Enhancement of Cell Ingrowth, Proliferation, and Early Differentiation in a Three-Dimensional Silicon Carbide Scaffold Using Low-Intensity Pulsed Ultrasound

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Concerns over the use of autografts or allografts have necessitated the development of biomaterials for bone regeneration. Various studies have been performed to optimize the cultivation of osteogenic cells using osteoconductive porous scaffolds. The aim of this study was to evaluate the osteogenic efficiency of bone cell ingrowth, proliferation, and early differentiation in a silicon carbide (SiC) porous ceramic scaffold promoted with low-intensity pulsed ultrasound. MC3T3-E1 mouse preosteoblasts were seeded onto scaffolds and cultured for 4 and 7 days with daily of 20-min ultrasound treatment. The cells were evaluated for cell attachment, morphology, viability, ingrowth depth, volumetric proliferation, and early differentiation. After 4 and 7 days of culture and ultrasound exposure, the cell density was higher in the ultrasound-treated group compared with the sham-treated group on SiC scaffolds. The cell ingrowth depths inside the SiC scaffolds were $149.2 \pm 27.3 \mu\text{m}$ at 1 day, $310.1 \pm 12.6 \mu\text{m}$ for the ultrasound-treated group and $248.0 \pm 19.7 \mu\text{m}$ for the sham control at 4 days, and $359.6 \pm 18.5 \mu\text{m}$ for the ultrasound-treated group and $280.0 \pm 17.7 \mu\text{m}$ for the sham control at 7 days. They were significantly increased, that is, 25% ($p=0.0029$) and 28% ($p=0.0008$) increase, respectively, with ultrasound radiation force as compared with those in sham control at 4 and 7 days postseeding. The dsDNA contents were $583.5 \pm 19.1 \text{ ng/scaffold}$ at 1 day, $2749.9 \pm 99.9 \text{ ng/scaffold}$ for the ultrasound-treated group and $2514.9 \pm 114.7 \text{ ng/scaffold}$ for the sham control at 4 days, and $3582.3 \pm 325.3 \text{ ng/scaffold}$ for the ultrasound-treated group and $2825.7 \pm 134.3 \text{ ng/scaffold}$ for the sham control at 7 days. There was a significant difference in the dsDNA content between the ultrasound- and sham-treated groups at 4 and 7 days. The ultrasound-treated group with the SiC construct showed a 9% ($p=0.00029$) and 27% ($p=0.00017$) increase in the average dsDNA content at 4 and 7 days over the sham control group, respectively. Alkaline phosphatase activity was significantly increased by the treatment of ultrasound at 4 ($p=0.012$) and 7 days ($p=0.035$). These results suggested that ultrasound treatment with low-intensity acoustic energy facilitated the cellular ingrowth and enhanced the proliferation and early differentiation of osteoblasts in SiC scaffolds.

Introduction

CRITICAL AND SEGMENTAL BONE DEFECTS are challenging and difficult to restore in the clinic. Autografts and allografts are the most widely used techniques to repair bone defects. However, concerns over the use of autografts or allografts have generated the need to develop biomaterials for bone replacement.¹ One of the most promising techniques is bone tissue engineering, which has advanced considerably and demonstrates a great potential for the improved regeneration of damaged bone compared to conventional therapies. Various studies have been performed to optimize the cultivation of osteogenic cells in an osteoconductive porous scaffold *in vitro*, such as the application of bone morphoge-

netic protein (BMP)² and bioreactor.³ Although these methods have been beneficial for the distribution, proliferation, and differentiation of cells in the scaffolds, the dosage of BMP or the flow rate of the bioreactor has not yet been determined.

Ultrasound has emerged as a noninvasive, relatively safe, practical, and effective treatment for fresh fracture, delayed union, nonunion, distraction, and osteogenesis, among others. Numerous clinical and experimental studies have confirmed the ability of ultrasound to enhance osteogenesis and thus facilitate bone regeneration and maturity.^{4–7} To date, low-intensity pulsed ultrasound (LIPUS) *in vivo* studies have demonstrated enhanced osteoconduction in porous implants.^{8–12} Our recent study has shown that low-intensity ultrasound can enhance matrix mineralization along with

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restoring formation of actin stress fibers under a cellular disuse osteopenia condition, indicating that acoustic wave can potentially be used as a means of countermeasure for disuse osteopenia.¹³ However, the mechanism of ultrasound on osteoblast behavior inside the pore of the porous scaffold remains unknown.

While the LIPUS passes through the scaffolds, we hypothesized that LIPUS can accelerate the migration and proliferation of osteoblasts in the 3D porous scaffolds. To evaluate the just-stated hypothesis, we evaluated the bony ingrowth and regenerative efficiency of cells into the silicon carbide (SiC) porous ceramic scaffold that has been demonstrated excellent biocompatibility.^{14,15} MC3T3-E1 mouse osteoblasts seeded onto the scaffolds were cultured for 4 and 7 days with daily 20-min ultrasound treatment and were evaluated for cell attachment, morphology, viability, ingrowth depth, proliferation, and early differentiation.

Materials and Methods

Preparation of the porous scaffolds

Porous SiC scaffolds were fabricated by the Institute of Metal Research, Chinese Academy of Sciences, and demonstrate 70–80% porosity and a pore size of 800–1000 μm (Fig. 1). Briefly, slurry mixed with carbon powder, bakelite resin, and alcohol was poured onto the polyurethane foams with structures of open cells. The excess slurry was then removed, and a slurry layer was deposited onto the surface of the polyurethane foam. The foam was dried and the process was repeated several times to obtain a sufficiently thick layer, corresponding to the apparent density required for the SiC foams. The green materials were then heated in a 99.99% nitrogen atmosphere using a pyrogenation process to obtain carbon foams. The carbon foams were then covered with slurry mixed with silicon powder and polyvinyl alcohol and dried several times. Finally, the foams were sintered at 1700°C for 0.5 h in a vacuum furnace to form the SiC foams. All of the samples were cut into disks with a 9-mm diameter and 5-mm thickness. Prior to the experiment, the samples were ultrasonically cleaned and sterilized at 121°C/0.21 MPa in an autoclave.

Cell culture and seeding

MC3T3-E1 osteoblasts (passage-16 cells at 80% confluency, which were used for all experiments) were cultured in media. The culture medium consisted of alpha-minimum essential medium (α -MEM; Gibco, Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco, Life Technologies) and 1% penicillin–streptomycin (Gibco, Life Technologies). The cells were maintained as subconfluent monolayers in vessels under standard conditions (95% humidity, 5% CO_2 , and 37°C). The cells were detached using a 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) solution and resuspended at a concentration of 1×10^7 cells/mL.

The scaffolds were prewetted in culture media for 4 h using vacuum equipment prior to cell seeding. Twenty microliters of cell suspension containing 2×10^5 cells was seeded onto the surface of each scaffold in the six-well plates (Corning Incorporated, Corning, NY) and incubated for 2 h. Next, 6 mL of media was added to each well, and the seeded scaffolds were further incubated for 24 h to enable firm attachment.

After 24 h of static culture, the samples in which the cells fell off were discarded, and the remaining samples were used in this study; 1-day samples (just after 24 h of static culture without ultrasound or sham stimulation) were harvested, and the other samples were placed into the fresh six-well plates for ultrasound or sham stimulation. Media were refreshed every 2 days. The samples were then harvested at 4 and 7 days ($n = 11$ for each type of scaffold, for each group at each time point; three scaffolds were used for the viability assay, three scaffolds were used for the DNA content assay, and the remaining five were used for the alkaline phosphatase [ALP] activity assay).

LIPUS stimulation

Ultrasound stimulation was applied using the Sonicator[®] 740 (Mettler Electronics[®] Corp., Anaheim, CA) with a gel-coupled planewave US applicator (ME 7410).¹⁶ The ultrasonic treatment group was treated with a sinusoidal ultrasound pulse of 1 ms in length and 1 MHz in frequency. The repetition frequency was 100 Hz with spatial-averaged and temporal-averaged intensities of 30 mW/cm². The daily exposure

FIG. 1. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) of the silicon carbide (SiC) porous scaffold. The porosity of the SiC scaffold is averaged at 70–80% and the pore size is 800–1000 μm . The XRD spectrum shows that the sample is composed of hexagonal phase SiC. This is consistent with the crystal morphology of the inset figure of the SEM image. Color images available online at www.liebertpub.com/tea

time was 20 min for 3 and 6 days for each culture. The samples were kept culturing for 24 h and harvested at 4 and 7 days. The sham control group was treated in the same manner, but with the ultrasound generator turned off. Ultrasound gel (Sonic Relief, Miami, FL) was used as a coupling medium to completely fill in the gap between the transducer and the plate to avoid reflection or attenuation of the ultrasound by air bubbles. On the basis of the manufacturer's protocol, the effective radiating area of the applicator was 10 cm²; thus, the entire well (~9.51 cm²) was contained within the ultrasound-signaling field. The six-well plate used in this experiment consisted of flat and uniform polystyrene with a low acoustic attenuation coefficient (0.13 dB/cm at 1 MHz). Thus, its effect on ultrasound propagation was negligible. The attenuating effect of the cell culture media was also negligible due to its low attenuation coefficient.

Cell attachment and morphology

The cell morphology on the SiC scaffolds was examined at 4-day postseeding using scanning electron microscopy (SEM; HITACHI S-3400N, Tokyo, Japan). Each scaffold was washed with phosphate-buffered saline (PBS) and fixed with 2% glutaraldehyde in PBS. Next, the scaffolds were dehydrated with a graded series of ethanol (60%, 70%, 80%, 95%, and 100%), dried at the critical point, mounted onto an aluminum stub, and sputter coated with gold-palladium. All samples were examined at 20-kV accelerating voltage.

Viability assay

Cell viability and the depth of ingrowth were measured using calcein acetoxymethyl ester (AM) staining (Molecular Probes, Life Technologies Corp., Grand Island, NY). In live cells, nonfluorescent calcein AM is converted into green-fluorescent calcein following acetoxymethyl ester hydrolysis by intracellular esterases. Briefly, at the indicated time points (1, 4, and 7 days), the constructs were washed with DPBS (Gibco, Life Technologies) three times. Next, the samples were incubated in working solution containing 2 μ M calcein AM for 45 min. After staining, fluorescence images of the stained samples were obtained using an Olympus DSU spinning disk confocal microscope (Olympus Corp., San Diego, CA) with SlideBook 4.2 software and a 10 $\times$ objective. Optical sectioning was achieved at 3- μ m thickness along the z -axis of the scaffolds, and 100 consecutive slices of the captured images were reconstructed into 3D images. The final depth of the cell ingrowth in the scaffolds was determined by the offset, which was the total distance traveled from top to bottom, where the cells were occupied.¹⁷ The offset number could be read in the Focus Window. Nine views were chosen for measurement from each sample ($n=3$), and because the depths in each of these views varied, the average product was used as the depth for the sample.

DNA content assay

Osteoblast proliferation was measured using the DNA content assay. The amount of DNA in the cells attached to the scaffolds was determined using Quant-iTTM PicoGreen[®] dsDNA Reagent and Kits (Molecular Probes, Life Technologies Corp.) according to the manufacturer's instructions. At the indicated time points (1, 4, and 7 days), the scaffolds were rinsed with PBS three times and submerged in 1 mL of lysis buffer containing 10 mM Tris (pH 8), 1 mM

EDTA, and 0.2% (v/v) Triton X-100. To release the DNA, the samples were vortexed for 10 s every 5 min for a total of 30 min and were kept on ice throughout the entire process. The samples were stored at -80°C until further analyses were performed. The samples were thawed on ice, and homogenized 10–15 times using a 21-gauge needle.¹⁸ Next, 100 μ L of the sample was mixed with 100 μ L of DNA-binding fluorescent dye solution, and the fluorescence intensity was measured at an excitation wavelength of 480 nm and an emission wavelength of 520 nm using the Tecan Infinite 200 Microplate Reader supported by the i-controlTM software (Tecan Deutschland GmbH, Crailsheim, Germany).¹⁹ Lambda DNA was used for the standard curve to calculate the amount of DNA. The samples ($n=3$) were prepared in triplicate.^{20,21}

ALP activity assay

ALP is secreted by matured osteoblasts. The ALP expression is generally associated with osteoblastic differentiation, and the level of ALP activity is an indication of the stage of osteoblastogenesis. At the indicated time points (1, 4, and 7 days), the scaffolds were rinsed with PBS three times and the cells were lysed with 0.2% Triton X-100. They were then briefly vortexed and centrifuged at 12,000 rpm for 5 min. The clear supernatant was used to measure ALP activity, which was determined using an ALP activity assay kit (KeyGEN Biotech, Nanjing, China). Total protein concentration of the supernatant was measured using a bicinchoninic acid protein assay kit (Dingguo Changsheng Biotech, Beijing, China). ALP activity was then calculated according to the formula, that is,

ALP (U/gprot) = [(absorbance of the measuring tube/absorbance of the standard tube) $\times$ amount of phenol in standard tube (0.003 mg)]/amount of the total protein in each sample (g).

Measurement of estimation of regional acoustic force

Acoustic radiation pressure was verified by using a capsule-type hydrophone (HGL-0200; ONDA Corp., Sunnyvale, CA) to measure the pressure from the surface of the ultrasound transducer along the z directions.

To calculate the shear stress experienced by the cells, we first employ Darcy's law to calculate the flux:

$$q = \frac{-k}{\mu} \nabla P.$$

Where k is the intrinsic permeability of the cell culture medium; 0.4×10^{-9} m² was chosen from the value reported by Grimm and Williams.²² μ is the viscosity and ∇P is the pressure gradient. Then τ , shear stress, is calculated using the following equation reported by Wang and Tarbell.²³

$$\tau = \frac{\mu(q/A)}{\sqrt{R}}$$

Where A is the cross-sectional area of the scaffold (considering the porosity factor).

Statistical analysis

Each experiment was repeated using three samples. The data were presented as the mean $\pm$ SD of three replicates and

FIG. 2. SEM images of MC3T3-E1 osteoblasts at 4 days postseeding on SiC porous scaffolds. The increased cell density was more evident on the SiC scaffolds with ultrasound radiation (**a, c**) compared with sham control (**b, d**). Scale bars represent 50 μm for magnification of 1000 $\times$ and 200 μm for magnification of 200 $\times$.

FIG. 3. Images of MC3T3-E1 osteoblasts cultured on SiC porous scaffolds at 1, 4, and 7 days and stained with calcein AM. An increased cell density was observed over time. And the cell density was higher in the ultrasound-treated group compared with the sham-treated group at 4 and 7 days. Scale bars represent 50 μm . AM, acetoxymethyl ester. Color images available online at www.liebertpub.com/tea

evaluated using Student's *t*-test. The level of statistical significance was defined at $p < 0.05$.

Results

Cell attachment and morphology

After 4-day postseeding, MC3T3-E1 osteoblasts formed long spindles or flat polygons with distinct pseudopodia. They extended and grew inside the pores on the scaffolds (Fig. 2a–d). The increased cell density was more evident on the SiC scaffolds with ultrasound radiation compared with the sham radiation. The cells connected to each other and formed a sheet on the surface of the SiC scaffolds in the ultrasound-treated group (Fig. 2a, c).

Cell viability

After 1, 4, and 7 days of culture, live cells were stained with calcein AM dye (Fig. 3). From the Figure 3 and the reconstructed 3D images (Fig. 4) taken from the center of the sample surface, (i) in addition to the increased cell density, most live cells were spatially distributed along the pore wall from the surface to the inside via interconnected channels over time, and (ii) after 4 and 7 days in culture, the cell density was higher in the ultrasound-treated group compared with the sham-treated group, as indicated by the larger area of the green fluorescence. From the side view of Figure 4, the cell ingrowth depths inside the SiC scaffolds were $149.2 \pm 27.3 \mu\text{m}$ at 1 day, $310.1 \pm 12.6 \mu\text{m}$ for the ultrasound-treated group and $248.0 \pm 19.7 \mu\text{m}$ for the sham

FIG. 4. Reconstructed 3D images of MC3T3-E1 osteoblasts cultured on SiC porous scaffolds at 1, 4, and 7 days and stained with calcein AM. Scale bars represent $100 \mu\text{m}$. T and S represent the top view and side view, respectively. The higher cell density and ingrowth depth were observed in the ultrasound-treated group compared with the sham-treated group at 4 and 7 days. Color images available online at www.liebertpub.com/tea

FIG. 5. The cell ingrowth depth of the SiC scaffold with ultrasound or sham simulation at different time points. The ultrasound-treated group for the SiC construct showed a 25% ($p=0.0029$) and 28% ($p=0.0008$) increase in the cell ingrowth depth at 4 and 7 days, respectively, compared with the sham control group. Color images available online at www.liebertpub.com/tea

control at 4 days, and $359.6 \pm 18.5 \mu\text{m}$ for the ultrasound-treated group and $280.0 \pm 17.7 \mu\text{m}$ for the sham control at 7 days. They were significantly increased, that is, 25% ($p=0.0029$) and 28% ($p=0.0008$) increase, respectively, with ultrasound radiation force as compared with those in sham control at 4 and 7 days postseeding (Fig. 5).

DNA content

Cell proliferation on the SiC scaffolds was quantified using dsDNA content. The dsDNA contents were $583.5 \pm 19.1 \text{ ng/scaffold}$ at 1 day, $2749.9 \pm 99.9 \text{ ng/scaffold}$ for the ultrasound-treated group and $2514.9 \pm 114.7 \text{ ng/scaffold}$ for the sham control at 4 days, and $3582.3 \pm 325.3 \text{ ng/scaffold}$ for the ultrasound-treated group and $2825.7 \pm 134.3 \text{ ng/scaffold}$ for

FIG. 6. DNA content of the SiC porous scaffold at 1, 4, and 7 days. The ultrasound-treated group for the SiC construct showed a 9% ($p=0.00029$) and 27% ($p=0.00017$) increase in the average dsDNA content at 4 and 7 days, respectively, compared with the sham control group. Color images available online at www.liebertpub.com/tea

FIG. 7. Alkaline phosphatase (ALP) activity of the SiC scaffold at 1, 4, and 7 days. There was a significant difference between the ultrasound- and sham-treated groups at 4 ($p=0.012$) and 7 days ($p=0.035$). Color images available online at www.liebertpub.com/tea

the sham control at 7 days. There was a significant difference in the dsDNA content between the ultrasound- and sham-treated groups at 4 and 7 days. The ultrasound-treated group with the SiC construct showed a 9% ($p=0.00029$) and 27% ($p=0.00017$) increase in the average dsDNA content at 4 and 7 days over the sham control group, respectively. In both the ultrasound- and sham-treated groups, the speed of the increased dsDNA content at the 1–4-day period was faster compared with the 4–7-day period (Fig. 6).

ALP activity

The ALP activity was $18.57 \pm 1.58 \text{ U/gprot}$ at 1 day, $43.84 \pm 4.76 \text{ U/gprot}$ for the ultrasound-treated group and $35.11 \pm 1.44 \text{ U/gprot}$ for the sham control at 4 days, and $47.59 \pm 2.7 \text{ U/gprot}$ for the ultrasound-treated group and $43.17 \pm 2.82 \text{ U/gprot}$ for the sham control at 7 days. There was a significant difference between the ultrasound- and sham-treated groups at 4 ($p=0.012$) and 7 days ($p=0.035$) (Fig. 7).

FIG. 8. Acoustic radiation pressure along the z -axis direction from the transducer surface. Peak value of acoustic radiation pressure of 140 kPa was measured within the range between 1 and 60 mm from the surface of the transducer.

Measurement and calculation for acoustic force

As shown in Figure 8, from 1 to 60 mm to the surface of the transducer, the highest recorded pressure was 140 kPa. Shear stress was calculated at 3.14 dyne/cm².

Discussion

We chose to use porous SiC as a scaffold because porous SiC ceramic has been demonstrated to be a potential bone-replacement material for large-area bone defects due to its stable physical and chemical properties, appropriate mechanical strength, controllable 3D microstructure, and biocompatibility.^{14,15} It has been also confirmed that SiC with 800–1000 µm pore size had good osteogenic and osteoconductive properties, and its promotion of adhesion, proliferation, and differentiation of primary culture osteoblasts in our previous research.^{24–26} We used the same materials without simulating the natural bone.

From the side view of 3D image, the depth only goes to 300 µm because the maximum offset of *z* travel for 3D image is 300 µm using SlideBook 4.2 software. This is the limitation of this study. However, the uniformity of cell density in the treatment group implied that the cell viability was enhanced by the ultrasound treatment. We found the clear differences between the experiment group and the control within the depth of 300 µm. Certainly, future testing using more advanced technology would help to further validate the results. We did plan to test whether there are cells in the center of the constructs in our future experiments.

Among various assays for assessing cultured cell proliferation, which often include measurement of metabolic activity (tetrazolium salts and alamarBlue), DNA quantification using fluorophores (Hoechst 33258 and PicoGreen), uptake of radioactively-labeled DNA precursors ([³H]thymidine), and manual cell counting (hemocytometer), it has been suggested that measuring the amount of DNA using PicoGreen assay is the most reliable strategy for quantifying cell proliferation in 3D cultures.^{20,21,27} In this study, the amount of DNA in the MC3T3-E1 osteoblasts attached to the scaffolds was determined using Quant-iT PicoGreen dsDNA Reagent and Kits, which indicated that LIPUS increased the proliferation of osteoblasts in SiC scaffolds. As far as the influence on the fluorescence intensity resulting from the dead cells was concerned, we found that there were several dead cells on the constructs of all the groups by the live/dead viability assay in our preliminary experiments. We thought that refreshing media every 2 days during cell culture on the scaffolds and rinsing the constructs with PBS three times prior to the assay (to remove or dilute serum esterase activity that could cause some increase in extracellular fluorescence by hydrolyzing calcein AM according to the manufacturer's instructions) might wash away most of the dead cells. In the PicoGreen assay, the constructs were rinsed with PBS three times before being submerged in the lysis buffer, so the number of dead cells on the scaffolds was insignificant relative to the live cells. Because of the aforementioned reasons, we did not take into account the impact of the dead cells on the results. The study was mainly focused on the live cell counting and analysis. In addition, in a parallel study, we have examined the effects of ultrasound signal, which was exactly the same setting as the one used in the current study, on osteoblastogenesis with focusing on cellular collagen contents,

proliferation, and mineralization.^{13,28} We were able to demonstrate the effects of high-frequency acoustic wave signals on several genetic markers of osteoblasts in a simulated microgravity (SMG) environment. The results indicated that LIPUS is capable to mitigate the detrimental effects generated by SMG. The LIPUS-induced changes were analyzed using the MTS assay for proliferation, Phalloidin for F-actin staining, Sirius red stain for collagen, and alizarin red for mineralization. The data showed that LIPUS stimulation significantly increased the rate of proliferation (~24%, *p* < 0.05), collagen content (~52%, *p* < 0.05), and matrix mineralization (~25%, *p* < 0.001) along with restoring formation of actin stress fibers in the SMG-exposed osteoblasts, in comparison with the untreated control. These data provided additional information parallel with current study, and suggested that the acoustic wave could potentially be used as a promoter for osteogenesis in tissue regeneration.

The results obtained in this study suggested that LIPUS increased the depth of the ingrowth, and enhanced the proliferation and early differentiation of the osteoblasts in the SiC scaffolds *in vitro*. The aforementioned results were similar to the animal studies by Iwai *et al.*, who demonstrated that LIPUS had a positive effect on the rate and extent of bone growth into porous implants *in vivo*.^{8–12} In our study, the highest recorded acoustic pressure was 140 kPa, which fell within the range reported in Kaya *et al.*,²⁹ and shear stress was calculated at 3.14 dyne/cm², comparable with and under the theorized physiological range for bone cells, 8–30 dyne/cm² for physiological loading magnitudes,³⁰ which meant (i) the absorbed energy might induce the osteoblasts in the scaffolds to initiate biochemical processes that played a critical role in accelerating bone formation. As we know, these processes included the increased production of prostaglandin E2 via the induction of cyclooxygenase-2 mRNA, ALP, osteopontin, and transforming growth factor-beta (TGF-β) synthesis in osteoblasts.^{31–34} (ii) Shear stress provided a convenient way to direct mechanical stimulation on to the cells, which was beneficial for the proliferation, differentiation, and generation of extracellular matrix of bone cells.³⁵ We considered that the aforementioned factors contributed to increased ingrowth depth and proliferation of osteoblasts in SiC scaffolds in this study.

Ultrasound exhibits wave propagation in the tissues, which can be reflected, refracted, scattered, or absorbed by the porous scaffold structure. The effect of ultrasound on the porous scaffolds is complex, which may relate to the composition, 3D geometric structures, and microstructure on the surface of the materials, resulting in the variable biological behavior of the cells on the scaffolds with ultrasound. The true mechanism requires further exploration.

Conclusions

Low-intensity ultrasound facilitates ingrowth, and enhances the proliferation and early differentiation of osteoblasts in the SiC scaffolds.

Acknowledgments

This study was kindly supported by the National Institute of Health (R01 AR52379, AR49286, and 61821), National Space Biomedical Research Institute through the NASA Cooperative Agreement NCC 9-58, NYSTAR, and US

Army Medical Research. The authors are grateful to Prof. Jinsong Zhang at the Institute of Metal Research, Chinese Academy of Sciences, for his support, technical assistance, and discussion. The authors also appreciate Dr. Jesse Muir for his assistance for the article.

Disclosure Statement

No competing financial interests exist.

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Received: September 20, 2013

Accepted: June 13, 2014

Online Publication Date: July 23, 2014