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Integrin expression by human osteoblasts cultured on degradable polymeric materials applicable for tissue engineered bone

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Abstract

The use of biodegradable polymers in the field of orthopaedic surgery has gained increased popularity, as surgical pins and screws, and as potential biological scaffolds for repairing cartilage and bone defects. One such group of polymers that has gained considerable attention are the polyesters, poly(lactide-co-glycolide) (PLAGA) and polylactic acid (PLA), because of their minimal tissue inflammatory response, favorable biocompatibility and degradation characteristics. The objective of this study was to evaluate human osteoblastic cell adherence and growth on PLAGA and PLA scaffolds by examining integrin receptor ($\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$ and β_1) expression. Primary human osteoblastic cells isolated from trabecular bone adhered efficiently to both PLAGA and PLA, with the rate of adherence on PLAGA comparable to that of control tissue culture polystyrene (TCPS), and significantly higher than on PLA polymers at 3, 6 and 12 h. Human osteoblastic phenotypic expression, alkaline phosphatase (ALP) activity was positive on both degradable matrices, whereas osteocalcin levels were significantly higher on cells grown on PLAGA than on PLA composites. Interestingly, the integrin subunits, $\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$ and β_1 were all expressed at higher levels by osteoblasts cultured on PLAGA than those on PLA as analyzed by westerns blots and by flow cytometry. Among the integrins, α_2, α_5 and β_1 showed the greatest difference in levels between the two surfaces. Thus, both PLA and PLAGA support osteoblastic adhesion and its accompanying engagement of integrin receptor and expression of osteocalcin and ALP. However PLAGA consistently appeared to be a better substrate for osteoblastic cells based on these parameters. This study is one of the first to investigate the ability of primary human osteoblastic cells isolated from trabecular bone to adhere to the biodegradable polymers PLAGA and PLA, and to examine the expression of their key adhesion receptors (integrins) on these substrates. © 2002 Orthopaedic Research Society. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Polymers; Human osteoblast; Integrins; Tissue engineering; Cell–polymer interaction

Introduction

Successful replacement of bone lost, due to trauma or diseases, poses a great challenge in the field of orthopaedics [14,27]. Conventional treatment continues to rely on biological grafts such as autografts and allograft, in spite of inherent limitations [8,14]. Allogenic grafts, i.e. bone tissues obtained from a cadaver [32], have the advantage that tissues are readily available and can be easily applied, especially when large segments of bone are to be reconstructed [8,14]. However, not only is the patient at risk for graft rejection, but allografts may also be a source of blood-borne pathogens and viral agents [4,14]. Autogenous grafts, tissues taken from the same individual and relocated to a different area, do not have the disadvantages associated with allografts [3,7,8,34]. Clinically, autografts have exhibited optimal biological behavior and interaction at the bone implant site [14,34]. Furthermore, there are no risks of disease transmission or graft rejection, which can be problematic for allografts [3,7,14]. However, donor site morbidity, and more

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importantly, limited availability and the additional procedure required for extraction of the graft make the use of autogenous bone less then ideal for osseous repair [14].

In order to circumvent the aforementioned limitations associated with biological grafts, our approach has been to develop biodegradable polymer and polymer-composite-based matrices for bone tissue engineering. Biodegradable polymers are ideal candidates for application in bone regeneration systems for several reasons. Firstly, polymers are readily available commercially and secondly, polymeric matrices can be engineered to promote optimal growth and compatibility with the tissue of interest [12,35]. Finally and most importantly, due to its biodegradable nature, removal of the polymeric implant is not required [25,30]. Clinically, biodegradable polymers have been utilized in orthopaedics as screws, pins and other devices for fracture fixation and repair [2,11,12,15,21,22,25,26,30,35]. In particular, one class of polymers known as polyesters, specifically poly(lactide-co-glycolide) (PLAGA) and polylactic acid (PLA) have been approved by the FDA for use as materials for wound repair and bone fixation pins [11,14,25]. Moreover, both PLA and PLAGA have been used successfully in implant models for fracture repair [30,35].

Little is known about the biomolecular events associated with successful osteoblastic cell growth on these materials. The initial cell–polymeric scaffold interaction represents an important process which directly influences stable cell adhesion and maturation on the surface of the matrix [41–43]. Stable osteoblastic cell adhesion is largely mediated by integrins, heterodimeric receptors that interact with extracellular matrix (ECM) proteins, such as fibronectin, vitronectin, fibrinogen and collagen [7,9,17,42], and allows the cell to respond to its extracellular environment as well as modulate cellular events that regulate remodeling. Thus, integrin transmembrane receptors have been shown to be involved in signal transduction, gene regulation, proliferation, differentiation, and apoptosis [1,5,7,9,10,16–18,23,24,38,41,42]. Integrins, are composed of α and β subunits that dimerize in specific combinations [9,40–44], with at least 12 different α and nine β subunits described to date [1,9,41–43]. A particular integrin subunit pair may bind to more than one ECM protein and conversely, an ECM protein may bind to more than one integrin receptor [16]. The importance of integrin–ECM interaction in osteoblastic formation is demonstrated by studies of human and rat osteoblastic cells, where plating on different collagen type I matrices affected cell morphology and maturation [9].

Several studies have examined osteoblastic cell integrin production in vitro and in vivo. Specifically, primary human osteoblast grown on tissue culture polystyrene (TCPS) expressed the integrin subunits $\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$, β_1 and β_3; however on surfaces such as titanium and cobalt-chrome, α_5, α_6 and β_1 [41,42]. These differences demonstrate that binding of osteoblasts to various surfaces is likely to be mediated by different integrin subunit combinations. However, to date, there have not been any studies examining the types of integrins expressed by osteoblasts grown on biodegradable polymers.

The objective of the current study is firstly to characterize the adhesion mechanism of primary human osteoblasts on the biodegradable polyester polymers, PLA and PLAGA. Secondly and most importantly, we will examine the expression of integrin subunits on these cells. We hope to gain an understanding of the mechanisms by which osteoblasts successfully attach and mature on biodegradable polymers.

Materials and methods

Biomaterials

Poly(lactide-co-glycolide) (PLAGA, 50:50, Mw = 50,000; American Cyanamid, Sunnyvale, CA, USA) and poly-L-(lactic acid) (PLA, Mw = 60,000; Purac, Netherlands) polymers were first dissolved in methylene chloride (Aldrich, 0.1 mg/ml), then stirred at a constant speed for 3 h. The dissolved polymer was poured into a Teflon-coated dish and placed under a vacuum hood for 2 h, and allowed to settle slowly overnight at −20°C. After evaporation of the solvent, the polymer was bored into discs 1.4 cm in diameter and 0.4 cm in height. The discs were subsequently lyophilized (Labconco, Kansas City, USA) for at least 24 h to remove residual solvent and stored at −20°C under argon.

Scanning electron microscopy

The polymeric surfaces (PLAGA and PLA) prior to and after cell attachment were examined by scanning electron microscopy (SEM). Matrices were fixed using 4% paraformaldehyde solution (Polysciences, Warrington, PA, USA) for 24 h, and washed three times in 0.1 M phosphate buffered saline (PBS) (pH 7.4) to remove residual fixative. The samples were then dried using a graded series of ethanol (50–100%) at 15 min intervals. After drying, the samples were sputter coated with gold using a Denton Desk-1 Sputter Coater, and examined at an accelerating voltage of 20 keV using an Amray 1830-D4 SEM equipped with a Tungsten electron gun.

Human osteoblastic culture

Human osteoblastic cells from trabecular bone were isolated from the femoral head of a patient undergoing hip arthroplasty using the method of Robey and Termine [37] as modified by Sinha et al. [41–43]. Briefly, under sterile conditions, trabecular bone was dissected from the femoral head using a surgical curette, placed in vials containing calcium-free DMEM/F 12K (Dulbeco's Modification of Eagles Essential Medium) (Specialty Media, Lavallette, USA), and minced into 0.3–0.5 cm bone chips. The bone chip suspension was then washed repeatedly in medium. The bone chips were then digested in a collagenase P enzyme medium (Sigma, St. Louis, MO, USA) while stirred constantly at 37°C in 5% CO_2 for 3 h to degrade the fibrous matrix. Digested chips were then washed repeatedly in sterile PBS, vortexed between washes at a low constant speed, and then placed in a culture flask (COSTAR, Cambridge, MA, USA) containing calcium-free DMEM/F 12K medium supplemented with 10% Fetal Bovine Sreum (FBS), 2 mM L-glutamine, 50 μg/ml ascorbate and 50 μg/ml penicillin/streptomycin at 37°C and 5% CO_2. Within two weeks, cells migrated from the bone chips and reached confluency at 3–4 weeks. At this time, the medium was supplemented with 110 mM $CaCl_2$. Cells

obtained under these conditions, consistently exhibited characteristic criteria for osteoblast such as morphology, the expression of osteocalcin and ALP production.

Cell adhesion

For adhesion studies, human osteoblastic cells were plated at 2×10^4 cells/disc on PLAGA, PLA and the control TCPS over a 12 h period. Confluent human osteoblastic cells were removed using 0.5% trypsin (Sigma Chemical, St. Louis, MO, USA). Cultures were then incubated at 37°C in 5% CO_2 for 12 h.

At 3, 6, and 12 h after plating, cell adhesion was measured using a fluorimetric dye, 2′7′-bis(2-carboxyethyl)5-carboxyfluorescein acetyoxymethylester (BCECF-AM), (Molecular Probes, Junction City, OR, USA) that is internalized within living cells [41]. Thirty min prior to harvesting, cells were washed in phosphate buffered saline (PBS, pH 7.4) and incubated with BCECF-AM in serum-free medium for 30 min at 37°C in 5% CO_2. Cells were washed twice with PBS and lysed using 1% Triton X-100 buffer (Fisher Scientific, Fair Lawns, NJ, USA). Dye release was determined spectrofluorimetrically (Spectrofluor Plus, Tecan, Triangle Park, NC, USA), at an excitation wavelength of 485 nm and emission wavelength of 535 nm. Cell number was determined by comparison to the dye intensities released from previously identified cell numbers.

Cell morphology studies

At 3, 6 and 12 h post-plating, human osteoblastic cells plated on PLAGA and PLA at 1.0×10^4 cells/disc surfaces were washed in PBS, fixed in a 2% paraformaldehyde solution for 15 min, washed twice in PBS and then stained with a 0.1% crystal violet solution for 20 min. The samples were washed again in PBS to remove residual stain and visualized using light microscopy (Hitech Instruments, PA, USA). In addition, parallel cultures were examined using SEM as described previously.

Osteocalcin and alkaline phosphatase

Human osteoblastic cells were grown to confluency, trypsinized and plated at 5×10^4 cells/cm^2 on PLAGA, PLA and TCPS surfaces, and then continuous maintenance for 72 h in serum-free DMEM/F12 medium containing 2 mM L-glutamine, 50 μg/ml ascorbate and 50 μg/ml penicillin/streptomycin at 37°C, 5% CO_2. An osteocalcin assay kit was used (Biological Technologies, Cambridge, MA, USA) to detect osteocalcin levels in the media.

To determine alkaline phosphatase (ALP) levels, human osteoblastic cells were plated on PLAGA and PLA matrices at a concentration of 1×10^5 cells/disc. At 72 h, cells were washed in phosphate buffered saline (PBS), and ALP activity was detected histochemically using the ALP diagnostic kit (Sigma Chemical, St. Louis, MO, USA). The stained samples were then mounted and visualized using a light microscope (Axiovert, Carl Zeis, Therwood, NY, USA). The percent of cells stained positive was determined as well.

Western blot analysis of integrin subunits

Human osteoblastic cells were plated at 5×10^4 cells/disc on PLAGA and PLA matrices for 24 h, trypsinized and the collected cell pellets were lysed in a 1% Triton X-100 solution in the presence of protease inhibitors at 4°C overnight. Total protein concentration was measured using a protein assay kit (Pierce, Rockford, USA). Sixty μg of protein was fractionated by a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 7% gel and electrotransferred to nitrocellulose paper (Schleicher and Schuell, Keene, USA). Non-specific protein–protein interactions were minimized by incubation of the blot in 3% bovine serum albumin in 0.5% Tween 20-Tris buffered saline solution for 2 h. The blots were incubated with rabbit polyclonal or monoclonal antibodies specific to the following integrins, $\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$ and β_1 at (1:100) (Chemicon International, Temecula, CA, USA) for 2 h. The blot was then washed, blocked and developed with ALP-conjugated anti-rabbit antibody using the Zymed Immunoblot SAP kit (Zymed Laboratories, San Francisco, CA, USA).

Flow cytometric analysis

Flow cytometric analysis was used to measure the number of cells expressing the integrin specific subunits on their surfaces. Confluent human osteoblastic cells were washed in PBS, removed with 0.05% trypsin, and plated on PLAGA and PLA matrices at 5×10^4 cell/cm^2 for 24 h. Cells were washed in PBS and trypsinized in 0.02% trypsin for 5 min in order to prevent removal of the cell surface proteins [23]. Cells were neutralized in medium, pelleted and incubated in 100 μl (1:100) of the desired integrin antibody $\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$ and β_1 (Chemicon International, Temecula, CA, USA) at 4°C for 1 h. The cell suspension was washed in PBS followed by incubation with a fluorescein isothiocyanate-conjugated goat anti-mouse antibody (1:100) (Sigma, St. Louis, MO, USA) in PBS at 4°C for 30 min. The cell suspension was then washed, pelleted and fixed in 2% paraformaldehyde solution for 2 h. The fixed cell samples were resuspended in 300 μl of PBS, and analyzed using a flow cytometer (Model 50H, Ortho Diagnostic Systems, Westwood, MA, USA). Fluorescence intensity was measured and the number of cells with positive surface proteins was determined.

Statistical analysis

Statistical analysis were performed for the experiments when deemed necessary using a one-tailed Student's *t*-test. All experiments were repeated.

Results

Scanning electron microscopy

The surfaces of PLAGA and PLA were examined using SEM with and without human osteoblastic cells after 24 h plating. Bioerodible polymers without human osteoblastic cells exhibited a uniform matrix, with a smooth surface (Fig. 1(a), PLA and (c), PLAGA). Human osteoblastic cells plated onto PLA and PLAGA matrices, exhibited morphologies consistent with the osteoblastic phenotype observed on various surfaces (Fig. 1(b), PLA and (d), PLAGA) [19]. The results showed that human osteoblastic cells could adhere to these smooth surfaces and exhibited their characteristic morphology.

Cell adhesion

The adhesion kinetics of human osteoblastic cells plated on biodegradable polymers PLAGA, PLA, and control TCPS are shown in Fig. 2. By 3 h, a significantly higher number of cells adhered to PLAGA matrices than to both PLA and TCPS. At 6 h, cell adhesion to PLAGA was similar to the control TCPS, but higher than on PLA matrices. By 12 h, the number of cells that adhered remained similar for PLAGA and TCPS but significantly less on PLA. Over the 3–12 h time course, cells consistently exhibited a high level of adherence to PLAGA.

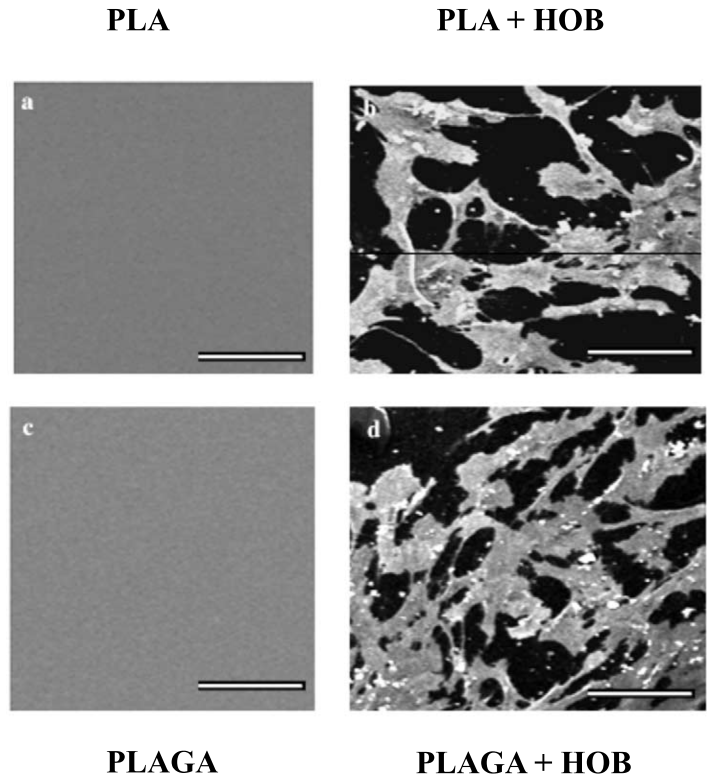

Fig. 1. SEM of surfaces of polylactic acid (PLA) and polylactide-co-glycolide acid (PLAGA) without and with human osteoblastic cells. Bar = 100 μm. A smooth even surface was seen for both matrices. Human osteoblasts were able to adhere and proliferate on the matrices: (a) PLA surface; (b) PLA with human osteoblast at 24 h; (c) PLAGA matrix surface; and (d) PLAGA surfaces covered by human osteoblastic cells at 24 h.

Osteocalcin production and alkaline phosphatase activity

As shown in Fig. 3 at 72 h post-plating, human osteoblastic cells seeded on PLAGA expressed osteocalcin levels comparable to cells grown on the control TCPS and was noticeably higher than the levels on PLA. In addition, ALP staining at 72 h was seen to be associated with the cell membrane as well as intracellular sites in osteoblasts attached on both PLAGA and PLA matrices (Fig. 4(a)) and more than 70% of the cells grown on both surfaces were positive for ALP staining (Fig. 4(b)). The continued expression of these two osteoblastic markers indicates the maintenance of the osteoblastic phenotype by cells attached to the biodegradable matrices.

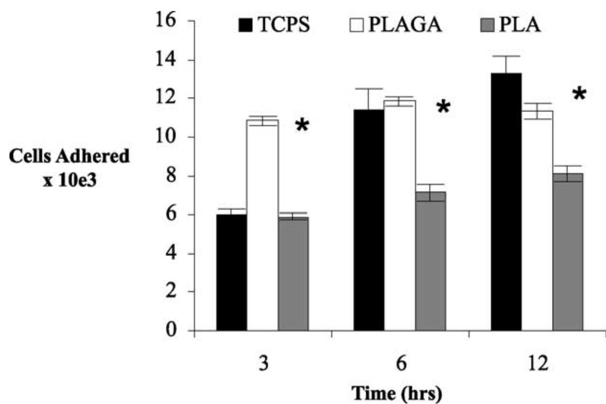

Fig. 2. Human osteoblastic cell adhesion on polymeric surfaces poly(lactide-co-glycolide) acid (PLAGA), poly(lactic acid) (PLA) and control tissue culture polystyrene (TCPS) at 3, 6 and 12 h. $N = 6$ and * denotes $P < 0.05$.

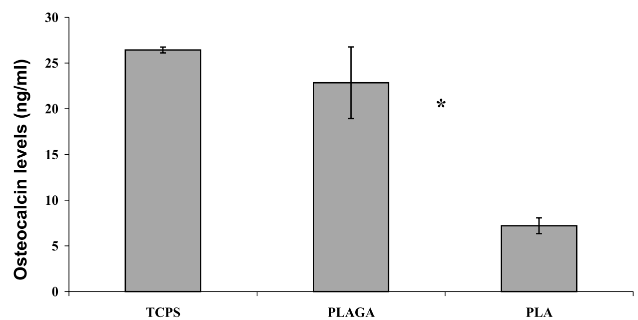

Fig. 3. Human osteoblastic osteocalcin expression on polymeric surfaces of poly (lactide-co-glycolide) acid (PLAGA), poly(lactic acid) (PLA) and the control tissue culture polystyrene (TCPS) at 72 h. $N = 3$ and * denotes $P < 0.05$.

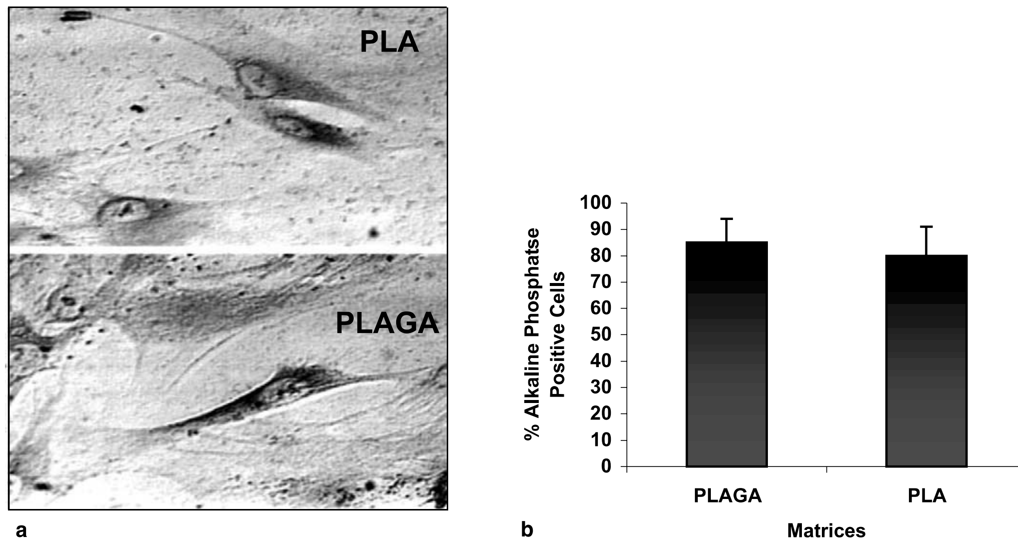

Fig. 4. Human osteoblastic phenotypic expression of alkaline phosphatase on polymeric surfaces of poly (lactide-co-glycolide) acid (PLAGA), and poly(lactic acid) (PLA) and the control tissue culture polystyrene (TCPS): (a) alkaline phosphatase staining in human osteoblastic cells grown on poly(lactide-co-glycolide) acid (PLAGA), and poly(lactic acid) (PLA) matrices at 72 h; (b) the percent of positive cells expressing alkaline phosphate on the matrices.

Human osteoblastic cell morphology

Human osteoblastic cells are described as polygonal, multilayered cells which form nodules and extend in a stellate-shaped fashion after attachment [39]. Cell morphology is a valuable parameter when evaluating cellular behavior on various materials. Human osteoblastic cells were grown on bioerodible matrices PLAGA and PLA to demonstrate their morphology and behavior on the surfaces. Fig. 5 represents the morphology at 3, 6 and 12 h on the various test substrates. At 3 h, cells appeared normal and maintained an equal distribution of their cytoplasm and membranous structure. By 6 h, cellular processes extended on the matrix and an increased appearance of stellate-shaped cells was noted, indicating early cytoskeletal organization. The appearance of this cellular morphology was more prevalent for cells seeded on PLAGA than on PLA surfaces. At 12 h, the morphology of the human osteoblast cell was largely polygonal, with cellular filopodia extending over the matrices, again, more prevalent for cells grown on PLAGA than on PLA matrices. In addition, cells grown on PLAGA matrices exhibited a monolayer formation as early as 6 h and continued through 12 h, respectively.

Integrin expression

We next measured levels of the integrin receptor subunits to determine relative expression on PLAGA and PLA. Expression of $\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$ and β_1 integrin subunit was determined in cells cultured on PLAGA and PLA matrices (Fig. 6). Human osteoblastic cells plated on PLAGA matrices expressed significantly higher levels of all integrins when compared to cells grown on PLA, determined by densitometry of western blots (Fig. 6(b)) and by flow cytometric analysis (Fig. 7). Western blot analysis showed increased intensities of the immunoreactive integrin protein bands in human osteoblastic cells grown on PLAGA (Fig. 7(a)) compared to cells seeded on PLA matrices. Quantitative densitometry confirmed the higher integrins subunit levels for PLAGA compared to PLA, with increases from α_2, 32%; α_3, 15%; α_4, 22%; α_5, 33%; α_6, 26% and β_1, 35% (Fig. 6(b)). Among the integrins, $\alpha_{2,}\alpha_5$ and β_1 were expressed at higher levels on the polymeric matrices studied. In addition, results confirmed by flow cytometric analysis revealed that human osteoblastic cells seeded on PLAGA matrices contained a higher number of cells positive for the cell surface integrin subunits relative to cells grown on PLA matrices (Fig. 7).

Discussion

The use of biodegradable polymeric matrices in the field of medicine has increased drastically over the last 30 years [2–4,12,27,30,35], and studies have illustrated their usefulness as implant devices for engineering scaffolds for both organ and tissue regeneration and for controlled delivery of macromolecules [11,12]. PLAGA and PLA represents one such class of biodegradable polymers that has been used widely and are being currently evaluated as candidate materials for our tissue

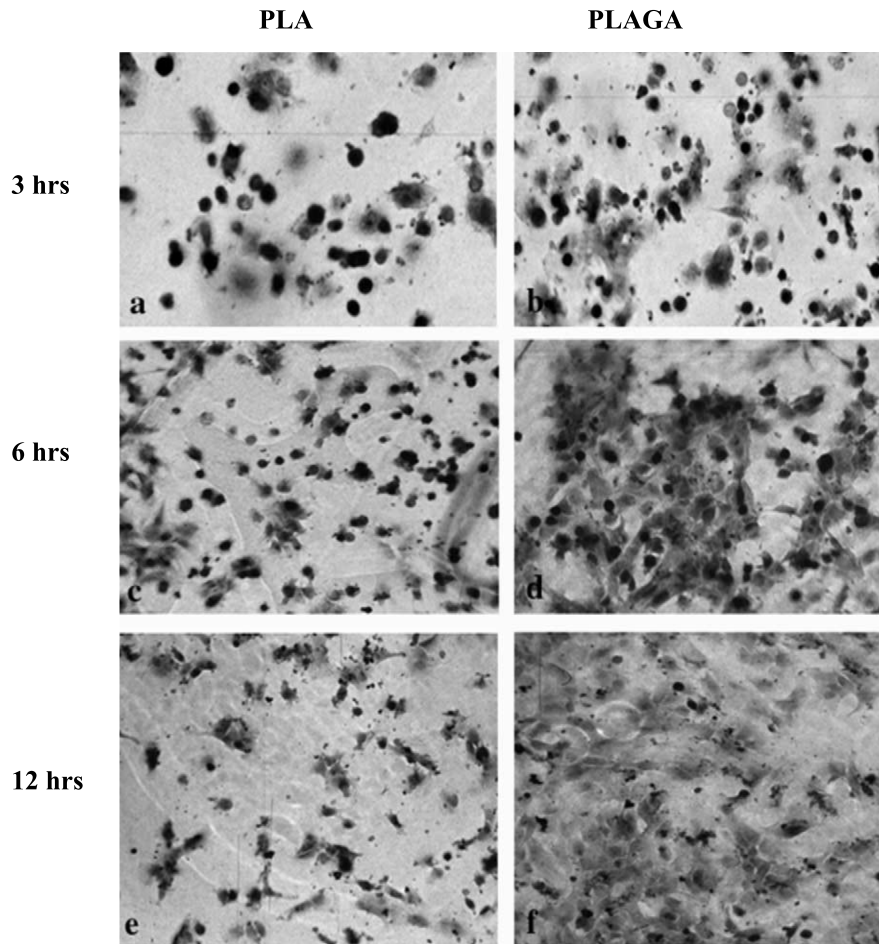

Fig. 5. Morphology of human osteoblastic cells grown on poly(lactide-co-glycolide) acid (PLAGA) and poly(lactic acid) (PLA) matrices at 3, 6 and 12 h: (a) PLA at 3 h; (b) PLAGA at 3 h; (c) PLA at 6 h; (d) PLAGA at 6 h; (e) PLA at 12 h; (f) PLAGA at 12 h.

engineered bone matrices. These polymers have been demonstrated to promote excellent biocompatibility and support osteoblast-like growth in vitro [14,28]. For example, Hollinger et al. have used PLA foams effectively as scaffolds for regeneration of bone defects of the mandible in vivo [21,22]. Our study here has provided crucial cell-based data to support the potential applicability of PLAGA and PLA for bone tissue engineering. This is one of the first studies to examine the morphology, phenotypic expression, adhesion characteristics and receptors of primary human osteoblastic cells on biodegradable polymers, PLAGA and PLA.

In this study, we initially focused on human osteoblastic cell adhesion on PLAGA and PLA. Adhesion was detected as early as 3 h on both degradable PLAGA and PLA, with a cellular morphological appearance consistent with studies reported on the behavior of osteoblasts cells grown on metal and TCPS surfaces [39]. However, by 12 h, cell adhesion was enhanced on PLAGA surfaces relative to the PLA matrices while comparable to the control substrate TCPS. Again, the osteoblastic morphology was maintained, especially on the PLAGA matrices, with a polygonal stellate-shaped appearance and an organized monolayer of interacting cells. These observations suggest that both PLAGA and PLA matrices are suitable materials for the support of osteoblast growth, particularly on PLAGA.

Supporting the osteoblastic phenotype of cells cultured on the biodegradable matrices, both osteoblast markers osteocalcin and ALP, were detected [6,31]. Osteocalcin is one of the most abundant proteins produced by bone [20], and is likely to be involved in bone remodeling through its interaction with other ECM molecules [6,13,36]. Human osteoblastic cells grown on both PLAGA and PLA matrices demonstrated osteocalcin expression, with levels higher on PLAGA relative to PLA, and similar to the control TCPS. The expression of osteocalcin on these materials indicate normal phenotypic behavior of osteoblastic cells seeded on these materials. For ALP activity, staining was positive on both PLAGA and PLA, suggesting that the ability of these cells to normalize is most likely not affected as result of interacting with these surfaces.

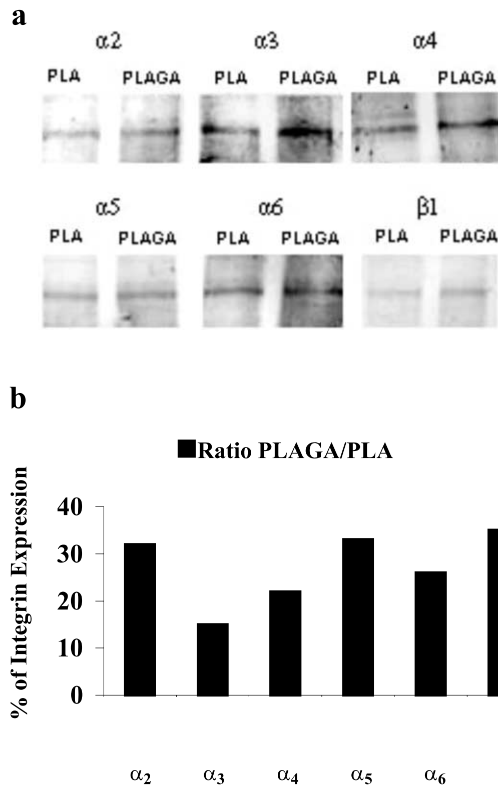

Fig. 6. Western blot analysis for the levels of integrins $\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$ and β_1 in human osteoblasts cultured on bioerodible polymers poly(lactide-co-glycolide) acid (PLAGA) and poly(lactic acid) (PLA): (a) immunoblot immunoreactive bands for all integrin subunits examined on both surfaces; (b) densitometry analysis for integrin subunits of human osteoblast cultured on biodegradable matrices. Results are presented as percent increase of band intensity in PLAGA samples over the PLA samples. Results indicate increase in the expression of integrin subunits, α_2, α_5 and β_1 in cells cultured on PLAGA relative to PLA.

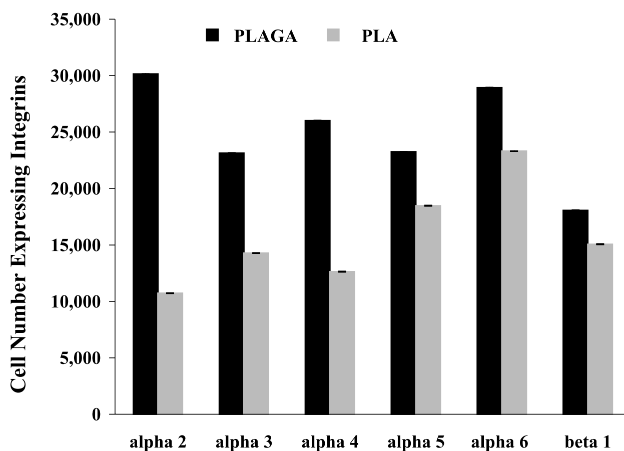

Fig. 7. Flow cytometric analysis of the number of cells positive for the specific cell surface integrin subunits in populations of osteoblasts cultured on poly(lactide-co-glycolide) acid (PLAGA), and poly(lactic acid) (PLA). Cells were analyzed at 24 h post-plating. Results report that integrin subunits expression was higher on cells cultured on PLAGA relative to PLA.

The expression of the key integrin subunits was identified on human osteoblastic cells seeded on matrices of PLAGA and PLA. Osteoblast integrin expression has been observed on cells isolated from both human and animal cell lines cultured in vitro [23,38,43], and these integrins have recently been demonstrated to play a key role in osteoblast adhesion to various orthopaedically relevant substrates [9,41–43]. Studies by Tuan et al. demonstrated that surface composition of orthopaedically relevant metallic surfaces affected human osteoblast integrin expression and cellular interaction [41,42]. Their findings revealed that human osteoblastic cells grown on various metal surfaces such as cobalt chrome or titanium expressed different integrins than osteoblasts seeded on TCPS [41,42]. Our results showed that human osteoblastic cells grown on polymeric surfaces (PLAGA and PLA) expressed integrin subunits $\alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6$ and β_1. In addition, integrin levels were greater on PLAGA, the substrate that supported increased cell adhesion. Furthermore, the integrin subunits expressed at the highest levels in osteoblastic cells adhered to degradable polymeric surfaces were the same integrins (α_2, α_5 and β_1) normally found to mediate adhesion to the ECM molecules collagen and fibronectin [9,19,38], indicating that the ligands responsible for receptor binding was properly produced by cells grown on these materials. However, future studies are needed to elucidate whether ECM production induces receptor production and stimulation on biodegradable matrices.

We believe the ability of the degradable polymeric material to promote cell adhesion of human osteoblasts may be directly dependent on the repertoire of integrins expressed on that material. This interaction has been shown to play a significant role in cell–cell adhesion, signaling and spreading in a variety of other settings [16,39,41]. The ability to induce integrin subunit expression via polymeric composition may be a key step in determining which materials are suitable candidates for tissue engineered application for bone and orthopaedically related circumstances. By investigating, the key receptors involved in adhesion, it offers a clear and intricate understanding of how cell–polymer interaction can be modulated and controlled. For instance, a polymer containing the proper osteogenic ligands or components may induce a higher binding affinity between the cell receptor and material of interest, thereby inducing enhanced osteointegration at the implant site. A number of other factors may affect integrin expression and cell adhesion on these materials. These factors include interaction with serum proteins, polymer surface charges and polymer hydrophobicity/hydrophilicity [29,31]. Further studies will continue to investigate the role of integrins in regulatory events surrounding human osteoblast adherence and growth on biodegradable materials suitable for bone tissue engineering.

We believe that understanding cell–material interaction will aid in the selection of materials suitable for applications in bone regeneration.

Acknowledgements

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