osteoclast cells.

As shown by this study, the bone cell counts changed significantly after adding SDCP particles (Fig. 1). If the particle size was larger than 177 μm, a significant increase in the osteoblast cell population was seen, while the osteoclast cell population decreased significantly (Fig. 1). When represented as a percentage of the control cell counts, the osteoblast counts increased by 140.1% to 153.8% compared with the control counts for a SDCP particles size larger than 177 μm (Fig. 2) and osteoclast counts decreased by 48.0% to 82.6% compared with the control counts (Fig. 2). The osteoblast and osteoclast cell population decreased significantly when SDCP particles were smaller than 53 μm (Fig. 1). When bone cells were cultured with SDCP particles smaller than 53 μm, the osteoblast counts decreased by 41.7% to 54.3% compared with the control counts for SDCP particle sizes smaller than 53 μm, and the osteoclasts decreased by 1.8% to 24.8% compared with the control counts (Fig. 2).

Although the larger SDCP particles may limit osteolysis, smaller particles may be generated in patients, and the newly-generated smaller SDCP particles will compromise the osteoblasts as well as osteoclasts. As shown previously, a decrease in cell number after exposure to the test material could be used as a marker for toxicity [23]. It has been suggested that toxicity due to direct contact of the particles with the cells only occurs with particles smaller than approximately 5 μm in diameter [24]. The decrease in cell population was more obvious in the osteoclast counts. This result suggests that the rates of osteoclast proliferation or differentiation decrease significantly after the addition of the SDCP particles into the culture.

Table 3. Changes in Intracellular and Extracellular Prostaglandin-E_2 Titer After Culture With Various Sized Sintered Dicalcium Pyrophosphate Particles at a Concentration of 1 mg/mL

Size	3 h (pg/mL)	1 d (pg/mL)	3 d (pg/mL)	7 d (pg/mL)
Medium Control	1290.9	2762.4	3997.7	2222.3
	SE: 133.8	SE: 394.3	SE: 821.5	SE: 87.4
420–841 μM	1356.9	2444.8	4687.0	2591.9
	SE: 138.5	SE: 367.3	SE: 1069.7	SE: 277.8
177–250 μM	1288.4	3172.2	5418.9*	3726.2*
	SE: 129.9	SE: 342.8	SE: 1323.5	SE: 484.6
37–53 μM	1553.6	4860.4*	10352.4‡	6510.6†
	SE: 160.4	SE: 989.4	SE: 1575.7	SE: 794.7
0.5–3.0 μM	1155.9	4603.6*	11200.8†	7040.4†
	SE: 152.8	SE: 616.8	SE: 1996.9	SE: 965.3
P value	.41	.016	.00086	3.41×10^{-7}
Cell Control	11607.1	4182.21	2776.0	1518.1
	SE: 835.1	SE: 360.9	SE: 318.6	SE: 198.9
420–841 μM	10659.0	5060.2*	3235.0	2121.8*
	SE: 924.5	SE: 373.4	SE: 438.3	SE: 272.7
177–250 μM	11916.8	6599.6*	3412.1*	2018.4*
	SE: 896.7	SE: 770.3	SE: 394.2	SE: 256.5
37–53 μM	12002.0	7888.2†	4156.0*	3629.9†
	SE: 1319.9	SE: 747.5	SE: 516.0	SE: 596.3
0.5–3.0 μM	12991.4	7208.7*	5648.1†	4755.7†
	SE: 1138.3	SE: 694.1	SE: 689.0	SE: 811.1
P value	.63	.00048	.0014	9.4×10^{-5}

NOTE. The differences between various sized sintered β-dicalcium pyrophosphate particles were evaluated by an analysis of variance statistical method. The post hoc test performed was Bonferroni's *t*-test.

Abbreviation: SE, standard error.

*$P < .05$.

†$P < .001$.

‡$P < 0.00001$.

All *P* values are when compared with the control group.

The changes in ALP and ACP secretions with the various sizes of SDCP particles did not attain a statistically significant level. However, the cytoplasmic ALP and ACP titer for various sizes of SDCP particles decreased significantly (Tables 1, 2). The changes in cytoplasmic ALP and ACP titer was more evident when bone cells were cultured with the smaller SDCP particles. This findings is in agreement with the changes in the cell counts of the bone cells.

Prostaglandin is produced in skeletal tissues, where it has complex effects on both the catabolic and anabolic activities of bone cells. Researchers have shown that it stimulates osteolysis in bone organ cultures [25–28] and this can cause an increase in bone loss [29, 30]. It has also been known to directly inhibit the cytoplasmic motility of and bone resorption by isolated osteoclasts [31, 32]. This apparently paradoxical behavior is thought to be due to prostaglandin-induced osteoclastic differentiation in bone marrow cultures and an inhibition of the function of the osteoclasts thus formed [32–34]. Prostaglandin's stimulation of osteoclastic activity in intact bone is thought to be mediated indirectly through the action of another cell type in bone, most likely the osteoblasts [35]. Colline and Chambers [36] reported that PGE_2 induces osteoclast differentiation in mouse spleen hemopoietic cell and bone marrow stromal cell cocultures.

In this study, the changes in PGE_2 titers corresponded to the changes in cell counts when the bone cells are cocultured with the various sizes of SDCP particles. The increase in PGE_2 titer in the culture medium and the cytoplasm of bone cells when cultured with the smaller sizes of SDCP particles were quite obvious. The PGE_2 titers increased by 2.39 to 3.13 times compared with the control (Table 3). It had been reported that PGE_2 inhibits osteoclast differentiation from precursor cells [37]. The smaller sized SDCP particles thus could affect osteoclast cells differentiation by the PGE_2 pathway. There could be an inhibition of osteoclastic recruitment to the bone surface and an inhibition of the activity of osteoclasts that have reached the surface, and this could shorten the life span of the osteoclasts, perhaps by inducing apoptosis [25]. The changes in osteoclast cell counts and the ratio of osteoclasts to osteoblasts after adding the SDCP

particles to the bone cells cultures are possibly mediated by the PGE_2 pathways.

Osteoclast differentiation and activation is controlled, at least in part, by the counterbalancing influences of receptor activator of nuclear factor-κ B ligand (RANKL) and osteoprotegerin (OPG). RANKL is the essential factor for osteoclast formation and activation and enhances bone resorption. By contrast, OPG, which is produced by osteoblastic lineage cells, acts as a decoy receptor that neutralizes RANKL and prevents bone loss [38]. Recombinant human OPG was found to cause osteoclasts to detach from the bone surface [39]. In this study, whether the inhibitory effect of SDCP on the osteoclasts was mediated by cyclo-oxygenase 2 (COX-2) induction in osteoblasts [40] needs to be further validated.

Periprosthetic osteolysis leading to aseptic loosening is a major cause of failure in total joint arthroplasty. The process of osteolysis begins with the generation of implant material wear particles. These particles stimulate macrophages and giant cells, which release mediators that stimulate the osteoclasts, leading to periprosthetic bone resorption. A net loss of bone at the tissue-implant interface thus occurs via an increase in bone resorption by osteoclasts or via a decrease in bone formation by osteoblasts. Bioceramics can contribute to bony growth and incorporation by inhibiting the functions of osteoclasts or accelerating the function of osteoblasts. The results of this study clearly show that larger sized SDCP particles are potentially useful for the prevention of osteoclast-mediated periprosthetic osteolysis. Further *in vivo* and clinical studies are needed to further analyze the effects of SDCP.

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