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Bone and Joint Protection Ability of Ceramic Material with Biological Effects

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Abstract

Ceramic materials with biological effects (bioceramic) have been found to modulate various biological effects, especially those effects involved in antioxidant activity and hydrogen peroxide scavenging. As arthropathy and osteopathy are the major chronic diseases of geriatric medicine, we explored the possible activity of bioceramic on these conditions using animal and cell models. Rabbits received intra-articular injections of lipopolysaccharides (LPS) to induce inflammation that mimic rheumatic arthritis. FDG isotopes were then IV injected for poistron emission tomography (PET) scan examinations at 16 hours and 7 days after the LPS injection. We examined and compared the bioceramic and control groups to see if bioceramic was capable of relieving inflammation in the joints by subtracting the final and initial uptake amount of FDG (max SUV). We studied the effects in prostaglandin E_2 (PGE_2) inhibition on the human chondrosarcoma (SW1353) cell line, and the effects on the murine osteoblast (MC3T3-E1) cell line under oxidative stress. All the subtractions between final and initial uptakes of FDG in the left knee joints of the rabbits after LPS injection indicated larger decreases in the bioceramic group than in the control group. This anti-arthritic or inflammatory effect was also demonstrated by the PGE_2 inhibition of the SW1353 cells. We further proved that bioceramic treatment of the MC3T3-E1 cells resulted in increased viability of osteoblast cells challenged with hydrogen peroxide toxicity, and increased alkaline phosphatase activity and the total protein production of MC3T3-E1 cells under oxidative stress. Since LPS-induced arthritis is an experimental model that mimics RA, the potential therapeutic effects of bioceramic on arthropathy merit discussion. Bioceramic may contribute to relieving inflammatory arthritis and maintaining bone health.

Key Words: bioceramic, rheumatic arthritis, PGE_2, alkaline phosphatase, murine osteoblast

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory disease that affects about 1% of the population in Western countries (13). It is a systemic autoimmune disease characterized by chronic synovitis and bone damage. Oral drugs, such as corticosteroids and NSAIDs, are the usual therapeutic treatment agents (33). Corticosteroids reduce the number of osteoblasts and the synthesis of bone matrix proteins. At the same time, corticosteroids enhance bone resorption which results in impaired bone remodeling. Therefore,

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bone manifestations of RA consist of joint destruction and systemic osteoporosis (40). Both joint destruction and systemic osteoporosis are caused by different mechanisms. Recent treatment strategies have improved management of RA-related joint destruction as well as corticosteroid-induced osteoporosis. Clinically, the trend of RA therapy is to treat joint destruction as an inflammatory disease and osteoporosis as a metabolic disease (39, 40).

Our earlier investigations and publications on bioceramic emitting ceramic material have shown that it promotes microcirculation and other effects by up-regulating calcium-dependent nitric oxide and calmodulin in different cell lines (21, 23). We have also demonstrated that this material produces an antioxidant effect by increasing hydrogen peroxide (H_2O_2) scavenging ability (24, 25). The purpose of this study was to investigate the effects of bioceramic irradiation using an *in vivo* animal model and to study its short-term effects on lipopolysaccharide (LPS)-induced inflammatory arthritis, which mimics an acute episode of inflammatory and RA. This animal model was designed for observing the results of exposure to bioceramic irradiation without an external heat source setting. The second focus of this study was to investigate the possible effects of bioceramic on the inflammatory index (prostaglandin E_2) of chondrosarcoma cells, osteoblast growth and bone differentiation. A cell model with oxidative stress induction was employed to test the possibility that bioceramic has the beneficial effect of preventing oxidation damage to bone tissues.

Materials and Methods

Bioceramic Ceramic Powder

The ceramic powder (obtained from the material laboratory of Taipei Medical University) was composed of micro-sized particles produced from several ingredients mainly mineral oxides, including aluminum oxide, ferric oxide and magnesium oxide. The average emissivity of the ceramic powder was 0.98 at wavelengths of 6-14 μm determined by a CI SR5000 spectroradiometer (CI systems Ltd., Migdal Ha'Emek, Israel) which represents an extremely high ratio of FIR intensity. Many physical, chemical and biological effects can be induced by the ceramic powder without direct contact. Many physiological effects can be induced by this ceramic powder at room temperature using indirect contact methods that have been previously described (21-24).

Determination of the Effect of Bioceramic on the Rabbit Model of Arthritis by Injection of LPS, and Monitoring under Positron Emission Tomography (PET) Scan

The experimental design was to induce arthritis in male New Zealand white (NZW) rabbits with an average weight of 2.0-2.5 kg. The rabbits were maintained at about 21 ± 2°C with food and water *ad libitum* and kept on a 12 h light/12 h dark cycle. Animal experiments were carried out at the animal facility of Taipei Medical University with the approval of the local and National Ethics Committees.

Two groups of rabbits were divided randomly into a bioceramic group (n = 3) and a control group (n = 3). The rabbits of the bioceramic group were placed in a cage surrounded by paper sheets covered with a thin layer of the ceramic powder, while the control group was surrounded by the same sheet without the material.

LPS-Induced Arthritis

The LPS arthritis induction was carried out according to published protocols (17, 22, 29, 31). Rabbits were anesthetized with zoletil 50 (0.25 mg/kg) intraperitoneally. LPS (10 ng/100 μg) (Sigma Chemical Co., St. Louis, MO, USA) was then injected intra-articularly into the left knee joint using a 30-gauge syringe. An FDG PET was performed 16 h after LPS administration.

PET Scanning Procedure

Before arthritis induction and FDG PET scanning, the animals were sedated and immobilized. In the PET study, a Concorde microPET R4 scanner was used (Concorde Microsystems, Knoxville, TN, USA) for the best resolution available. An average 1 mCi was given by intravenous bolus injection into one of the veins of the ear of each rabbit. A 20-min emission PET scan was initiated about two hours after FDG administration, similar to the protocol described by Hsieh *et al*. (16) and Lin *et al*. (27).

Data Analysis

We reconstructed the PET images through Fourier rebinning and ordered-subset expectation maximization (OSEM) using default corrections for radioactive decay, dead time and attenuation provided by the vendor. To evaluate the arthritis level, standard uptake value (SUV) was used as quantization parameter of PET. All the parameter settings were referenced from a previous experiments (16, 28). FDG uptake was assessed only in the most metabolically active portions of both knees of each rabbit (16, 31, 32). During the post-processing of results, we used the ASIProVM analysis software to draw the regions of interest (ROIs) and to determine the SUVs. Differences between the SUVs of the arthritic and the control knees and the corresponding

ratios were calculated as PET arthritis severity indexes as previously described (16, 26). The maximum SUV (MaxSUV) was used as the base data to compare the change of radioactivity during the periods of study (16 h after LPS administration to the 7th day).

Cell Model Determination of the Effect of Bioceramic on the Chondrosarcoma (SW1353) Cell Line for Prostaglandin E_2 (PGE_2) Production under LPS Induction

SW1353 cells were seeded into a 24-well cell culture plate (GeneDireX, Inc., Flint Place Poway, CA, USA) 1 day before the experiment. They were then stimulated with 20 ng/ml LPS for 48 h with or without the bioceramic material placed beneath the culture medium discs. The supernatant was harvested and used to measure PGE_2 production by ELISA (R&D Systems, Minneapolis, MN, USA).

Determination of the Effect of Bioceramic on the Cell Proliferation Curve of Osteoblastic Cells (MC3T3-E1)

For the bioceramic groups, bioceramic ceramic powder enclosed in plastic bags was inserted beneath the cell culture plates and uniformly distributed. The bioceramic and control plates were incubated at 37°C in a CO_2 incubator for 0, 1, 4, 7, 10 and 14 days. Cells were counted by the MTT [3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyl tetrazolium bromide] method. The absorbance of each well was recorded on a microplate spectrophotometer at 595 nm.

Determination of the Effect of Bioceramic on Cell Viability of MC3T3-E1 under H_2O_2-Mediated Oxidative Stress

MC3T3-E1 cells were seeded in six-well tissue culture plates at a density of 4×10^5 cells per well. After 16 h of culturing, the medium was changed and various concentrations of H_2O_2 were added. For the bioceramic groups, bioceramic ceramic powder enclosed in plastic bags was inserted beneath the cell culture tissue plates and uniformly distributed. In this way, the cells constantly received even bioceramic irradiation. The control group received H_2O_2 treatment without any bioceramic exposure. After 24 h of treatment, viable cells were counted by the MTT method.

Determination of the Effect of Bioceramic on MC3T3-E1 for Alkaline Phosphatase Production with or without H_2O_2-Mediated Oxidative Stress

The MC3T3-E1 cell suspension containing 5×10^3 cells was then added to the individual wells of 24-well plates (GeneDireX, Inc., Flint Place Poway, CA, USA). Cells were separated into a bioceramic material-treated group and a control group. For the bioceramic groups, bioceramic ceramic powder enclosed in plastic bags was inserted beneath the culture tissue plates and uniformly distributed. After 0, 4, 7, 10, 14 and 17 days, the cells were collected and stored at -80°C. The cells were lysed. Clear supernatant was used to measure the alkaline phosphatase activity determined by using an alkaline phosphatase activity assay kit (Biovision Inc., Mountain View, CA, USA).

Determination of the Effect of Bioceramic on MC3T3-E1 for Total Protein Concentration with H_2O_2-Mediated Oxidative Stress

The process was the same as described above. The protein concentration was measured using a protein assay kit (Biorad Laboratories, Hercules, CA, USA).

Statistical Analysis

Three data sets were obtained by subtracting the maximum SUV ratios on the 7th day from that observed 16 h after the LPS injection. This was done separately for the control and bioceramic groups. For the parameters of different cell lines, data from both the bioceramic and control groups were collected. The statistical relationships between the groups were determined using the *t*-test method, with *P* values smaller than 0.05 considered significant.

Results

PET Scan Study

The knees of rabbits of both control and bioceramic groups were evaluated sequentially by MicroPET system at 16 h after LPS injection and the 7th day. Six knees with inflammatory arthritis were examined.

Fig. 1 presents three standard views of typical PET image volume taken on the 7th day after LPS injection by coronal, axial and sagittal views of the knee and joints of the control and bioceramic groups. Brighter and reddish areas indicate higher radioactivity, while a yellow-greenish color represents lesser degrees of radioactivity. FDG uptake was assessed in the most metabolically active portions of both knees of each rabbit. The PET scan images for the two groups showed two out of three amongst the control group rabbits, with their LPS-induced knee joints, were found further increase in FDG uptake. The results reflected inflammation exacerbate from 16 h to

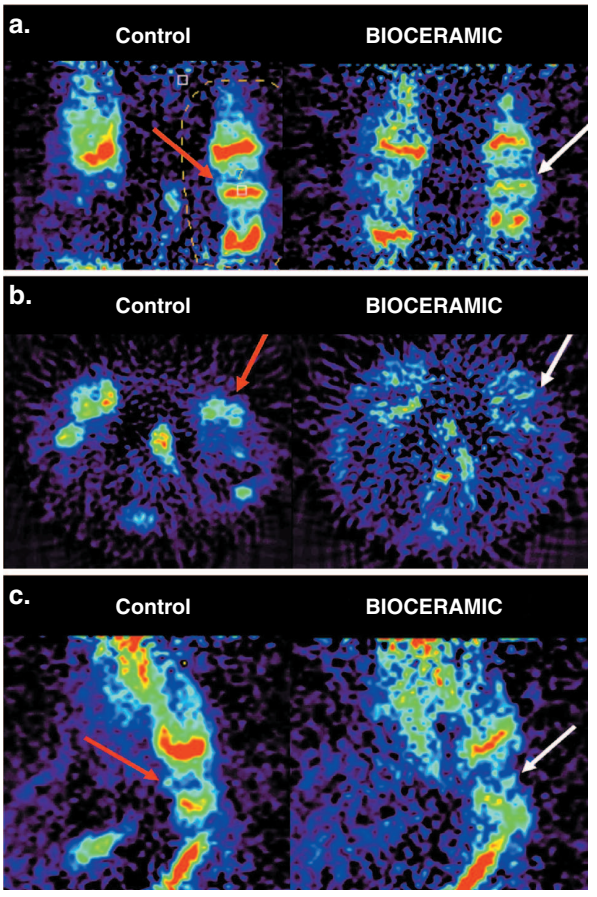

Fig. 1. Example pictures of PET scan on experimental rabbits, which showed the comparison of the control [left figures, with red arrows, in coronal (a), axial (b) and sagital (c) views]; and bioceramic group [right figures, with white arrows, in coronal (a), axial (b) and sagital (c) views], after 7 days of LPS intra-articular injection of left knees.

the 7th day. On the contrary, every LPS-injected knee in the bioceramic-treated group exhibited significant relief of arthritis. The statistical relationship between the control and bioceramic groups using mean of maxSUVs on the 7th day and mean of the subtraction values (maxSUV) by 7th day to 16 h were obtained. The *P*-value was smaller than 0.05 and 0.01 respectively, which were statistically significant. These indicate that the bioceramic group recovered faster from inflammatory arthritis after seven days of intra-articular LPS injections than the control group (Tables 1 and 2).

The Effect of Bioceramic on the Chondrosarcoma Cell Line with Prostaglandin E_2 (PGE_2) Production

To investigate the effect of bioceramic material on PGE_2 production, the accumulation of PGE_2 in the culture media was measured after 48 h of 20 ng/ml LPS induction with or without bioceramic material placed beneath the cell medium discs.

In the culture disc of the experimental group, treatment of LPS caused lower amounts of PGE_2 production than the LPS-treated control group (Fig. 2). This result reflects a significant suppression of the LPS-induced PGE_2 of cells by the bioceramic material.

The Effect of Bioceramic on Cell Proliferation of Osteoblastic Cells (MC3T3-E1)

The proliferation curve of cells treated with bioceramic showed significant higher than the control group on the 10th day ($P < 0.05$; three experiments and 2/3 of the data were significant) (Fig. 3).

The Effect of Bioceramic on the Cell Viability of MC3T3-E1 under H_2O_2-Mediated Oxidative Stress

The viability of cells treated with bioceramic under H_2O_2-mediated oxidative stress was greater than that of the control counterparts receiving H_2O_2 treatment without bioceramic (Fig. 4). Additional bioceramic treatment resulted in 23.02% and 18.77% increases in osteoblastic cell viability in the 200 M and 800 M H_2O_2 concentration groups, respectively ($P < 0.05$). The *t*-test confirmed the significance of these findings and suggests that bioceramic treatment reduced cytotoxicity from H_2O_2-induced oxidative stress.

The Effect of Bioceramic on Alkaline Phosphatase Activity in Osteoblastic Cells (MC3T3-E1) with or without H_2O_2-Mediated Oxidative Stress

The effect of bioceramic material on alkaline phosphatase activity in osteoblastic MC3T3-E1 cells was examined (Fig. 5). The results demonstrated higher alkaline phosphatase activity of the bioceramic group on the 10th day ($P < 0.05$; two experiments and 1/2 of the data were significant).

The effect of bioceramic on alkaline phosphatase content in osteoblastic MC3T3-E1 cells under oxidative stress is shown in Fig. 6. The presence of bioceramic caused an increase in alkaline phosphatase content from the 10th to 14th day and was statistically significant on the 14th day ($P < 0.05$; five experiments and all of the data were significant on day 14).

The Effect of Bioceramic on Total Protein Production in MC3T3-E1 with H_2O_2-Mediated Oxidative Stress

The effect of bioceramic on total protein content in osteoblastic MC3T3-E1 cells was examined (Fig. 7). The results show that bioceramic caused an increase in total protein content on the 10th day ($P <$

Table 1. MaxSUVs of LPS-injected rabbits' knees

Groups	16-h after LPS Injection	7^{th} Day after LPS Injection	Change of maxSUVs
Control-1	1.33575	1.60000	Increase
Control-2	0.756942	1.16618	Increase
Control-3	1.71334	1.2413	Decrease
Bioceramic-1	1.43541	1.01615	Decrease
Bioceramic-2	1.18309	0.875833	Decrease
Bioceramic-3	1.96363	0.87509	Decrease

Table 2. Mean of maxSUVs on 7^{th} day and of subtractions by 7^{th} day on 16-hour after intra-articular LPS injections in the control and bioceramic groups

	Control (n = 3)	Bioceramic (n = 3)	*P* value
Mean of maxSUVs on 7^{th} day	1.34 ± 0.23	0.92 ± 0.81	0.0420*
Mean of subtraction	-0.07 ± 0.47	0.61 ± 0.42	0.0022**

*$P < 0.05$; **$P < 0.01$, bioceramic group found significantly different compared with the control group.

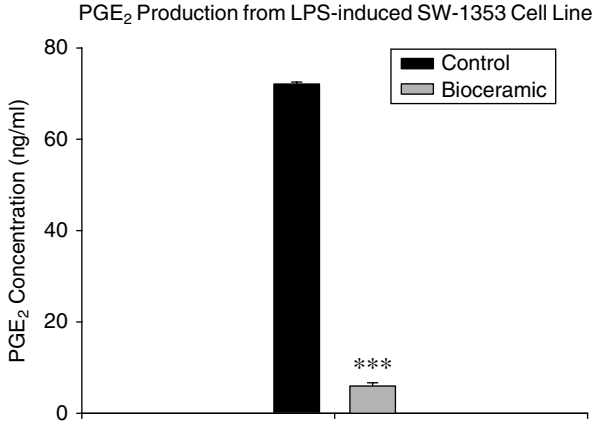

Fig. 2. Comparison of LPS induced PGE_2 (ng/ml) of SW1353 cell line in the control and experimental groups (n = 5). ***$P < 0.0001$, significantly different compared with the control group.

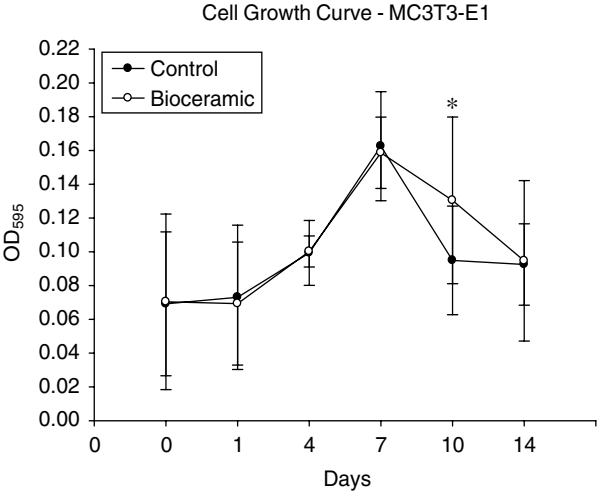

Fig. 3. Effect of bioceramic on osteoblast cell growth curve, compared with the control group (n = 12). *$P < 0.05$, significantly different compared with the control group.

0.05; two experiments and all of the data were significant).

Discussion

A number of factors, including inflammation and oxidative stress, are believed to play a role in the development of chronic joint disease. Inflammation induced by LPS is the most widely used biological model for arthritis. LPS preparations are used to induce inflammatory arthritis by stimulating formation of antibodies against intra-articular tissues after local injection. Such conditions are used as experimental models of inflammation that mimic RA (35).

In inflammatory arthritis, such as rheumatoid arthritis, large numbers of polymorphonuclear leucocytes and macrophages infiltrate the joint space of the synovium. When activated, these phagocytic cells produce oxidative stresses accompanied by burst generation. The phygocytic cells also produce reactive oxygen species and hydrogen peroxide (15). There are strong evidences for the role of oxidative stress in the pathogenesis of RA (42). Epidemiologic studies have also shown an inverse association between antioxidants and RA incidences (2, 15, 20, 21, 31, 35, 41).

RA is a chronic inflammatory disorder leading to bone and cartilage destruction. PGE_2 may contribute to the pathogenesis of RA (14). Aspirin and other $NSAID_s$ are inhibitors of the synthesis of PGE_2 and are used in the treatment of related diseases that

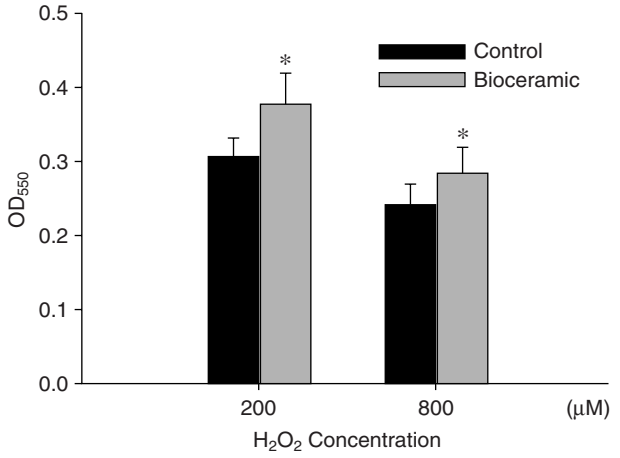

Fig. 4. Effect of bioceramic on osteoblast cell viability under H_2O_2-induced cytotoxicity (n = 5). *$P < 0.05$, significantly different compared with the control group.

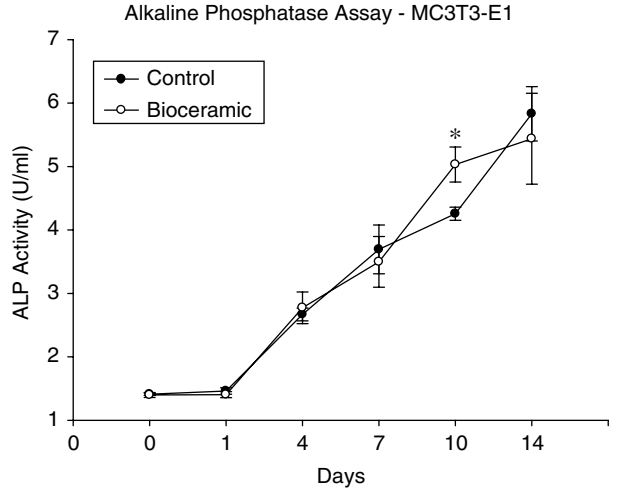

Fig. 5. Effect of bioceramic on MCF3T3E-1 cell line for alkaline phosphatase activity (n = 4). *$P < 0.05$, significantly different compared with the control group.

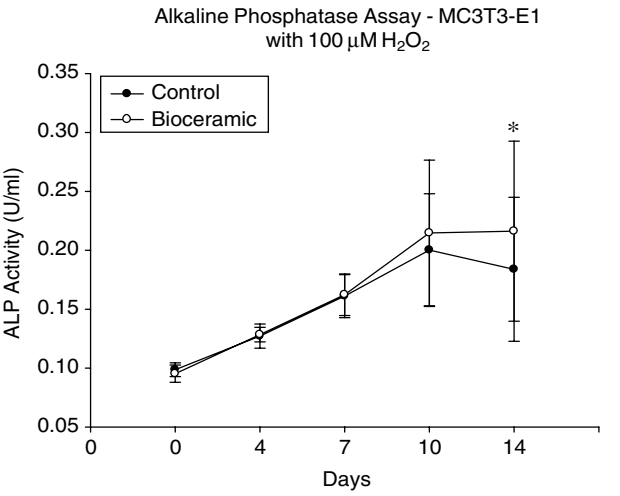

Fig. 6. Effect of bioceramic on MC3T3-E1 cell line for alkaline phosphatase activity under H_2O_2-induced oxidative stress (n = 20). *$P < 0.05$, significantly different compared with the control group.

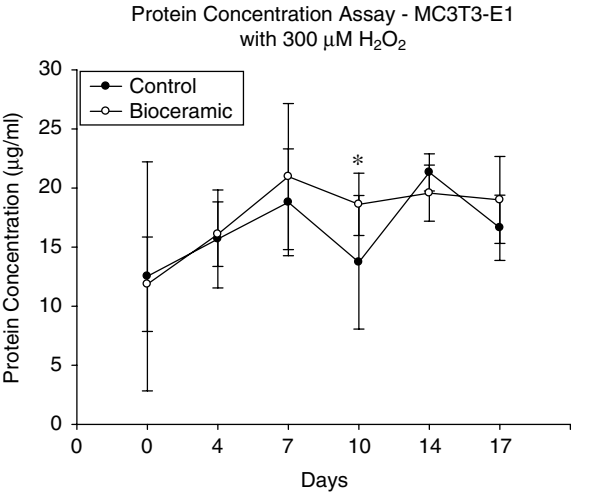

Fig. 7. Effect of bioceramic on MC3T3-E1 cell for total protein production under H_2O_2-induced cytotoxicity (n = 8). *$P < 0.05$, significantly different compared with the control group.

produce over production of PGE_2 (6, 9, 29). Thus, we established an *in vitro* model using the SW1353 cell line to determine the potential anti-inflammatory ability of bioceramic. We found a significant inhibitory effect on PGE_2 expression by LPS-stimulated SW1353. This result suggests a chondroprotective effect of bioceramic on inflammatory tissue and, subsequently, on pain relief (30). A close association exists between acute inflammation and pain sensation. The role of PGE_2 as an inflammatory mediator is also well established. A direct link also exists between the specific concentration of PGE_2 at the site of local inflammation and the pain experienced by a patient (29, 36, 37). Hydrogen peroxide-mediated oxidative stress is a major cause of cellular damage and osteoblast death, which significantly decreases bone formation and results in net bone loss. (2, 3, 5, 13, 18, 19).

Bone formation requires differentiated and active osteoblasts to synthesize the extracellular matrix that supports the mineralizing process. Cultures of the MC3T3-E1 cell line have been used as suitable *in vitro* models to study some aspects of bone formation, proliferation and differentiation patterns, representing *in vivo* osteoblast populations (12). Alkaline phosphatase and total protein production are the markers of early osteoblast differentiation and are associated with organic bone matrix synthesis before its mineralization (7), and were in measurements in this study.

In this study, we found that bioceramic did not express higher cell proliferation rates under normal circumstances. However, bioceramic increase cell viability, cell production of alkaline phosphatase activity and total protein content in osteoblasts under H_2O_2 toxicity (4, 11, 16, 26, 33). Thus, bioceramic has an anabolic effect in osteoblastic MC3T3-E1 cells. Bioceramic may, then, increase osteoblast differentiation *in vitro* and protect against oxidative stress-induced toxicity, which may promote bone recovery under inflammatory bone disease and the regenerative process (1, 10, 16, 38). Our previous published data showed bioceramic's effects on enhancement of intracellular nitric oxide and calmodulin (8, 28) which may also play the role of regulation of proliferative and differentiation of osteoblasts.

Bioceramic produced an anti-inflammatory effect on joints by reversing LPS-induced arthritis in an animal model and inhibiting PGE_2 in a cell model (34). We proved that bioceramic could scavenge H_2O_2, increase cell survival and preserve bone differentiation under H_2O_2-induced oxidative stress. Thus, bioceramic could potentially protect bone tissue in aging degeneration and the osteoporotic process. Bioceramic may help relieve the pain of acute inflammatory joint disease and help to maintain bone health.

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