Enhancement of osteogenesis by poly(lactide-co-glycolide) sponges loaded with surface-embedded hydroxyapatite particles and rhBMP-2

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Abstract: The bone mesenchymal stem cells (BMSCs) were seeded on [poly(lactide-co-glycolide) scaffolds with hydroxyapatite (HA) coating, and “s” stands for surface] (PLGA/HA-S), PLGA/HA-M (containing the same HA amount in the matrix as that of the PLGA/HA-S and “m” stands for matrix), and PLGA scaffolds, which were then cultured in a medium-containing Escherichia coli-derived recombinant human bone morphogenetic protein-2 (ErhBMP-2). In vitro culture of rat BMSCs found no different cell morphology in all the scaffolds, but the alkaline phosphatase activity and osteogenic gene expression of type I collagen (COL I) and osteocalcin (OCN) in the PLGA/HA-S scaffolds were always highest and were significantly improved in comparison with those in the PLGA scaffolds. In a rat calvarial defect model, new bone formation was enhanced in the PLGA/HA-S/ErhBMP-2 implants at 4 and 8 weeks after implantation too. Therefore, the PLGA/HA-S scaffold can better enhance the ErhBMP-2-induced osteogenic differentiation of BMSCs in vitro and osteogenesis in vivo. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 100B: 1103–1113, 2012.

Key Words: poly(lactide-co-glycolide), hydroxyapatite, bone morphogenetic protein, BMSCs, bone regeneration


INTRODUCTION
Scaffolds play central roles in regenerative medicine and tissue engineering, which promote tissue regeneration by directing cellular behaviors and functions. Enhancement of scaffold bioactivity by surface modification is a major focus. In particular, improvements in the cell-material surface interactions by surface modification appear highly promising to facilitate the bone-healing process.1–4 As the main inorganic component of bone, hydroxyapatite (HA) has been shown to promote bone healing and regeneration due to its good osteoinductivity, osteoconductivity, and osteointegration.5–7 The traditional fabrication method obtains polymer/HA nanoparticles composite scaffolds with uniform distribution of HA particles in the polymer matrix,8–10 implying that most HA particles cannot directly interact with bioactive molecules such as cell growth factors especially at the very earlier stage before the degradation of the polymer matrix. Therefore, these HA particles are more likely used as reinforcing agent but not bioactive additives. On the contrary, HA coating on the pore walls of scaffolds could improve the interactions between osteoblast cells and polymeric scaffolds.11 Using the HA-coated paraffin spheres obtained by “Pickering emulsion” as the porogens, the poly(lactide-co-glycolide) (PLGA) scaffolds embedded spatially with HA particles on pore walls (PLGA/HA-S) have been fabricated by a porogen-leaching method, which has shown to stimulate osteoblast differentiation of bone mesenchymal stem cells (BMSCs) in dexamethasone (Dex) containing medium, compared to PLGA/HA-M (HA nanoparticles dispersed in PLGA matrix) and PLGA scaffolds.12

As one of the glucocorticoids, Dex increases the capacity to form mineralized bone nodules in vitro but suppresses bone-forming cells in vivo and induces osteoporosis.13,14 Bone morphogenetic proteins (BMPs), on the other hand, are most effective in inducing bone morphogenesis and have been widely studied and used in bone repair.15–18 Accordingly, studies of the influence of scaffolds on osteogenic differentiation of BMSCs in BMP-containing medium are necessary. Most biologically active rhBMP-2 was produced in mammalian Chinese hamster ovary cells (C-rhBMP-2). This eukaryotic protein expression system results in the shortcomings of the low yield and high cost in rhBMP-2.
production. As the development of recombinant DNA technology, bioactive rhBMP-2 can also be generated in large quantity from *Escherichia coli* (ErhBMP-2),\(^{19,20}\) yet their bioactivity especially in combination with scaffolds needs to be substantiated. Therefore, in this study, attention shall be paid to the osteogenic differentiation of BMSCs on PLGA/HA-S, PLGA/HA-M, and PLGA scaffolds in ErhBMP-2-containing medium and osteogenesis of scaffolds/ErhBMP-2 implants in rat calvarial defects. The aims of this study are (i) to evaluate whether PLGA/HA-S scaffolds enhance the osteogenic differentiation of rat BMSCs in ErhBMP-2-containing medium and (ii) to determine if PLGA/HA-S scaffolds combined with ErhBMP-2 more efficiently induces new bone formation in rat calvarial defects than PLGA/HA-M and PLGA scaffolds.

**MATERIALS AND METHODS**

**Materials**

PLGA (75/25 lactide/glycolide, \(M_w = 78.5 \text{ kDa}, M_m = 132.4 \text{ kDa}\)) was purchased from China Textile Academy. Poly(vinyl alcohol) with a molecular weight of 25 kDa, paraffin (melting point 53–56°C), 1,4-dioxane, hexane, and ethylene diamine tetraacetic acid (EDTA) disodium salt were obtained from Shanghai Chemical Industries Co. Fluorescein diacetate (FDA) was obtained from Sigma. *Escherichia coli*-derived recombinant human BMP-2 (ErhBMP-2) was obtained from Shanghai Rebone Biomaterials Co. (Shanghai, China). Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) were obtained from GIBCO. Millipore water was used throughout the study. All other reagents were of analytical grade and used as received.

**Preparation and characterization of PLGA/HA-S, PLGA/HA-M, and PLGA scaffolds**

PLGA/HA-S scaffolds were fabricated by a porogen-leaching method. HA-coated paraffin spheres obtained by “Pickering emulsion” were used as the porogens. HA nanoparticles (20–40 nm in width and 100–300 nm in length) were synthesized according to previous report.\(^{21}\) To fabricate HA nanoparticles-coated paraffin spheres, 0.02 g of the HA nanoparticles was dispersed uniformly in 10 mL water, into which 1 g paraffin wax was added at 70°C. After the emulsion was stirred at 500 rpm for 30 min, it was cooled to room temperature to solidify the paraffin microspheres. The paraffin spheres were washed with water to remove those weakly attached HA nanoparticles. The sieved HA-coated paraffin spheres (450–600 μm) were added into a Teflon mold and tapped gently, which was then incubated in an oven at 45°C for 30 min to form a loosely bound spheres assembly. After being cooled to room temperature, about 1 mL 12% PLGA/1,4-dioxane solution was cast dropwise onto the HA-coated paraffin spheres assembly. The system was subsequently frozen at −25°C for 2 h and followed by freeze-drying to remove 1,4-dioxane. The PLGA/HA porous scaffolds (PLGA/HA-S) were finally obtained by leaching the paraffin assembly in 400 mL hexane at 37°C for 2 days, with changing the hexane every 4 h. By this method, the HA nanoparticles (2% determined by thermal gravimetric analysis) were spatially embedded on pore walls of PLGA/HA-S scaffolds but not buried in the matrix.

For comparisons, pure PLGA scaffolds and PLGA/HA-M scaffolds containing the same HA amount (2%) in the matrix as that of the PLGA/HA-S were also prepared according to the method reported previously.\(^{22}\) To prepare the PLGA/HA-M scaffolds (HA nanoparticles were buried in the PLGA matrix), the HA nanoparticles were premixed with 12% PLGA/1,4-dioxane solution. By this method, the HA particles dispersed evenly in the PLGA matrix of PLGA/HA-M scaffolds. The pure PLGA scaffolds were prepared by using paraffin spheres as the porogens. The pore size of all scaffolds is between 450 and 600 μm.

The microstructure and pore morphology of the scaffolds were characterized by scanning electron microscopy (SEM, FEI SIRION100). The compressive strength and modulus of the scaffolds (10 mm in height and 5 mm in diameter) were measured using a Universal Testing Machine (Instron 5543A) at a crosshead speed of 2 mm/min. Calcium ion release from PLGA/HA-S and PLGA/HA-M scaffolds was assessed by immersing the samples in phosphate-buffered saline (PBS) with a pH of 7.4 at 37°C. After the scaffolds (Φ5 × 2 mm) were sterilized in 70% ethanol for 30 min, they were washed with PBS for three times to remove the residual ethanol, each for 30 min. Scaffolds were placed in sterile Falcon tubes containing 1 mL of PBS and incubated at 37°C for 14 days. The calcium ion in PBS was detected by Hitachi 180-50 atomic absorption spectrometer.

**Morphology and alkaline phosphatase activity of BMSCs on scaffolds**

Rat bone marrow stem cells (BMSCs) were isolated from bone marrow of young adult male Sprague–Dawley rats as described previously.\(^{23}\) The procedures were performed in accordance with the guidelines for animal experimentation by the Institutional Animal Care and Use Committee, Zhejiang University. The isolated BMSCs were maintained and expanded for an additional passage in growth medium-containing low-glucose DMEM supplemented with 10% FBS, 100 μg/mL penicillin, and 100 U/mL streptomycin.

The scaffolds (Φ5 × 2 mm) were sterilized in 70% ethanol for 30 min and washed with PBS (Invitrogen) thrice (30 min each) to remove the residual ethanol. Into each scaffold placed in a 24-well plate, 1 × 10\(^5\) BMSCs were seeded. After 24 h, the growth medium was changed to ErhBMP-2-containing medium (high-glucose DMEM containing 10% FBS, 50 mM ascorbic acid 2-phosphate, 10 mM β-glycerophosphate, and 50 ng/mL ErhBMP-2). The medium was changed every 2 days.

After incubation for 7 days, the constructs were fixed in 2.5% glutaraldehyde and then dried by a CO\(_2\) critical-point drying method for SEM observation. The cell morphology was also investigated by confocal microscopy afterFDA staining.\(^{24}\)

On days 3, 7, and 14, the constructs were removed and washed with PBS. The cells on the scaffolds were homogenized in 500 μL of Millipore water with 0.05% Triton X-100. Supernatant was collected for alkaline phosphatase
(ALP) assay according to the manufacturer’s instructions. The amount of ALP in the cells was normalized against reaction time and total protein content. The total protein content was measured by a protein assay kit (KeyGEN Biotechnology, Nanjing, China). Three replicate samples were used for the assay (n = 3).

Quantitative reverse transcription-polymerase chain reaction analysis

The effect of scaffolds on BMSC osteogenic differentiation was further assessed by real-time quantitative RT-PCR (RT-qPCR) to measure the mRNA expression of type I collagen (COL I, NM_053304) and osteocalcin (OCN, NM_013414) (Table I). The primer sequences specific for the target genes and the internal control gene [glyceraldehyde-3-phosphate dehydrogenase (GAPDH), NM_017008] used for qRT-PCR were listed in Table I. Onto each scaffold (5 mm in diameter) in parietal bone using a slow speed dental drill. Constant saline irrigation was applied, and the dura mater was kept intact during the procedure. After placement of the scaffold/ErhBMP-2 implants into the defects, the skin was closed by suturing.

Microcomputed tomography analysis

The rats were euthanized at 4 or 8 weeks after implantation. After the block samples including the surgical sites were removed and fixed in 4% paraformaldehyde at 37°C for 48 h, they were scanned using a micro-CT system (μCT-80; Scanco Medical AG) in a high-resolution scanning mode (a voxel size and slice thickness of 20 μm). Three-dimensional images were reconstructed, and the volume of the regenerated bone within the defects was calculated using its auxiliary software (Scanco Medical AG). Three samples in each group were harvested for the micro-CT analysis after implantation for 4 and 8 weeks, respectively.

Histology and histomorphometry

After μCT measurement, the specimens were decalcified in 10% EDTA for 3 weeks at room temperature and dehydrated through ascending alcohol gradients and embedded in paraffin. Longitudinal sections were stained with hematoxylin and eosin (H&E) and Masson’s trichrome, respectively. The stained sections were visualized by an optical microscope (Digital Camera DXM2000F, Nikon, Japan). Semi-quantitative image analysis was used to estimate the regenerated bone within the calvarial defect after implantation for 4 and 8 weeks, respectively, by using the automated image analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD). Each H&E-staining image was equalized and converted into image format (TIFF) suitable for the measurement of area. Areas of newly formed bone were delineated manually and then quantified. The bone formation ratios were quantified from calculating newly formed bone area divided by the total area available for bone in growth. The results were reported as a percentage of bone volume per tissue volume (% BV/TV). Six samples per group at each time point were used for analysis.

Statistics

Data are expressed as mean ± standard deviation (SD). The statistical significance of the differences among the experimental groups was evaluated by a one-way analysis of variance (ANOVA). All statistical computations were performed using SPSS version 16.0 (SPSS, Chicago). The significant level was set as p < 0.05.

### Table I. Nucleotide Primers Used for qRT-PCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Oligonucleotide Sequence (5’–3’)</th>
<th>Product size (bp)</th>
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<tr>
<td>Rat collagen type I</td>
<td>Forward: 5’ACCTCGGCTCCTGCTCTTCTTAG 3’ Reverse: 5’GACAGCAGCCAGGCGGCTTCTG 3’</td>
<td>234</td>
</tr>
<tr>
<td>Rat osteocalcin</td>
<td>Forward: 5’CTACCTCTGCTGCTCCTCTGAC 3’ Reverse: 5’CACCTTTACTGCCCCCTGCTTTC 3’</td>
<td>111</td>
</tr>
<tr>
<td>Rat-GAPDH</td>
<td>Forward: 5’GGTGAGCCTCTATGCGGCTACAT 3’ Reverse: 5’GCTCTCTCTTTCGCTCAGTATCCT 3’</td>
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</tr>
</tbody>
</table>
RESULTS

The scaffolds

As shown in Figure 1, PLGA/HA-S, PLGA/HA-M, and PLGA scaffolds had a similar macroporous structure with a pore size of 450–600 μm and a porosity of >90% measured by ethanol inhalation. Unlike their counterparts [Figure 1(e,f)], the inner pore surface of PLGA/HA-S scaffolds was covered densely by needle-like HA nanoparticles [Figure 1(d)] due to the use of HA-covered paraffin spheres as the porogens. As reported in previous report, the compressive modulus of PLGA/HA-S scaffold is about 3.2 MPa, larger than PLGA/HA-M and PLGA scaffolds. About 9.9 and 9.1 μM of Ca²⁺ were detected for PLGA/HA-S and PLGA/HA-M scaffolds, respectively, after immersing in PBS for 14 days. The unique strategy is thus successful to expose the HA particles on the pore surface of the PLGA/HA-S scaffolds, but not bury them inside the matrix as traditionally reported in the composite scaffolds like PLGA/HA-M. The difference in surface chemical compositions, such as polymer in PLGA/HA-M and inorganic particles in PLGA/HA-S, will inevitably bring different biological performance in terms of BMSCs differentiation and osteogenesis, because only those exposed HA particles can actively participate in the bone-related events during the initial stage. For example, the exposed HA particles are expected to adsorb more bone-related cell growth factors or better maintain their activity, so that better bone regeneration can be achieved compared to their counterparts.

In vitro BMSCs culture on the scaffolds

Seven days after cell seeding, the SEM images showed that the BMSCs attached and fully spread on the pore surfaces of all the scaffolds [Figure 2(a–c)]. Confocal microscopy images confirmed that the cells mainly distributed on the pore walls of the scaffolds [Figure 2(d–f)]. No noticeable difference in cell morphology was observed among the cells on these three types of scaffolds.

The ALP activity gradually increased in all three scaffolds (Figure 3). Although no significant difference was found at 3 days, significantly higher ALP activity was found for the PLGA/HA-S scaffolds at 7 and 14 days compared to the PLGA scaffolds ($p < 0.05$). However, no significant difference ($p > 0.05$) was found between the PLGA/HA-S and PLGA/HA-M scaffolds, although the former had a higher value of ALP activity.

Expression of genes associated with the osteoblastic differentiation (COL I and OCN) was examined using real-time PCR among the three groups. Repeated experiments showed that the expression levels of these factors were consistently higher in the cells cultured on the PLGA/HA-S scaffolds than those in the cells cultured on the PLGA/HA-M and PLGA scaffolds [Figure 4(a,b)]. There was significant difference between the type-I collagen and OCN mRNA levels on PLGA/HA-S (or PLGA/HA-M) scaffolds and PLGA scaffolds ($p < 0.05$) [Figure 4(a,b)]. The gene expression level of type-I collagen in the PLGA/HA-S scaffolds was significantly higher than that in the PLGA/HA-M scaffolds ($p < 0.05$), but no significant difference was observed for the OCN.

Calvarial defect implantation

As determined by μCT, bone-healing efficacy was increased along with the sequence of PLGA/ErhBMP-2, PLGA/HA-M/ErhBMP-2, and PLGA/HA-S/ErhBMP-2 (Figure 5). At 4 weeks, the PLGA/HA-S/ErhBMP-2 implants demonstrated clear defect bridging from the host bone [Figure 5(a)], stimulating more new bone formation compared to the PLGA/HA-M/ErhBMP-2 [Figure 5(b)] and PLGA/ErhBMP-2 implants [Figure 5(c)]. After 8 weeks, new bones became denser in all the defects [Figure 5(d–f)], but, apparently, the PLGA/HA-S/ErhBMP-2 had the minimum volume of...
remained bone defect [Figure 5(d)]. Quantitative analysis [Figure 5(g)] showed that the new bone volume in the PLGA/HA-S/ErhBMP-2 implants was significantly higher than that in the PLGA/ErhBMP-2 implants at 4 and 8 weeks ($p < 0.05$). No significant difference was found between PLGA/HA-M/ErhBMP-2 and PLGA/ErhBMP-2 implants ($p > 0.05$).

Representative H&E staining confirmed defect bridging from the host bone (Figure 6). After 4 weeks, the new bone (marked as B) has grown into the pores of the scaffolds. Many osteocytes (marked as black arrow) were trapped in the lacunae. The new bone regenerated within the calvarial defects was directly adjacent to the scaffolds (osteointegration) with minimal fibrous layer (marked as F). The scaffolds (marked as S) had been degraded significantly but were still detectable after 8 weeks (Figure 7). No obviously adverse reaction can be observed, and the new bone had a more similar morphology as the host bone after a longer healing time. The newly formed bone contained osteocytes and mature marrow spaces (marked as BM). If preadsorption of ErhBMP-2 was not applied, only fibrous tissue could be observed even in the PLGA/HA-S scaffolds (image not shown). Masson's trichrome was also used to identify new bone formation within the calvarial defects (mineralized bone stains dark blue). It is quite clear that bone healing was obvious within calvarial defects at 4 and 8 weeks after implantation (Figures 8 and 9).

Semiquantitative measurements of sections of H&E staining are shown in Figure 10. Consistent with the μCT measurements, the fraction of new bone formation was significantly increased at 8 weeks than at 4 weeks for all three groups ($p < 0.05$). The PLGA/HA-S/ErhBMP-2 group had $45\% \pm 3\%$ and $66\% \pm 5\%$ bone-healing percentages at 4 and 8 weeks, respectively, which were significantly higher than those of PLGA/ErhBMP-2 group. No significant difference was found for the PLGA/HA-M/ErhBMP-2 and PLGA/ErhBMP-2 groups at both 4 and 8 weeks.

**DISCUSSION**

HA are known to enhance osteogenic differentiation of stem cells\textsuperscript{27–29} and are generally blended with degradable polyesters in a homogenous way for bone repair. From the viewpoint of regulation of stem-cell differentiation, the resultant composite materials are less efficient especially in the very earlier stage of applications, since only a few HA particles exposing on the very top surface. Previous study has shown that the PLGA/HA-S scaffolds stimulated osteoblast differentiation of MSCs in Dex-supplemented medium.\textsuperscript{12} As one of the glucocorticoids, Dex increases the capacity to form mineralized bone nodules in vitro but Dex suppresses bone-forming cells in vivo and induces osteoporosis.\textsuperscript{14} BMPs actively recruit stem cells from the surrounding tissue to

![FIGURE 2. SEM (a–c) and CLSM images (d–f) of rat MSCs cultured in ErhBMP-2 containing medium on (a,d) PLGA/HA-S, (b,e) PLGA/HA-M, and (c,f) PLGA scaffolds for 7 days, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

![FIGURE 3. ALP activity assay of rat MSCs cultured in ErhBMP-2 containing medium on various scaffolds for different time periods. * $p < 0.05$.]
the bone defect area and initiate new bone formation. ErhBMP-2, which could be produced at low cost in large quantity, has a good prospect for clinical application. Accordingly, studies of the influence of scaffolds on the osteogenic differentiation of MSCs in ErhBMP-2 containing medium are necessary. The osteogenic efficacy of PLGA/HA-S, PLGA/HA-M, and PLGA scaffolds after loading ErhBMP-2 was also investigated in rat calvarial defects.

Differentiation of MSCs is one of the key processes for bone regeneration. The ALP is an indication of osteoblastic phenotype expression and differentiation of BMSCs toward the osteogenic lineage and is involved in the process of

FIGURE 4. Quantitative PCR analysis of (a) collagen type I and (b) osteocalcin after 14 days culture in ErhBMP-2 containing medium. *p < 0.05.

FIGURE 5. Representative in vivo µCT images of rat calvarial defects treated with (a,d) PLGA/HA-S/ErhBMP-2, (b,e) PLGA/HA-M/ErhBMP-2, and (c,f) PLGA/ErhBMP-2 for 4 and 8 weeks, respectively. (g) Quantitative bone volume analysis by micro-CT. At each time point, PLGA/HA-S/ErhBMP-2 implants stimulated more new bone formation within the defects. *p < 0.05. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
FIGURE 6. H&E staining photomicrographs of calvarial defect sites treated with (a,d,g) PLGA/HA-S/ErhBMP-2, (b,e,h) PLGA/HA-M/ErhBMP-2, and (c,f,i) PLGA/ErhBMP-2 for 4 weeks, respectively (B, BM, F, and S represent new bone, bone marrow, fibrous tissue, and residual scaffolds, respectively). Black arrow represents osteocytes. Triangle indicates the margin of the bone defects. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 7. H&E staining photomicrographs of calvarial defect sites treated with (a,d,g) PLGA/HA-S/ErhBMP-2, (b,e,h) PLGA/HA-M/ErhBMP-2, and (c,f,i) PLGA/ErhBMP-2 for 8 weeks, respectively (B, BM, F, and S represent new bone, bone marrow, fibrous tissue, and residual scaffolds, respectively). Black arrow represents osteocytes. Triangle indicates the margin of the bone defects. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
minerals’ deposition. In this study, all three types of scaffolds supported osteogenic differentiation of BMSCs in ErhBMP-2-containing medium, because the ALP activity gradually increased during the 14 days of culture (Figure 3). The higher values of ALP activity in the PLGA/HA-S scaffolds indicated that the early osteogenic differentiation of BMSCs was enhanced compared to the PLGA/HA-M and PLGA scaffolds. These results are consistent with their physical structures (Figure 1), because the exposed HA particles in the PLGA/HA-S scaffolds may adsorb more bone-related cell growth factors or better maintain their activity.

Surface properties of biomaterials regulate stem-cell differentiation.28,36–38 Gene-expression levels were also analyzed to assess the osteogenic behavior of stem cells on the three types of scaffolds. Type-I collagen is the most abundant protein found in the bone matrix and is indicative of cell differentiation toward osteoblast lineage.39 Higher expression of Type-I collagen on PLGA/HA-S scaffolds
demonstrated that the HA coating on the scaffold surface could more efficiently regulate the BMSCs differentiation toward osteoblasts. Osteocalcin is synthesized by mature osteoblasts and is most specific for the osteoblast differentiation and mineralization. Osteocalcin is expressed during the postproliferative period and reaches its maximum expression during mineralization. Higher expression levels of OCN in the PLGA/HA-S scaffolds demonstrate the enhancement of PLGA/HA-S scaffolds on the BMSCs osteoblastic differentiation in ErhBMP-2-containing medium. HA has been shown to be able to absorb multiple proteins and growth factors from the cell-culture medium. These biological molecules may be responsible for promoting BMSCs on PLGA/HA-S scaffolds to differentiate into osteoblasts. Local increase in Ca²⁺ concentration resulted from HA coating of PLGA/HA-S scaffolds may contribute to the enhanced osteoblastic differentiation of BMSCs. Previous results show that expression of the osteoblast differentiation markers is increased when the cells are exposed to high-calcium ion concentration. BMPs act on MSCs through BMP-Smad pathway, which can be further regulated by interacting with other signaling pathways either synergistically or antagonistically. The extracellular calcium-sensing receptor (CaR) has shown to reciprocally regulate the secretion of BMP-2 and the BMP antagonist Noggin in colonic myofibroblasts. Further studies are underway to understand the insight mechanisms of enhancement of MSC osteogenic differentiation by PLGA/HA-S scaffolds in ErhBMP-2-containing medium.

Although the PLGA/HA-S scaffolds enhanced MSC osteogenic differentiation in ErhBMP-2 containing medium in vitro, the potential of scaffolds/ErhBMP-2 implants for bone regeneration needs to be verified in vivo. Generally, the dosage of BMP-2 used for rat calvarial defects was in the range of micrograms, and 3 μg of ErhBMP-2 was loaded onto each scaffold in this study. Four weeks after implantation, no obvious inflammation reactions were observed in our experiments (Figure 6). As reported previously, BMP-2 can attenuate the inflammatory response that is triggered by the native polymers. Obvious new bone formation was observed at 4 weeks after implantation of the scaffolds/ErhBMP-2 implants in rat calvarial defects. However, no chondrocyte-like cells were observed in the newly formed bone in the present study at 4 weeks (Figure 6). Remarkable new bone formation in the PLGA/HA-S/ErhBMP-2 implants indicates that the HA coating of PLGA/HA-S scaffolds has a positive influence on new bone formation in rat calvarial defects. PLGA degrades into lactic and glycolic acids in vivo that can be metabolized by the tricarboxylic acid cycle and finally eliminated from the body as carbon dioxide and water. If the capacity of clearance of the degradation products was not adequate, the acidic degradation products would cause a decrease in pH, resulting in local inflammatory reactions and potentially poor tissue development. It can be assumed that the HA nanoparticles may neutralize the acidic degradation products of PLGA to a certain extent and release calcium and phosphate ions. The increase in the total concentrations of calcium and phosphate ions may stimulate the cell chemotaxis and could favor new bone formation in the PLGA/HA-S/ErhBMP-2 implants. Furthermore, HA has been reported to adsorb BMP-2 through electrostatic interaction and slow down the BMP-2 release rate. Therefore, the PLGA/HA-S with HA nanoparticles exposed to the scaffold surface may adsorb ErhBMP-2 more efficiently, resulting in enhancement of new bone formation.

CONCLUSION

The PLGA/HA-S scaffolds with HA nanoparticles embedded spatially on pore walls could enhance osteogenic differentiation of BMSCs in ErhBMP-2-containing medium in vitro. ErhBMP-2 induced new bone formation in rat calvarial defects, which was enhanced by PLGA/HA-S scaffolds due to the largely exposed HA particles on the pore walls of the composite scaffolds. Therefore, the PLGA/HA-S scaffolds render a more favorable niche for osteoblastic differentiation of BMSCs in ErhBMP-2-containing medium and an effective carrier for ErhBMP-2 for bone regeneration.

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