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Biocompatibility of Bioresorbable Poly(L-lactic acid) Composite Scaffolds Obtained by Supercritical Gas Foaming with Human Fetal Bone Cells

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ABSTRACT

The aim of this investigation was to test the biocompatibility of three-dimensional bioresorbable foams made of poly(L-lactic acid) (PLA), alone or filled with hydroxyapatite (HA) or β-tricalcium phosphate (β-TCP), with human primary osteoblasts, using a direct contact method. Porous constructs were processed by supercritical gas foaming, after a melt-extrusion of ceramic/polymer mixture. Three neat polymer foams, with pore sizes of 170, 310, and 600 μm, and two composite foams, PLA/5 wt% HA and PLA/5 wt% β-TCP, were examined over a 4-week culture period. The targeted application is the bone tissue-engineering field. For this purpose, human fetal and adult bone cells were chosen because of their highly osteogenic potential. The association of fetal bone cells and composite scaffold should lead to *in vitro* bone formation. The polymer and composite foams supported adhesion and intense proliferation of seeded cells, as revealed by scanning electron microscopy. Cell differentiation toward osteoblasts was demonstrated by alkaline phosphatase (ALP) enzymatic activity, γ-carboxylated Gla-osteocalcin production, and the onset of mineralization. The addition of HA or β-TCP resulted in higher ALP enzymatic activity for fetal bone cells and a stronger production of Gla-osteocalcin for adult bone cells.

INTRODUCTION

BONE IS THE MOST commonly replaced tissue of the body, with nearly 800,000 surgical procedures performed per year in the United States alone.[1] Autograft has major disadvantages that are well documented and include donor site morbidity, increased operative time, blood loss, and often limited quantity,[2–4] whereas allograft can expose patients to viral contaminations and complications, such as infections,[5] fractures,[6] or intense immune response.[7–9] For clinical bone transplantations, tissue-engineering techniques based on the delivery of cells to the defect through the use of three-dimensional porous materials are being investigated.

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Scaffolds in bone tissue engineering are currently made of either soft bioresorbable polymers such as poly(α-hydroxy acids), chitosan, or collagen,[10,11] or of brittle osteoconductive ceramics such as synthetic calcium phosphates or natural coral.[12,13] Ceramic–polymer composites have gained increased interest.[14–16] The combination of ceramic and polymer should result in porous composite structures that maintain their shape, with improved mechanical properties, enhanced bioactivity, and controlled resorption rates.[17] To process scaffolds, several techniques have been reported such as solvent casting/particulate leaching,[18] emulsion freeze-drying or thermally induced phase separation,[19,20] three-dimensional printing,[21] and gas foaming.[22–24] It was previously shown that supercritical gas foaming of poly(L-lactic acid) (PLA) polymers can be used to obtain porous structures with controlled parameters particularly important for bone tissue engineering, such as porosity, pore size, and connectivity.[25] Ceramic fillers were added to the polymer matrix by melt-extrusion in order to obtain a homogeneous ceramic–polymer mixture before foaming. Composite foams were thus processed by a solvent-free technique, from raw materials to final porous structures. The behavior of bone cells seeded directly on them remained to be demonstrated. The use of primary human bone cells to study the *in vitro* biocompatibility of orthopedic materials is an adequate model, undoubtedly more relevant than experiments based on osteogenic cell lines.[26] The interesting potential of fetal bone cells for tissue engineering was demonstrated on the basis of their rapid growth rate and their responsiveness to differentiation factors.[27] In this study, fetal and adult bone cells were each seeded on the same batches of scaffolds to compare their interactions. We demonstrate here for the first time that once seeded with human fetal bone cells, PLA/ceramic composite osteoconductive scaffolds—processed by supercritical gas foaming—give a promising approach in tissue engineering due to the high osteogenic potential of these cells.

MATERIALS AND METHODS

Materials

A commercial bioresorbable polymer, poly(L-lactic acid) (PLA; Boehringer Ingelheim, Ingelheim, Germany), was used without further purification. It was characterized by an intrinsic viscosity of 1.6 dL/g and a melting temperature of 181.7°C. Two ceramic powders were added to the polymer: hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) (a gift from the Dr. Robert Mathys Foundation, Bettlach, Switzerland). HA particles were of nanometric size, with a high specific surface area (50 m^2/g), whereas β-TCP particles were of micrometric size, with a much smaller specific surface area (1–2 m^2/g). Both PLA and ceramics were dried overnight at 105°C under vacuum before processing in order to prevent polymer degradation by hydrolysis. Foaming was carried out with supercritical CO_2 (pure, >99.995%; Sauerstoffwerk Lenzburg, Lenzburg, Switzerland). PLA pellets and 5 wt% ceramic particles were mixed in the dry state before extrusion. A microcompounder (Micro 5 compounder; DSM, Heerlen, The Netherlands) with two conical corotating screws, of small capacity (5 cm^3), was used to prepare ceramic–polymer blends, under a flow of nitrogen to limit polymer degradation. A set temperature of 205°C, a screw rotation speed of 100 rpm, and a residence time of 4 min were considered. These parameters were optimized in order to obtain a homogeneous distribution of fillers in PLA. The supercritical gas-foaming technique used in this study has been described.[25] The foaming equipment was composed of a custom-made high-pressure chamber (Autoclave France, Rantigny, France) and a computerized data acquisition system. PLA pellets or extruded composite rods were placed into cylindrical open molds (inner diameter, 35 mm) and loaded in the pressure vessel. Pressure was increased up to saturation pressure P_{sat}, and temperature was increased up to 195°C, above the PLA melting temperature. Polymer saturation by CO_2 was completed after 10 min. Foaming was then achieved by sudden gas release, with simultaneous gas cooling and consequently foam cooling also. Initial depressurization rate *dP/dt*, controlled by a back-pressure regulator, and maximum cooling rate *dT/dt* are significant parameters that affect pore expansion and stabilization. Four processing conditions were investigated to manufacture the scaffolds tested in this study (Table 1).

For cell culture, scaffolds were machined into half-cylinders (4 mm high, 8 mm in diameter). Samples were sterilized in 24-well plates overnight in phosphate-buffered saline (PBS), pH 7.4, containing penicillin and streptomycin at concentrations of 1000 units/mL and 1000 μg/mL, respectively (Invitrogen, Carlsbad, CA).

Scaffold characterization

Polymer and composite foams were cut with a razor blade and coated with gold to avoid charge accumulation

Table 1. Foaming Conditions Investigated to Process Tested Scaffolds

Condition	*Saturation pressure* P_{sat} *(bars)*	*Cooling rate* dT/dt *(°C/s)*	*Gas release rate* dP/dt *(bars)*
C1	242	4.4	13.3
C2	100–150	5.1	12.1
C3	208	3.4	2.3
C4	225	4.7	4.5

and prevent sample damage. Specimens were observed by scanning electron microscopy (SEM) (Philips XL30; FEI, Acht, The Netherlands), under a high tension of 5 kV with secondary electrons for simple topographic observation. Average pore diameter *d* was evaluated by image analysis. Foam samples were first embedded with EpoFix resin (Struers, Balterup, Denmark) under vacuum. Polished surfaces were then observed with an optical microscope (BH2; Olympus America, Melville, NY), to detect the maximum number of pores. AnalySIS software (Soft Imaging System, Münster, Germany) was used to estimate pore diameter. Porosity ε^* was evaluated by apparent density measurement, by weighing a sample of known volume. Compression behavior of foam was evaluated with specimens of measured density. Samples were prepared, paying special attention to obtaining parallel surfaces, perpendicular to the testing direction. Measurements were carried out with compression equipment (UTS Testsysteme, Ulm, Germany), with a cross-head speed of 0.5 mm/min. Elastic modulus E^* was evaluated from the initial linear elastic part of the stress–strain curve. Results of architectural and mechanical analyses are given in Table 2.

Cell sources

Human fetal and adult bone cells were obtained from our bank of bone cells comprising 32 adult donors (18 females and 14 males) and 5 fetal donors (1 female and 4 males) at the end of January 2005. More than 250 primary total hip replacements with potentially interesting orthopedic tissue samples are performed each year at the Hôpital Orthopédique de la Suisse Romande (Lausanne, Switzerland). Documents relative to the patients were consulted to confirm the absence of major disease, medications, and alcohol consumption. Bone samples were extensively collected and care was taken to obtain healthy bone samples. In particular, the selected adult bone cells were from donors without bone necrosis. In this study, human trabecular bone biopsies at femoral locations obtained from a 60-year-old adult woman subsequent to orthopedic surgery, and from a fetus (gestational age, 16 weeks) after voluntary interruption of pregnancy, were used. Biopsies were obtained in accordance with the Ethics Committee of University Hospital at Lausanne (Ethical Protocol 51/01). Primary osteoblast cultures were established by rinsing the tissue first with PBS (containing penicillin and streptomycin). Afterward, bone samples were mechanically dissociated with a scalpel blade and transferred to 10-cm culture-grade plates, where cell outgrowth was seen within 2 to 5 days under normal culture conditions: Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA), 10% fetal calf serum (FCS; Sigma-Aldrich, St. Louis, MO), and 4 mM L-glutamine (Invitrogen).

Cell culture

Fetal and adult bone cells were maintained in culture by passaging once per week and culturing at 37°C in a humidified, 5% CO_2 atmosphere in DMEM, 10% FCS, 4 mM L-glutamine. Culture medium was changed twice per week. Cells were used in the third passage for all experiments.

Biocompatibility test preparation

Scaffolds were placed in new 24-well plates and washed with complete culture medium. Cells (120,000), prepared as described above, were then seeded on samples by direct pipetting. After 1 h, culture medium was added around the scaffolds and renewed every second day. At these time points, osteogenic factors 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, 10 nM; Alexis Biochemicals/MP Biomedicals, Carlsbad, CA), 10 nM dexamethasone (Sigma-Aldrich), in addition to L-ascorbic acid (50 μg/mL; Sigma-Aldrich) and 1 mM β-glycerophosphate (Sigma-Aldrich), were added to the growth medium, to promote cell differentiation. Cell growth was observed in the presence of the scaffolds during the 4-week exposure time. Blanks consisted of scaffolds placed in the culture medium but without cells. As controls, polystyrene (PS) wells, which were not used for polymer samples, were seeded. The experiment was performed twice, in triplicate for each scaffold.

Antibodies and immunofluorescence microscopy

Fetal and adult cells seeded directly on PLA/HA IV and PLA/β-TCP IV composites were allowed to grow for 72 h. Cells were then observed in close proximity to the

Table 2. Properties of Scaffolds Tested in Biocompatibility Study

Sample	*Condition*	*Material*	*Porosity* ε^* *(%)*	*Pore diameter* d *(μm)*	*Modulus* E* *(MPa)*
PLA I	C1	PLA	91.9 ± 2.1	170 and 1030	5.2 ± 4.8
PLA II	C2	PLA	83.1 ± 0.8	600 ± 90	52.2 ± 4.5
PLA III	C3	PLA	77.4 ± 0.6	310 ± 100	82.9 ± 18.6
PLA/HA IV	C4	PLA+5%HA	79.2 ± 0.9	400 ± 250	133.2 ± 15.2
PLA/β-TCP IV	C4	PLA+5%β-TCP	83.4 ± 2.5	390 ± 180	121.0 ± 12.1

different materials. Immunostaining was performed as described previously.[28] Briefly, cells were permeabilized for 5 min with 0.2% Triton X-100 (TX-100) in 3% paraformaldehyde (PFA) and fixed with 3% PFA–PBS for 10 min. We used primary antibody against alkaline phosphatase (R&D Systems, Minneapolis, MN) and as secondary antibody Alexa 488-conjugated goat anti-mouse antibody (Molecular Probes, Eugene, OR); F-actin was probed with phalloidin–Alexa 568 (Molecular Probes) and DNA with 4′,6-diamidino-2-phenylindole (DAPI; Fluka, Buchs, Switzerland). Images were acquired with a ×63 objective (Neofluar, NA 1.4; Carl Zeiss, Feldbach, Switzerland) on an inverted microscope (Zeiss Axiovert 135), equipped with a digital charge-coupled device (CCD) camera (Hamamatsu C4742-95-12ERG; Bucher Biotec, Basel, Switzerland) and Openlab 3.1.2 software (Improvision, Basel, Switzerland).

Alkaline phosphatase activity

Alkaline phosphatase (ALP) activity was determined at the end of the exposure time, using *p*-nitrophenol tablets as substrate (Sigma-Aldrich), and values were quantified by comparison with a standard curve obtained with serial dilutions of alkaline phosphatase from *Escherichia coli* (Sigma-Aldrich). Enzymatic activity values were then normalized to the total protein content determined by total cellular protein assay.

Total cellular protein

Scaffolds colonized by cells were rinsed twice with PBS, and total protein was determined in cell lysates by protein assay (*RC DC* protein assay kit II; Bio-Rad, Hercules, CA). Total protein values were quantified by comparison with a standard curve obtained with serial dilutions of bovine serum albumin contained in the Bio-Rad protein assay kit II (Bio-Rad).

Osteocalcin synthesis

Quantitative determination of human γ-carboxylated Gla-osteocalcin (Gla-OC) was achieved in culture supernatants, using an *in vitro* immunoassay Gla-type osteocalcin EIA kit (Cambrex Bio Science, Walkersville, Walkersville, MD). Measurements were done according to the procedure furnished and sample concentrations were determined by comparing their specific absorbance with those obtained for the standards. Values represent accumulation of the polypeptide during 72 h at the end of the 4-week exposure to the scaffolds. They were then normalized to the total protein content of the cell lysates determined by total cellular protein assay.

In vitro *mineralization*

Porous constructs were rinsed twice with PBS. The degree of *in vitro* calcium deposition was determined by von

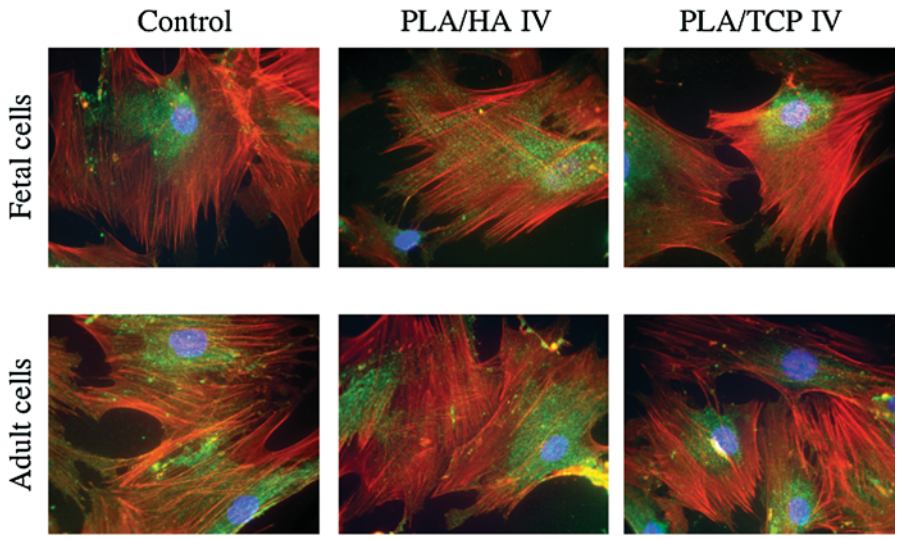

FIG. 1. Primary human fetal cells (*top row*) and adult osteoblasts (*bottom row*). Cells were seeded in the absence (*left*) or presence of composites PLA/HA IV (*middle*) and PLA/β-TCP (*right*). Specific immunostaining for ALP (green), F-actin (red), and DNA (blue) were used: primary antibody against alkaline phosphatase (R&D Systems, Minneapolis, MN) and as secondary antibody Alexa 488-conjugated goat anti-mouse antibody (Molecular Probes, Eugene, OR); F-actin was probed with phalloidin–Alexa 568 (Molecular Probes) and DNA with DAPI (Fluka, Buchs, Switzerland). Images were acquired with a ×63 objective (Neofluar, NA 1.4; Carl Zeiss, Feldbach, Switzerland) on an inverted microscope (Zeiss Axiovert 135), equipped with a digital CCD camera (Hamamatsu C4742-95-12ERG; Bucher Biotec, Basel, Switzerland) and Openlab 3.1.2 software (Improvision, Basel, Switzerland). In all cases, adherent osteoblasts with normal morphologies and expressing alkaline phosphatase were observed, indicating perfect biocompatibility of the tested scaffolds.

Kossa staining. This test is used extensively to reveal *in vitro* mineralization. Briefly, the cells were fixed for 5 min in 10% neutral formalin and washed three times with deionized water. Samples were then stained with 5% $AgNO_3$ (in water). The plates were exposed for 1 h under ultraviolet (UV) light and washed three times with water. A solution of 5% sodium thiosulfate was then added for 2 min. Finally, scaffolds were washed three times with water and examined for black clusters by light microscopy. The experiment was performed twice, in triplicate.

Scanning electron microscopy

Cells were seeded as previously described and exposed for 4 weeks to the osteogenic factors. Subsequently, cells were fixed with glutaraldehyde, followed by a dehydration procedure in ethanol. The sample surfaces were coated with gold for 2 min under 1 kV and observed with a Philips XL30 scanning electron microscope. Using a voltage of 5 kV and secondary electrons, surface topography was examined. During SEM observations, energy-dispersive X-ray microanalyses were carried out locally at a high tension of 10 kV. Selective microanalysis enables the detection of specific elements present on the surface. For example, phosphorus and calcium, which are present in calcium phosphate compounds produced during mineralization by osteoblasts,[29] can be analyzed.

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was carried out to confirm the presence of phosphorus. Moreover, it can indicate to which chemical group the element phosphorus belongs, and can also lead to a quantitative analysis. However, XPS is a technique sensitive to surface roughness. Therefore bulk PLA platelets were prepared by compression molding (195°C, 10 MPa, 10 min) and then sterilized as previously described. Cells were seeded and cultured under conditions similar to those used for scaffolds. Subsequently, cells were fixed with glutaraldehyde, followed by a dehydration procedure in ethanol. XPS spectra were acquired on a Kratos AXIS Ultra spectrophotometer operating at a base pressure of 10^{-9} mbar and equipped with a monochromatized Al Kα source. Acquisition was done with a pass energy of 80 eV (acquisition time, 120 ms). Samples were investigated with a charge compensation gun (emission current, 0.15 μA). Chemical elements present on the surface can thus be analyzed.

Statistical analysis

ALP and Gla-OC assays were carried out twice, each time in triplicate. Similar results were obtained and data are expressed as the mean of one experiment ± SEM.

RESULTS

Cell proliferation and differentiation

When seeded directly on scaffolds, fetal and adult bone cells did proliferate and differentiate in their immediate vicinity (Fig. 1). Furthermore, both adult and fetal cells

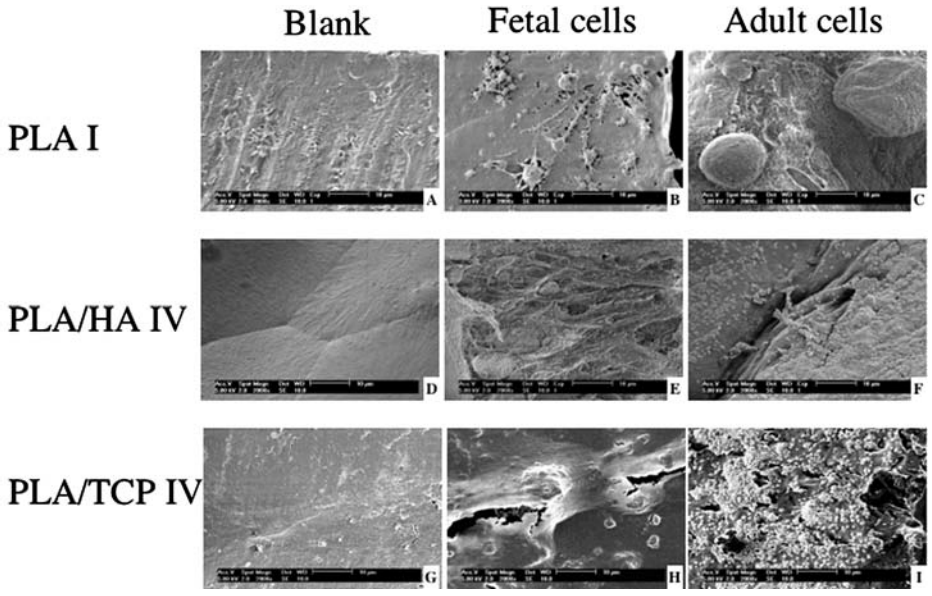

FIG. 2. SEM micrographs of uncolonized scaffolds (*left*) and of scaffolds colonized by fetal cells (*middle*) and adult cells (*right*). After 4 weeks of treatment with osteogenic factors, cells were visible on the scaffold surfaces, with extracellular matrix deposition and mineral crystals. The cells exhibited multiple cytoplasmic extensions that interconnected, wrapping the polymer surface. Macropores were gradually obturated by a multilayered cover of cells. The width scale was chosen in order to focus on cells lying on pore wall cross-sections and inside pores. Scale bars: 10 μm.

were able to spread on scaffold surfaces, independently of the foaming condition and of the presence or absence of ceramic fillers (Fig. 2). Some cells covered the macropore surfaces, whereas others sent cytoplasmic extensions across the apertures. Thus, the macropores were gradually obturated by a multilayered coat of cells growing toward the center of the pores. Cross-sections revealed that fetal cells penetrated inside the scaffolds better than did adult bone cells after 4 weeks of culture. Moreover, the inner colonization was more pronounced with composite scaffolds than with neat polymer foams. The results of ALP and Gla-OC biochemical assays indicated that the osteoblastic phenotype was either expressed by fetal cells or maintained by adult osteoblasts in all scaffolds (Figs. 3 and 4). In the case of ALP activity of fetal cells, there were two distinct groups of materials: PLA I and PLA II induced a lower enzymatic activity level than did PLA/HA IV and PLA/β-TCP IV. With adult cells a low level of ALP activity was measured for all materials tested. In the case of osteocalcin synthesis, a similar low level was observed with fetal cells in all scaffolds, whereas for adult cells, neat PLAs led to less Gla-OC synthesis than did composite scaffolds.

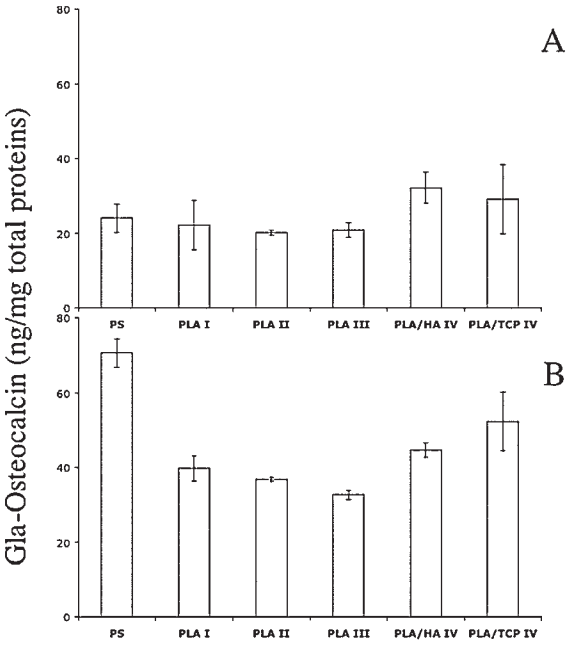

FIG. 4. Comparison of γ-carboxylated Gla-osteocalcin synthesized by fetal (**A**) and adult (**B**) osteoblasts seeded on PLA scaffolds after 4 weeks of exposure to osteogenic factors. The Gla-OC assay was carried out twice, each time in triplicate, and results are expressed as the mean of one experiment ± SEM. Representative data of one experiment are shown.

In vitro *mineralization*

The latest stage of osteoblast maturation is indicated by extracellular matrix mineralization. Maturing cells may start to induce mineralization, that is, the precipitation of small calcium phosphate crystals on the scaffold surface. Small, rounded or more polygonally shaped crystals were observed by scanning electron microscopy (Fig. 2). To determine their chemical nature, several approaches were taken.

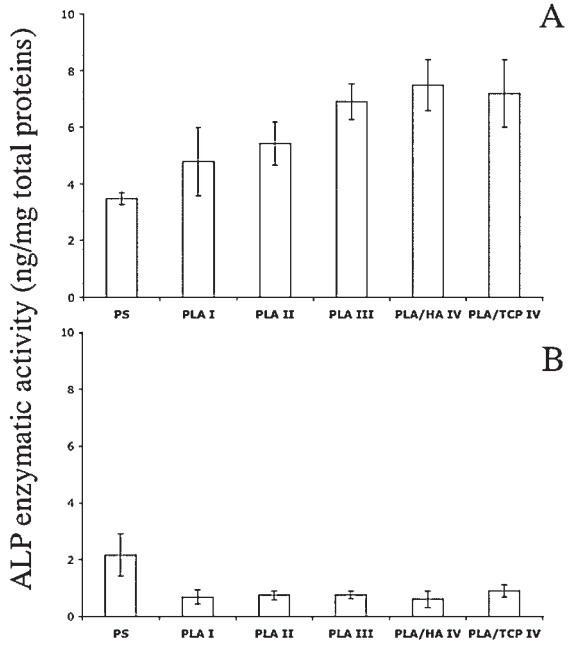

FIG. 3. Comparison of alkaline phosphatase enzymatic activity from fetal (**A**) and adult (**B**) osteoblasts seeded on PLA scaffolds after 4 weeks of exposition to osteogenic factors. The ALP assay was carried out twice, each time in triplicate, and results are expressed as the mean of one experiment ± SEM. Representative data of one experiment are shown.

von Kossa coloration of sample surfaces resulted in dark coloration, which indicates the presence of calcium (Fig. 5). All scaffolds in the presence of the two cell types were stained. SEM topographic observations, associ-

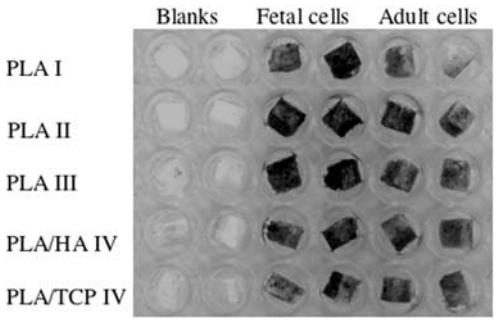

FIG. 5. von Kossa staining of PLA scaffolds. Fetal or adult bone cells were seeded on PLA samples as described in Materials and Methods. After 4 weeks of treatment with osteogenic factors, cells were fixed and samples were stained to reveal *in vitro* mineralization.

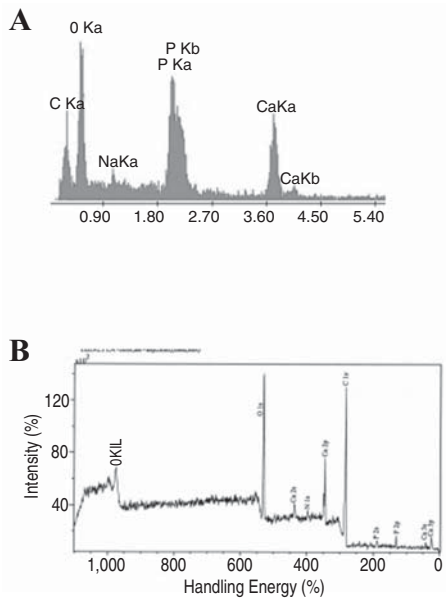

FIG. 6. Identification of mineral crystal deposits observed on a PLA I sample colonized by fetal bone cells: (**A**) energy-dispersive X-ray microanalysis and (**B**) X-ray photoelectron spectroscopy. Similar results were obtained with adult cells, and with PLA/HA IV and PLA/β-TCP IV surfaces. Samples analyzed refer to sample B of Fig. 2.

ated with X-ray microanalysis, were conducted on gold-coated samples with a high tension of 10 kV (Fig. 6A). On PLA scaffolds, crystals containing calcium (peaks at 3.71 and 4.02 keV) and probably phosphorus (peaks at 2.02 and 2.12 keV) were detected. However, phosphorus peaks were in the same area as those of gold, from coating (peak at 2.30 keV). To confirm the presence of phosphorus on the scaffold surface after cell proliferation and differentiation, X-ray photoelectron spectroscopy (XPS) was carried out on flat PLA samples after 4 weeks of cell culture (Fig. 6B). Composite samples were not tested, because they already contained calcium and phosphorus, which would have been detected, along with potential elements created by mineralization, by XPS. Phosphorus and calcium peaks are clearly visible, therefore confirming the presence of calcium phosphates on the PLA surface after fetal cell proliferation and differentiation. Oxygen, carbon, and nitrogen peaks result from the presence of extracellular matrix proteins on the surface.

DISCUSSION

The use of the extrusion method, to mix ceramic and polymer, followed by gas foaming, leads to scaffolds with controlled porosity and morphology, and targeted mechanical properties[25] without the use of potentially toxic organic solvents.[30,31] Fillers are mixed with the polymer at a given content, and homogeneously distributed in the composite preform before foaming. They are also well dispersed in pore walls in the final composite scaffolds, as evidenced by two-dimensional micro-computed tomography analysis (data not shown). Importantly, the filler content is not modified by the gas-foaming process. Compared with scaffolds described in the literature, highly porous scaffolds were prepared, with improved resistance in compression (Table 3). Scaffolds developed in this study showed high osteoconductive potential, allowing proliferation of bone cells through the porous structures. Fetal bone cells were already characterized for their high osteogenic potential,[27] and their proliferative aptitude observed on artificial structures is of major interest from the perspective of the use of human primary fetal bone cells in tissue engineering. During the exposure, fetal and adult bone cells seeded on the scaffolds were pushed toward differentiation, and osteoblastic genes such as cbfa-1, α_1 chain of type I collagen, alkaline phosphatase, and osteocalcin were expressed (data not shown). The cells seeded on these engineered scaffolds held their ability to differentiate into mature osteoblasts. On all scaffolds investigated, ALP and Gla-OC markers were expressed by fetal and adult cells. However, these two parameters follow different kinetics: alkaline phosphatase is expressed at an early stage of cell development,[32–36] whereas *in vitro* studies showed that osteocalcin is a marker of late osteoblast differentiation.[37–39] Therefore, the higher level of ALP enzymatic activity observed in this article with fetal cells compared with adult bone cells is not surprising. Likewise, the level of Gla-OC synthesis was higher in adult bone cells compared with fetal bone cells. As mature osteoblasts, adult bone cells are already involved in extracellular matrix deposition. Synthesis of osteocalcin confirmed their fully differentiated osteoblastic phenotype. With adult or fetal cells, no significant differences between the three polymers PLA I, PLA II, and PLA III were observed. Therefore cell development did not seem to be affected by a difference in porosity or pore size.

As far as composite scaffolds PLA/HA IV and PLA/β-TCP IV are concerned, a higher level of ALP activity was observed for fetal cells, and a higher level of Gla-OC synthesis was measured for adult cells. The presence of ceramic fillers, either HA or β-TCP, therefore favored differentiation of fetal and adult bone cells owing to their osteoconductive properties.[16,26,40] Finally, extracellular matrix deposition and *in vitro* mineralization were observed on all scaffolds, indicating that complete differentiation did occur. Fetal bone cells colonized the whole of PLA and composite scaffolds, whereas adult bone cells showed mainly superficial growth. von Kossa staining corroborated these observations. Composites seeded with fetal cells displayed more extensive proliferation than did neat PLA. X-ray microanalyses and XPS detected calcium and phosphorus on cell-cultured surfaces but not on

Table 3. Comparison between Foam Properties Obtained in This Study and in the Literature[a]

Study	*Material*	*Porosity (%)*	*Modulus (MPa)*	*Elastic collapse stress (MPa)*	*Average diameter (μm)*	*Anisotropy*	*Structure/ foaming technique*
Targeted properties	Cancellous bone	75[b]	100–300[c]	5–10[c]	200–400[d]	Yes	Anisotropy, interconnected
This study	Neat PLA	70–90	20–180	2–3.5	200–100	Yes	Pore size gradient
This study	PLA–ceramic	75–90	30–200	2–6	200–100	Yes	Pore size gradient
Marra *et al.* (Ref. 15)	PLGA	—	2.5 ± 0.7	—	—	No	Solvent casting
	PLGA–10% HA	—	12.5 ± 3.2	—	—	No	
Ma *et al.* (Ref. 40)	L-PLA	92.7	6.42 ± 1.44	0.32 ± 0.04	10–100	Yes	Microtubular/TIPS
	PLA–50% HA	89.2	10.87 ± 3.2	0.39 ± 0.01	10–100	Yes	Microtubular/TIPS
Walsh *et al.* (Ref. 50)	HA	—	—	9	—	No	HA coated with PCL
	HA–PCL	—	—	37	—	No	HA coated with PCL
Boccaccini and Maquet (Ref. 41)	PLGA	94.3	8	—	—	Yes	TIPS
	PLGA–50% Bioglass	89.9	23	—	—	Yes	TIPS
Wei and Ma (Ref. 51)	L-PLA	93	4.3	—	—	Yes	Nano-HA/TIPS
	PLA + 50% HA	91.8	8.3	—	—		

Abbreviations: HA, hydroxyapatite; PCL, polycaprolactone; PLA/L-PLA, poly(L-lactic acid); PLGA, poly(lactic-*co*-glycolic acid); TIPS, thermally induced phase separation.

[a]From Refs. 15, 40, 41, 50, and 51.

[b]See Ref. 52.

[c]See Ref. 53 and 54.

[d]See Ref. 55.

control specimens, indicating that crystals were produced only in the presence of cells proliferating and differentiating on the scaffolds, and were not the result of simple chemical precipitation. After 8 weeks of culture with fetal cells, no sign of polymer degradation was noticed, either structurally or by acidification of the culture medium. Moreover, no alteration of mechanical properties was observed (data not shown). This is particularly worthwhile for orthopedic implants, which must support significant loading.

We conclude that porous structures obtained by supercritical gas foaming of PLA offer suitable conditions for osteoblasts to achieve full differentiation. This solvent-free method may be of great interest for bone tissue engineering, to obtain open frames with controlled parameters, such as porosity, pore size, and connectivity. This procedure enables mass production of biocompatible samples with improved mechanical properties, as expected, and as has also been obtained by other methods.[15,41] Furthermore, we demonstrate for the first time that once seeded with human fetal bone cells, such osteoconductive scaffolds are promising because of the high osteogenic potential of these cells. Fetal-associated tissues such as placenta, amniotic liquid, and umbilical cord are potential sources of cells for tissue engineering.[42–45] Human fetal liver cells have been used for transplantation when there was no perfectly matched donor for marrow transplantation.[46] Neuronal conditions such as Huntington's[47] or Parkinson's disease[48] have been treated with fetal neuroblasts. Concerning bone grafts, the decreasing ability of adult mesenchymal stem cells derived from osteoporotic donors to differentiate into the osteogenic lineage was demonstrated.[49] Thus, a bank of fetal bone cells could be of great interest for tissue-engineering applications.

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