Engineering new bone tissue *in vitro* on highly porous poly(α-hydroxyl acids)/hydroxyapatite composite scaffolds

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Abstract: Engineering new bone tissue with cells and a synthetic extracellular matrix (scaffolding) represents a new approach for the regeneration of mineralized tissues compared with the transplantation of bone (autografts or allografts). In the present work, highly porous poly(L-lactic acid) (PLLA) and PLLA/hydroxyapatite (HAP) composite scaffolds were prepared with a thermally induced phase separation technique. The scaffolds were seeded with osteoblastic cells and cultured *in vitro.* In the pure PLLA scaffolds, the osteoblasts attached primarily on the outer surface of the polymer. In contrast, the osteoblasts penetrated deep into the PLLA/HAP scaffolds and were uniformly distributed. The osteoblast survival percentage in the PLLA/HAP scaffolds was superior to that in the PLLA scaffolds. The osteoblasts proliferated in both types of the scaffolds, but the cell number was always higher in the PLLA/HAP composite scaffolds during 6 weeks of *in vitro* cultivation. Bone-specific markers (mRNAs encoding bone sialoprotein and osteocalcin) were expressed more abundantly in the PLLA/HAP composite scaffolds than in the PLLA scaffolds. The new tissue increased continuously in the PLLA/HAP composite scaffolds, whereas new tissue formed only near the surface of pure PLLA scaffolds. These results demonstrate that HAP imparts osteoconductivity and the highly porous PLLA/HAP composite scaffolds are superior to pure PLLA scaffolds for bone tissue engineering. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res 54: 284–293, 2001

Key words: bone; tissue engineering; hydroxyapatite; biodegradable polymers; scaffolding; osteoblast

INTRODUCTION

Bone fractures and damage result in more than 1.3 million surgical procedures each year in the United States.[1] Approximately 500,000 vertebral fractures occur annually in the United States, along with 250,000 proximal femur and 200,000 distal radius fractures.[2] In addition, over 120,000 total hip arthroplasties are performed there each year.[3,4] In dentistry, plastic and reconstructive surgeries are performed daily for craniofacial bone reconstruction and replacement.[5] Bone grafting is increasing and the failure rate is unacceptable.[6,7] In patients who are managed with various bone grafts, a failure rate ranging from 16% to 50% is reported.[6,8,9] The failure rate of autografts is at the lower end of the spectrum,[10] but the need for second site of surgery, limited supply, inadequate size and shape, and the morbidity associated with donor site are all major concerns.[11]

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Engineering osseous tissue with cells and a synthetic extracellular matrix is a new approach compared to transplantation of harvested tissues. In this approach, a highly porous scaffold serves as the growth substrate for osteoblasts or osteoprogenitor cells. This scaffold should allow nutrients and metabolites to permeate, should be conducive to vascularization, and should serve as a guide for cell growth and new bone tissue formation in three dimensions. Biodegradable polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic acid-*co*-glycolic acid) (PLGA) are widely used as scaffolding materials for a variety of tissue engineering applications because they degrade and resorb after fulfilling the template function so that there will be no long-term foreign-body reaction and complications.[1,12–15]

Poly(lactic acid-*co*-glycolic acids) have been studied as scaffolding materials for bone tissue engineering.[16,17] A salt-leaching technique has been used to generate porous foams of degradable polymers.[12,14,18,19] PLGA (75:25) was processed into porous foam using this technique and cultured with stromal osteoblastic cells by Ishaug et al.[17] One of the

limitations was that the new tissue matrix was formed only at the surface layer of the foam (<240 μm) with only a minimum number of cells located in the center.

The scaffolding materials for bone tissue engineering should be osteoconductive so that osteoblasts and osteoprogenitor cells can adhere, migrate on the scaffolds, differentiate, and synthesize new bone matrix. With this approach, we can potentially use the patient's own cells so that immunosuppression is not needed. The new tissue should also have the potential to grow and remodel, which is especially important for pediatric patients. We hypothesize that hydroxyapatite can improve osteoconductivity of biodegradable polymer scaffolds; and as a mineral existing in natural bone, it will be a site for new bone deposition and therefore be integrated within new bone after the polymer degrades.[20]

Previously, a composite scaffold of PLGA (50:50) and hydroxyapatite (HAP) was fabricated with the salt-leaching technique by Laurencin and coworkers.[21,22] In a 21-day primary osteoblast culture, the HAP-containing matrix showed some promising features in cell attachment and differentiated function. However, the matrix porosity was quite low (based on the mixture used to make the matrix: PLGA:HAP: NaCl = 1:1:1), which might not be ideal for long-term cell survival, proliferation, and tissue formation owing to mass transport limitations. We used a novel solid–liquid phase separation technique to create highly porous (as high as 95% porosity) poly(α-hydroxyl acids)/HAP composite scaffolds.[20] It was demonstrated that the porosity, pore size, and pore morphology of these composite foams could be controlled by the polymer concentration, chemical structure of the polymer, HAP content, phase-separation temperature, and solvent used.[20] The high porosity was expected to better satisfy the cell penetration and mass transport requirements (for nutrient, metabolites, and soluble signals) for tissue engineering.[13,14,23] Therefore, highly porous biodegradable polymer/HAP composite scaffolds were investigated for bone tissue engineering in this work.

MATERIALS AND METHODS

Materials

Poly(L-lactic acid) with an inherent viscosity of approximately 1.6 was purchased from Boehringer Ingelheim (Ingelheim, Germany). The polymer was used without further purification. Dioxane and HAP [$Ca_{10}(PO_4)_6(OH)_2$] were obtained from Aldrich Chemical Company (Milwaukee, WI). Fetal bovine serum, α-minimum essential medium (α-MEM), ascorbic acid-free α-MEM (Formula 94-5049EL), penicillin–streptomycin, Dulbecco's phosphate-buffered saline (PBS), trypsin-EDTA, and sodium bicarbonate were purchased from Gibco BRL Products, Life Technologies (Grand Island, NY). Ascorbic acid was purchased from Fisher Scientific (Pittsburgh, PA). Neutral-buffered formalin, Trypan blue, and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO). Ethylene oxide was purchased from H. W. Anderson Products (Chapel Hill, NC).

Polymer and polymer/HAP scaffolds

The preparation and morphology of PLLA and PLLA/HAP scaffolds have been reported in detail earlier.[20] Briefly, PLLA was dissolved in dioxane to make a 5% (w/v) solution. To prepare the composite scaffolds, hydroxyapatite powder (platelets ranging from 10 to 100 μm in size) was added into the prepared PLLA solution. A solid-liquid phase separation technique and a subsequent solvent sublimation process were used to generate the highly porous PLLA and PLLA/HAP composite scaffolds. The PLLA/dioxane solution and PLLA/HAP/dioxane mixture were cooled to −18°C to induce solid–liquid phase separation. The phase-separated samples were dried under vacuum (0.5 mmHg) at between −5°C and −10°C for 7 days to remove the solvent completely.[20]

The density and porosity of the porous PLLA and PLLA/HAP composite scaffolds were determined with a liquid displacement method as reported in detail earlier.[20,24] Ethanol was used as the displacing liquid because it penetrated easily into the pores and did not induce shrinkage or swelling. With this method, the total pore volume, polymer or polymer/HAP skeleton volume, and the overall scaffold volume were determined to calculate the density and porosity.[20,24]

The compressive mechanical properties of the foams were characterized with an Instron mechanical tester (Model 4502; Instron Co., Canton, MA). Circular disk specimens (16 mm in diameter, and 3 mm thick) were tested with a crosshead speed of 0.5 mm/min. Five or more specimens were tested for each sample, and the averages and standard deviations were calculated. A two-tailed Student t test was performed to determine the statistical significance ($p < .05$) of the differences between samples.

The porous morphologies of the PLLA and PLLA/HAP composite foams were examined with scanning electron microscopy (SEM; S-3200N, Hitachi, Japan) at 15 kV. The specimens were cut with a razor blade after being frozen in liquid nitrogen for 5 min. The cut specimens were then coated with gold using a sputter coater (Desk-II, Denton Vacuum Inc.) for 200 s. The gas pressure was <50 mtorr and the current was approximately 40 mA.

Osteoblast passage, seeding, and culture

The thawed MC3T3-E1 osteoblasts (clone 4)[25] were cultured in a supplemented ascorbic acid-free α-MEM [Formula 94-5049EL; 10% fetal bovine serum (FBS), 50 U/mL penicillin, and 50 μg/mL streptomycin] in a humidified in-

cubator at 37°C with a CO_2/air ratio of 5:95. The medium was changed every other day. The cells of passages 3 and 4 were seeded on to the PLLA and PLLA/HAP scaffolds. The viability of the cells before seeding was higher than 90% determined with the Trypan blue exclusion assay.

The porous PLLA and PLLA/HAP disks with a diameter of 10 mm and thickness of 1.5 mm were prepared. These scaffolds were assembled on the bottoms of custom-made 12-well Teflon culture plates with a well diameter of 10 mm. The scaffold-containing culture plates were sterilized with ethylene oxide. The sterilized PLLA and PLLA/HAP scaffolds (assembled in the wells) were soaked in ethanol for 30 min and then exchanged with phosphate-buffered saline three times (30 min each). The scaffolds were then washed with a complete medium (α-MEM, 10% FBS, 50 U/mL penicillin, 50 μg/mL streptomycin, and an additional 50 mg/L of L-ascorbic acid) two times (2 h each). The medium was then decanted and 2 million cells (suspended in 0.5 mL of the complete medium) were seeded on each scaffold. The cell-scaffold constructs (in the Teflon plates) were cultured on an orbital shaker (Model 3520; Lab-Line Instruments, Melrose Park, IL) at 75 rpm in a humidified incubator with a CO_2/air ratio of 5:95. The medium was changed two times a day (0.5 mL) for 2 days.

After the 48-h cell seeding, the polymer–osteoblast constructs were removed from the Teflon plates and transferred into six-well tissue culture plates. The constructs were cultured with the complete medium on the orbital shaker at 75 rpm in the humidified incubator. Four-milliliter medium was used for each construct and the medium was changed every other day.

Histology

Osteoblast–PLLA and osteoblast–PLLA/HAP constructs were cultured *in vitro* for varying time intervals (the culture time was calculated from the day of cell seeding), and then fixed in 10% neutral-buffered formalin. Paraffin-embedded disk specimens were cut into 5-μm-thick cross sections and stained with hematoxylin and eosin, or von Kossa's silver nitrate.

RNA extraction and Northern blot analysis

To obtain as much RNA as possible from the osteoblast–PLLA and osteoblast–PLLA/HAP constructs that were mechanically strong, samples were homogenized with a Polytron homogenizor (Brinkmann Easycare Generator; POLYTRON-Aggregate, Switzerland) for 30 s at top speed (VI) for three times before preparing RNA according to the method of Chomczynski and Sacchi.[26] Aliquots of total RNA were fractionated on 1.0% agarose-formaldehyde gels and blotted onto nitrocellulose paper as described by Thomas.[27] The mouse cDNA probes used for hybridization were obtained from the following sources: osteocalcin from Dr. John Wozney (Genetics Institute, Boston, MA)[28] and bone sialoprotein from Dr. Marion Young (NIDR, Bethesda, MD).[29] All cDNA inserts were excised from plasmid DNA with the appropriate restriction enzymes and purified by agarose gel electrophoresis before labeling with α-[^{32}P]-dCTP using a random primer kit (Boehringer-Mannheim, Indianapolis, IN). Hybridizations were performed as previously described using a Bellco Autoblot hybridization oven[30] and quantitatively scanned using a Packard A2024 InstantImager. All values were normalized for RNA loading by probing blots with cDNA to 18S rRNA.[31] At least three repeats were analyzed for each sample, and representative results are reported.

DNA assay

Osteoblast adhesion and proliferation on the scaffolds were quantified with a previously described DNA assay.[25,32] To remove the large amounts of HAP that may interfere with the assay, the osteoblast–PLLA/HAP constructs were homogenized as described above and precipitated in 0.4 N perchloric acid (PCA). The DNA assay was then performed using the method of Schneider.[33] Two to four repeats were analyzed for each sample, and the averages and standard deviations were plotted.

RESULTS

The PLLA and PLLA/HAP porous scaffolds were prepared with a solid–liquid phase separation technique similar to that detailed previously.[20] Foams prepared from a 5% PLLA/dioxane solution and a suspension of HAP in 5% PLLA/dioxane (HAP:PLLA = 1:1) were interconnected with pore size ranging from tens to hundreds of micrometers (Fig. 1). The morphology of the PLLA foam was different from that of the PLLA/HAP foam. The pores of PLLA foam were relatively regular in shape, whereas the pores of the PLLA/HAP foam were irregular in shape. These observations were consistent with those reported earlier.[20] The porosity of the PLLA/HAP composite foam (89.2%) was slightly lower than that of the pure PLLA foam (92.7%). The mechanical properties of the PLLA/HAP foam were improved over those of the pure PLLA foam (Table I). Both compressive modulus and compressive yield strength of the PLLA/HAP composite foam were significantly higher than those of the pure PLLA foam ($p < .05$).

Osteoblasts were more uniformly distributed in the PLLA/HAP scaffolds than in pure PLLA scaffolds (Fig. 2). For both PLLA and PLLA/HAP scaffolds, osteoblasts were more abundant in the surface regions than in the central regions 1 week after cell seeding. However, repeated histological observations showed that a large amount of osteoblasts penetrated deep into and relatively uniformly distributed in the central area of the PLLA/HAP scaffolds, whereas there were

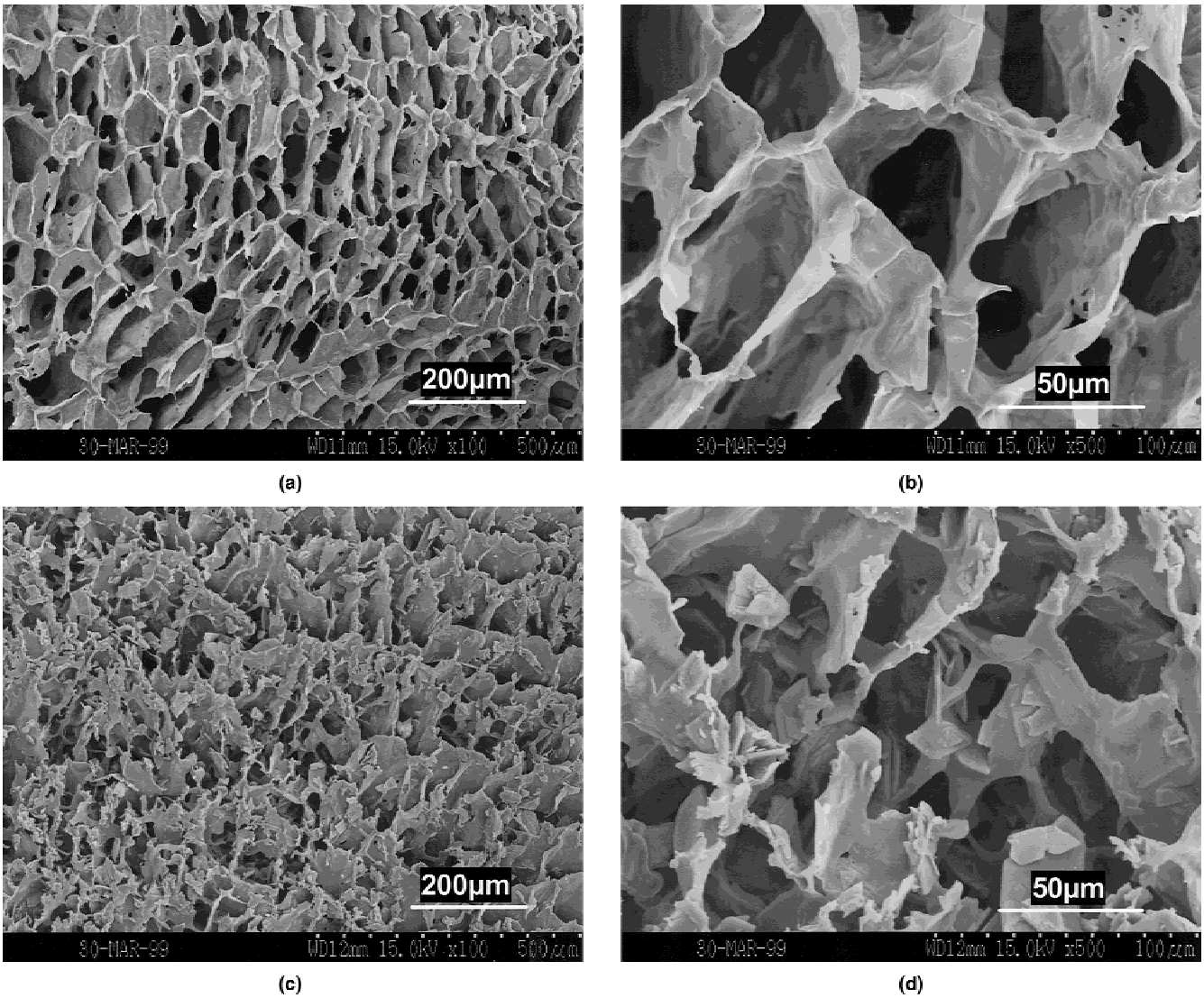

Figure 1. Scanning electron micrographs of PLLA and PLLA/HAP (PLLA/HAP: 50:50) foams prepared from a 5% (w/v) PLLA/dioxane solution: (a) PLLA, ×100; (b) PLLA, ×500; (c) PLLA/HAP, ×100; (d) PLLA/HAP, ×500.

only scattered osteoblasts in the central area of the pure PLLA scaffolds.

The DNA content of the osteoblast–scaffold constructs was measured to quantify the osteoblast adhesion and proliferation on the PLLA and PLLA/HAP scaffolds. Control experiments showed that neither scaffold interfered with the DNA assay. The cell numbers adhered on the two types of scaffolds after 5 h of cell seeding were statistically identical (Table II). One week after cell seeding, the cell numbers on the PLLA/HAP scaffolds were significantly higher than those on the PLLA scaffolds (Fig. 3). The average cell number on the PLLA/HAP scaffolds was slightly higher than that adhered after the initial 5 h of cell seeding (DNA: 28.5 μg vs. 25.8 μg), whereas the cell number on the PLLA scaffolds was significantly lower than that adhered after the initial 5 h of cell seeding (DNA: 9.9 μg vs. 25.6 μg). It was obvious that the cell survival rate (and growth) during the first week of culture in the PLLA/HAP scaffolds was much higher than that in the pure PLLA scaffolds. The osteoblasts proliferated over the *in vitro* cultivation time in both types of scaffolds during the 6-week *in vitro* cultivation. The cell number in the PLLA/HAP scaffolds was always higher than that in the PLLA scaffolds during the 6 weeks of culture (Fig. 3).

TABLE I
Compressive Mechanical Properties of PLLA and PLLA/HAP Composite Scaffolds Made from 5% PLLA/Dioxane Solution

	Modulus (MPa)	Yield Strength (MPa)
PLLA	6.42 ± 1.44	0.32 ± 0.04
PLLA/HAP (1:1)	10.87 ± 3.20	0.39 ± 0.01

The cell distribution and morphogenesis were visualized by histologic analysis (Fig. 4). In the central areas of the PLLA scaffolds, the cell number and tissue mass did not change significantly in the first 4 weeks,

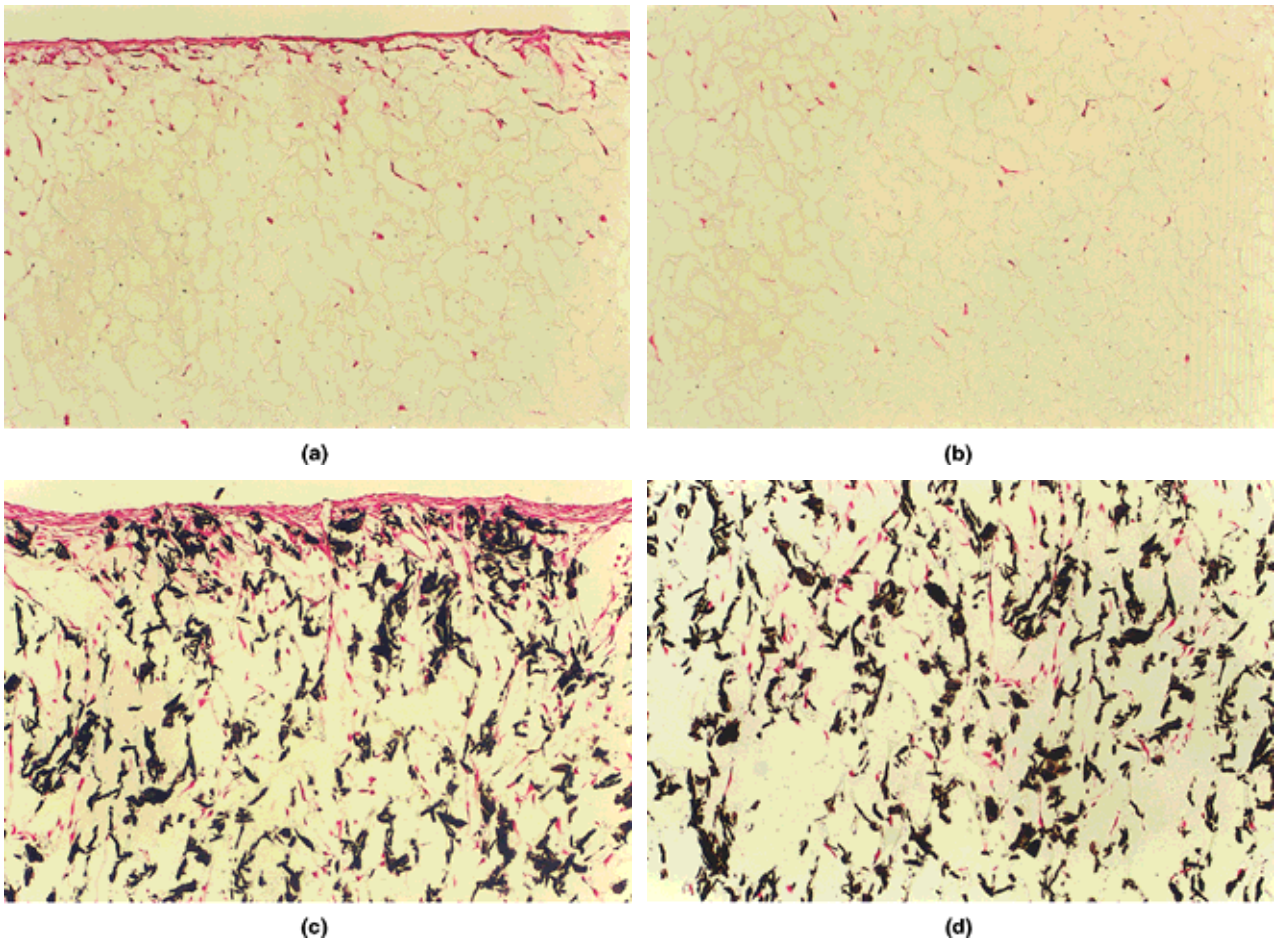

Figure 2. Osteoblastic cell distribution in highly porous PLLA and PLLA/HAP composite scaffolds 1 week after cell seeding (von Kossa's silver nitrate staining; original magnification ×100): (a) the surface area of an osteoblast–PLLA construct; (b) the center of an osteoblast–PLLA construct; (c) the surface area of an osteoblast–PLLA/HAP construct; and (d) the center of an osteoblast–PLLA/HAP construct.

but some small tissue domains formed after 8 weeks of *in vitro* culture. In contrast, cell number and new tissue mass seemed to increase continuously in the PLLA/HAP scaffolds. After 8 weeks of *in vitro* cultivation, the new tissue became nearly continuous (Figs. 4 and 5). However, the tissue mass was slightly denser in the areas closer to the surfaces of the osteoblast–PLLA/HAP constructs than in the very central areas. Some ossification was also observed (dark areas) but the relative cellular contribution to this process could not be evaluated accurately because of interference from the HAP particles incorporated into the scaffolds. More detailed experiments are needed to study the ossification process. *In vivo* implantation experiments are also being conducted to enhance ossification with the desired vascularization.

TABLE II
Adhesion of MC3T3-E1 Cells onto PLLA and PLLA/HAP Composite Scaffolds after 5 h Cell Seeding

	DNA (μg)
PLLA	25.63 ± 1.57
PLLA/HAP (1:1)	25.80 ± 0.75

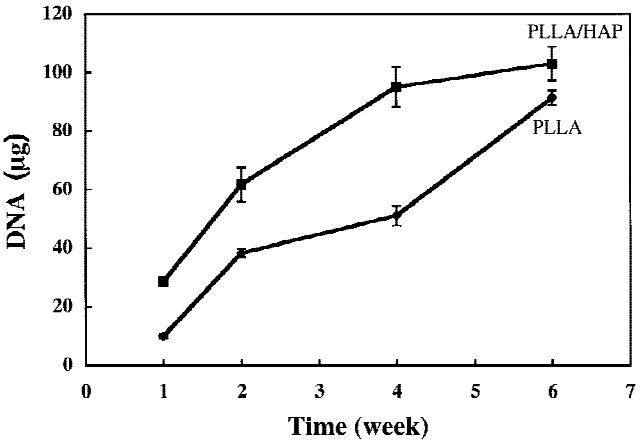

Figure 3. Osteoblast proliferation in the PLLA and PLLA/HAP scaffolds versus *in vitro* cultivation time; 2×10^6 MC3T3-E1 subclone 4 cells were seeded into each scaffold and fed every other day until harvest. DNA contents at each time point were determined as described in Materials and Methods.

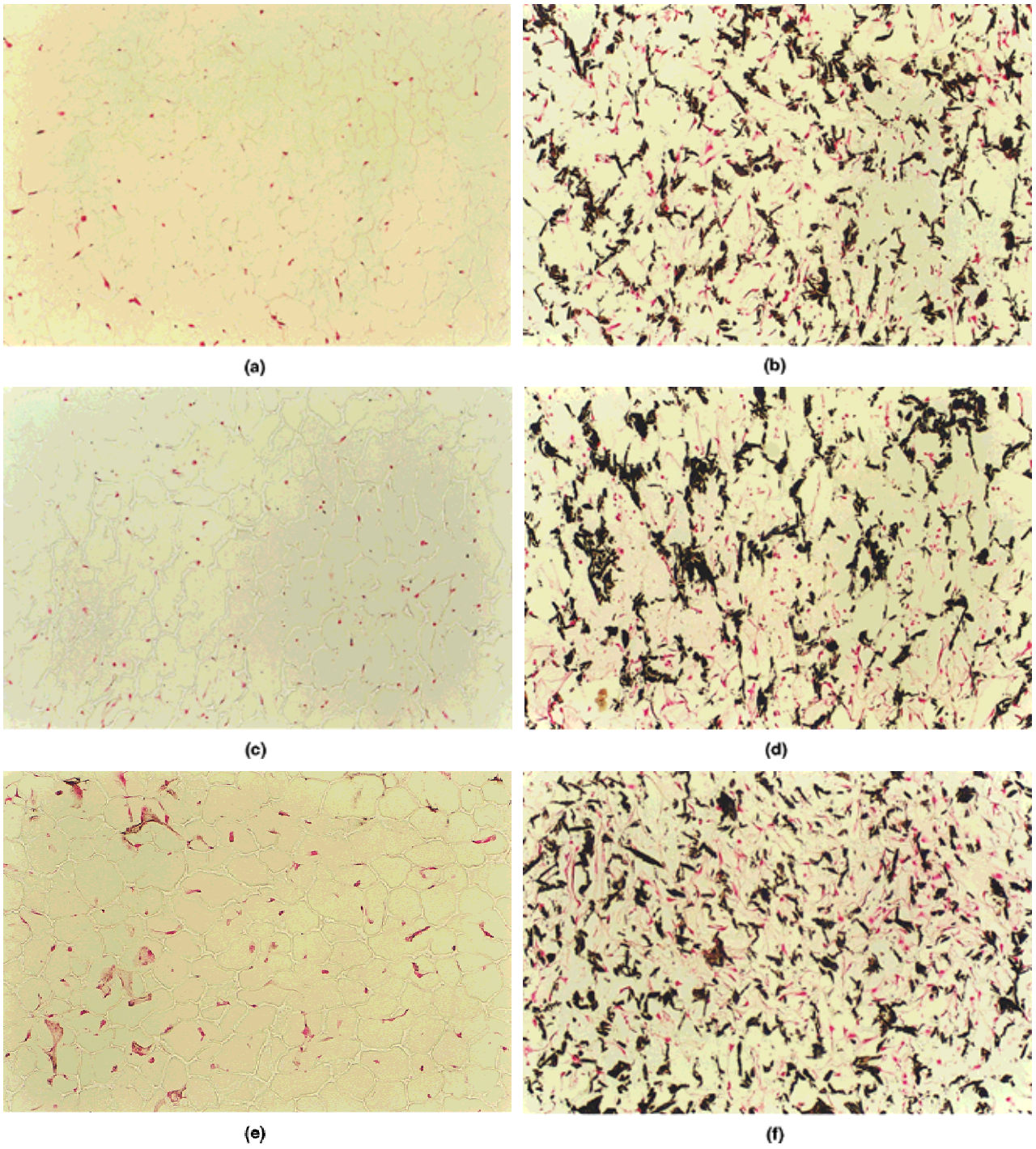

Figure 4. Osteoblast–PLLA and osteoblast–PLLA/HAP constructs cultured *in vitro* for varying times (von Kossa's silver nitrate staining, original magnification ×100): (a) PLLA, 2 weeks; (b) PLLA/HAP, 2 weeks; (c) PLLA, 4 weeks; (d) PLLA/HAP, 4 weeks; (e) PLLA, 8 weeks; (f) PLLA/HAP, 8 weeks.

The osteoblasts in the PLLA/HAP scaffolds showed a highly differentiated phenotype. Bone specific gene expression was analyzed in both osteoblast–PLLA and osteoblast–PLLA/HAP constructs. MC3T3-E1 cells, like other osteoblast cell lines, are phenotypically heterogeneous; i.e., only a fraction of the cells exhibit osteoblast characteristics. To obtain a highly responsive cell population, MC3T3-E1 cells were subcloned as previously described.[25] Highly responsive clone 4 cells were used in the current investigation. Northern blots were probed for mRNAs encoding bone sialoprotein (BSP) and osteocalcin (OCN), two well-characterized osteoblast-specific gene products, in the osteoblast-scaffold constructs (Fig. 6). The values were normalized for RNA loading by probing blots with cDNA to 18S rRNA (Fig. 7). The mRNAs of both BSP

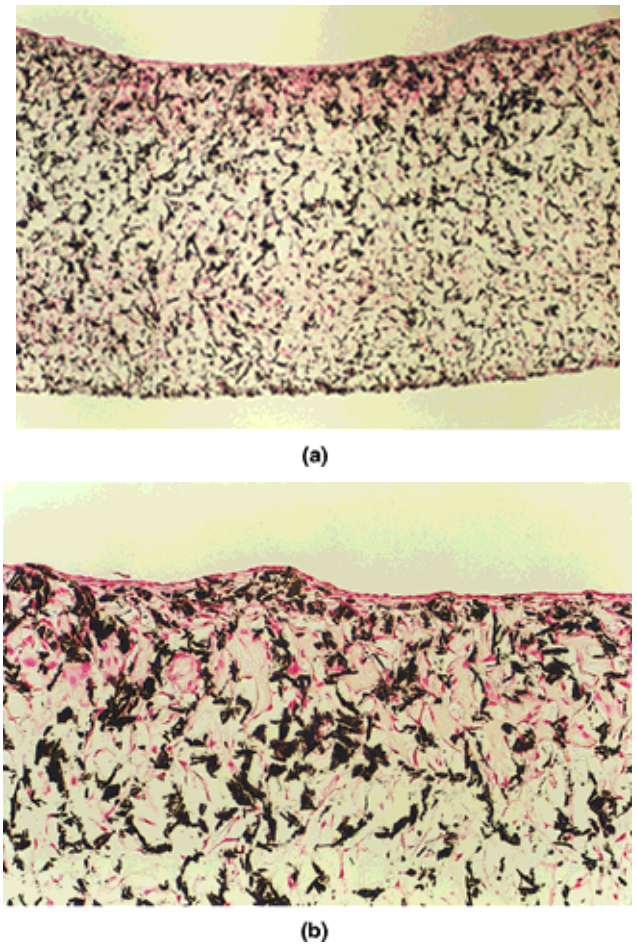

Figure 5. Osteoblast–PLLA/HAP constructs after 8 weeks of *in vitro* culture (von Kossa's silver nitrate staining): (a) overall cross-section, original magnification ×40; (b) surface area, original magnification ×100.

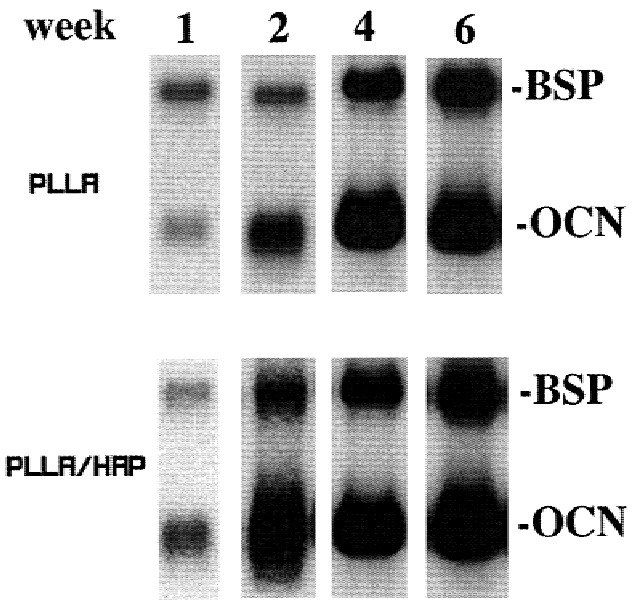

Figure 6. Northern blot analysis of the mRNA from MC3T3-E1 subclone 4 cells cultured on PLLA and PLLA/HAP scaffolds at varying *in vitro* culture times: 10 μg total RNA from each group was used for Northern blot hybridization using cDNA probes to bone sialoprotein (BSP) and osteocalcin (OCN).

and OCN increased with cultivation time, which demonstrated that the scaffolding architecture was suitable for osteoblast differentiation. The mRNA levels for both BSP and OCN were higher in the osteoblast–PLLA/HAP constructs than in the osteoblast–PLLA constructs, which showed the advantages of the osteoconductivity imparted by HAP. The differences in the expression levels of bone-specific markers were more significant at the later stage of the culture. The drop in the marker expression levels at week 6 in the PLLA scaffolds might be caused by the worse mass transport limitations owing to the surface tissue growth in the PLLA scaffolds as opposed to the more homogeneous three-dimensional tissue formation in the PLLA/HAP scaffolds.

DISCUSSION

Bone is the primary constituent of the human skeleton. Because of its rigidity and strength, bone is the framework on which soft tissues are attached and act against to produce movement. Its four main functions are to provide mechanical support (e.g., ribs), to permit locomotion (e.g., long bones), to provide protection (e.g., skull), and to provide a reservoir of calcium, phosphorous, and other ions (bone contains about 99% of the body's calcium) to control and balance the ionic content in body fluids (homeostasis). Bone matrix is composed of an organic portion and an inorganic (calcified) portion. The inorganic portion is composed of calcium, phosphate, bicarbonate, citrate, magnesium, potassium, and sodium. This portion represents 50% of the dry weight of bone, and consists primarily of HAP crystals, which have the composition $Ca_{10}(PO_4)_6(OH)_2$. The organic portion consists primarily of type I collagen (95%) and small amount of ground substance that contains glycoproteins, proteoglycans, and glycosaminoglycans. Some of these molecules have been shown to bind calcium and may play a role in matrix calcification. Among the noncollagenous glycoproteins are osteocalcin (OCN), osteopontin, and bone sialoprotein (BSP). Osteocalcin and bone sialoprotein are considered the most sensitive markers of bone-specific tissue formation.[34–39]

To engineer new bone tissue, a porous scaffold is needed to guide cell attachment, migration, proliferation, differentiated function, and tissue regeneration in three dimensions. Synthetic aliphatic polyesters such as PLA, PGA, and their copolymers are biocompatible and biodegradable, satisfy the basic scaffolding material requirements,[13,14] and have been ap-

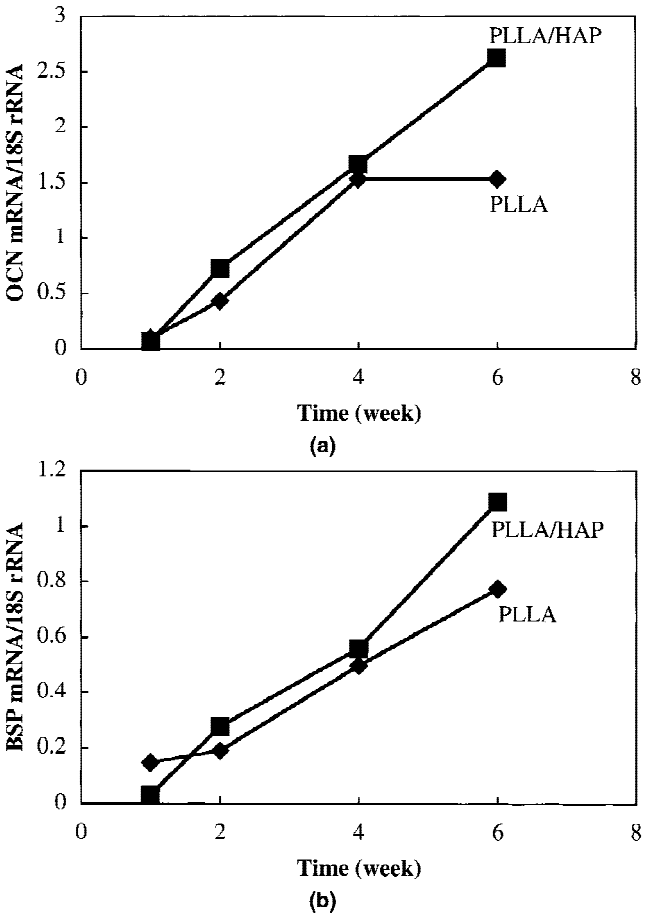

Figure 7. Normalized values of (a) osteocalcin (OCN) mRNA, and (b) bone sialoprotein (BSP) mRNA, using 18S rRNA cDNA as a probe as described in Materials and Methods.

proved by the Federal Food and Drug Administration (FDA) for certain human clinical applications such as surgical sutures and some implantable devices. Therefore, these polymers have been widely explored for tissue engineering applications. They have been processed into woven or nonwoven fabrics to engineer a variety of tissues such as cartilage, tendon, heart valves, and blood vessels.[13,23,40–49] They have also been processed into foams with a well-documented salt-leaching technique to engineer bone, liver, and other tissues.[12,14,18,19,50–52] In addition, they have been processed into foam structures with thermally induced phase separation or emulsion-freeze-drying techniques as tissue engineering or controlled-release matrices.[20,24,53–59]

One of the disadvantages of these materials is that the degradation products reduce the local pH, which may accelerate the polymer degradation rate[60] and induce an inflammation. In addition, the mechanical properties of these highly porous polymer scaffolds are weak, which is disadvantageous for structural tissue engineering applications. Ishaug et al. demonstrated that stromal osteoblasts grew on PLGA foams but the cell penetration and tissue regeneration occurred only in the surface areas.[17] We hypothesized that the incorporation of a bonelike mineral—synthetic HAP—into a highly porous biodegradable polymer scaffold may provide the following advantages for bone tissue engineering: (a) A better environment for cell seeding, survival, growth, and differentiated function was expected because of the osteoconductive properties imparted by HAP; (b) the acidic degradation by-products from polyesters might be buffered; and (c) the mechanical properties might be improved.[20]

In this work, a novel phase separation technique is used to fabricate the scaffolds, which have moduli about 20 times higher than those of the same porosity made with the well-documented salt-leaching technique. The mechanical properties of the PLLA/HAP scaffolds are further significantly improved over those of pure PLLA scaffolds. More importantly, it has been demonstrated that the cell survival percentage on the PLLA/HAP scaffolds is significantly improved over that on the pure PLLA scaffolds. Moreover, the seeded osteoblasts are more uniformly distributed in the entire PLLA/HAP scaffolds compared with the cell attachment primarily in the surface area of the PLLA scaffolds. Cells also proliferate over cultivation time in the scaffolds, which show that the scaffolding architecture is suitable for osteoblast seeding and growth. The cell number is always higher in the PLLA/HAP scaffolds than in the pure PLLA scaffolds during the entire *in vitro* cultivation period. The bone-specific markers (mRNAs encoding bone sialoprotein and osteocalcin) are expressed in both pure PLLA scaffolds and PLLA/HAP composite scaffolds, whereas the expression levels are higher in the PLLA/HAP scaffolds. The new bone tissue formation is significantly enhanced and is more uniformly distributed in the PLLA/HAP scaffolds in three dimensions than in the PLLA scaffolds. These results demonstrated that HAP imparted osteoconductivity to the scaffolds. The long-term cell survival, growth, and highly differentiated state in the center of the osteoblast-scaffold constructs demonstrated the advantages of the high porosity, which lead to high permeability for nutrients, metabolic wastes, and the soluble signal molecules. The adsorption of proteins and other biologically active molecules to HAP is likely different from that to polymers. These proteins and biologically active molecules may mediate the interactions between the osteoblastic cells and the scaffolds. The varied interactions from the scaffolds to the osteoblastic cells might have resulted in some of the changes in cellular behavior (osteoconductivity of the scaffolds).

The calcification was not highly appreciable in the osteoblast–scaffold constructs partially because of the interference of the HAP particles incorporated. There were also several factors that might not be ideal for

ossification. One of them could be the cell source. Although a single cell type might not be ideal for calcified bone matrix formation, the *in vitro* cultivation of this well-characterized osteoblastic cell line allowed for well-controlled comparison between different types of scaffolds without complications from mixed cell types. The lack of blood supply in an *in vitro* culture was known to be a disadvantage for calcified bone formation, but was deliberately chosen to avoid complicating systemic factors for a more controlled comparison of the scaffolding materials. Other possible factors include not-optimized culture medium composition, cultivation conditions, and so forth. However, the advantages of the highly porous poly(α-hydroxyl acids)/HAP composite scaffolds for bone tissue engineering have been clearly demonstrated in this work. The studies on scaffold degradation and acidity, protein adsorption, effects of cell types, tissue culture conditions, *in vivo* implantation, host tissue reaction, and integration are under way and will be reported separately.

CONCLUSIONS

Highly porous foams fabricated with a thermally induced phase separation technique are mechanically strong and possess architectures suitable for osteoblast seeding and growth. The mechanical properties of the polymer/HAP scaffolds are significantly higher than those of the pure polymer scaffolds. The bone-mineral–mimicking synthetic HAP imparts osteoconductivity to the polymer scaffolds. The polymer/HAP composite scaffolds have been shown to have higher osteoblast survival rate, more uniform cell distribution and growth, improved new tissue formation, and enhanced bone specific gene expression *in vitro.* These results have demonstrated that the polymer/HAP scaffolds are superior to the pure polymer scaffolds for osseous tissue engineering. Studies on the composite scaffold degradation behavior, pH variations, protein adsorption, and *in vivo* tissue formation are under way to understand the mechanisms and explore the potentials of these new scaffolds for clinical application.

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