Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Biomaterials 24 (2003) 1213–1221
Biomaterials
www.elsevier.com/locate/biomaterials

Extracellular matrix production by human osteoblasts cultured on biodegradable polymers applicable for tissue engineering

S.F. El-Amin[a], H.H. Lu[a], Y. Khan[a], J. Burems[a], J. Mitchell[a], R.S. Tuan[b], C.T. Laurencin[a,c,*]

[a] *Center for Advanced Biomaterials and Tissue Engineering, Department of Chemical Engineering, Drexel University, Room # 383, CAT Building, 3141 Chestnut Street, Philadelphia, PA 19104, USA*
[b] *Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA, USA*
[c] *Department of Orthopaedic Surgery, Drexel University, College of Medicine, Philadelphia, PA, USA*

Received 25 May 2002; accepted 28 August 2002

Abstract

The nature of the extracellular matrix (ECM) is crucial in regulating cell functions via cell–matrix interactions, cytoskeletal organization, and integrin-mediated signaling. In bone, the ECM is composed of proteins such as collagen (CO), fibronectin (FN), laminin (LM), vitronectin (VN), osteopontin (OP) and osteonectin (ON). For bone tissue engineering, the ECM should also be considered in terms of its function in mediating cell adhesion to biomaterials. This study examined ECM production, cytoskeletal organization, and adhesion of primary human osteoblastic cells on biodegradable matrices applicable for tissue engineering, namely polylactic-*co*-glycolic acid 50:50 (PLAGA) and polylactic acid (PLA). We hypothesized that the osteocompatible, biodegradable polymer surfaces promote the production of bone-specific ECM proteins in a manner dependent on polymer composition.

We first examined whether the PLAGA and PLA matrices could support human osteoblastic cell growth by measuring cell adhesion at 3, 6 and 12 h post-plating. Adhesion on PLAGA was consistently higher than on PLA throughout the duration of the experiment, and comparable to tissue culture polystyrene (TCPS). ECM components, including CO, FN, LM, ON, OP and VN, produced on the surface of the polymers were quantified by ELISA and localized by immunofluorescence staining. All of these proteins were present at significantly higher levels on PLAGA compared to PLA or TCPS surfaces. On PLAGA, OP and ON were the most abundant ECM components, followed by CO, FN, VN and LN. Immunofluorescence revealed an extracellular distribution for CO and FN, whereas OP and ON were found both intracellularly as well as extracellularly on the polymer. In addition, the actin cytoskeletal network was more extensive in osteoblasts cultured on PLAGA than on PLA or TCPS.

In summary, we found that osteoblasts plated on PLAGA adhered better to the substrate, produced higher levels of ECM molecules, and showed greater cytoskeletal organization than on PLA and TCPS. We propose that this difference in ECM composition is functionally related to the enhanced cell adhesion observed on PLAGA. There is initial evidence that specific composition of the PLAGA polymer favors the ECM. Future studies will seek to optimize ECM production on these matrices for bone tissue engineering applications.
© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Extracellular matrix molecules; Polymers; Human osteoblast; Tissue engineering; Cell adhesion

1. Introduction

Biodegradable materials offer several advantages over metals and other non-degradable materials currently in use as orthopaedic implants [1]. Once placed in vivo, these materials obviate the removal of the implant by surgeons, and their degradation profile can be modulated to fit intended applications [1–4]. Studies have also demonstrated that by adding growth factors, such as bone morphogenetic protein to the polymeric systems, cell growth and bone formation increase at the defect site over time, leading to accelerated osseous repair [5,6]. Another significant advantage of biodegradable polymers is their ability to offer researchers the

*Corresponding author. Center for Advanced Biomaterials and Tissue Engineering, Department of Chemical Engineering, Drexel University, Room # 383, CAT Building, 3141 Chestnut Street, Philadelphia, PA 19104, USA. Tel.: +1-215-895-6645; fax: +1-215-895-6219.
E-mail address: laurencin@drexel.edu (C.T. Laurencin).

0142-9612/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0142-9612(02)00451-9

opportunity to control their surface properties and subsequently direct cellular adhesion and cellular response [2,7].

Cell adhesion is an important parameter by which proposed tissue engineered surfaces may be evaluated to determine suitability for use. The extracellular matrix (ECM) is crucial in mediating cell adhesion to biomaterials, and its organization and production modulate the degree of cell attachment to these materials [6]. A cell's behavior and interaction with a biomaterial surface is dependent on properties such as topography, charge, chemistry and surface energy [7–9]. Collectively, these properties play an important role in the cell's ability to attach, adhere and spread on polymer surface, and this has been referred to as the first phase of cell/material interaction [7].

Cell/material interactions can be closely evaluated by examining the cellular receptors responsible for adhesion and the ligands that they bind to, specifically ECM proteins [8–10]. The receptors that are responsible for osteoblast adhesion are the integrins, which are transmembrane, heterodimeric proteins composed of α and β subunits [11–16]. Depending on the combination, pairing of the integrin subunits has been shown to be specific for binding to various ECM proteins. For example, $\alpha_2\beta_1$ binds to the abundant ECM protein, collagen (CO), whereas $\alpha_4\beta_1$ binds to FB [9]. In bone, the ECM is largely composed of several molecular complexes of proteins, such as CO, FB, laminin (LM), vitronectin (VN), osteopontin (OP), osteonectin (ON) and other glycoproteins [7,9,11]. All of these proteins are synthesized by osteoblasts and play various roles in biological bone formation. CO proteins are involved in structural stabilization as well as binding points for osteocalcin [7], while FB and VN have been shown to be involved in osteoblast adhesion in vitro [9]. Other glycoproteins and plasma proteins are believed to play a role in mineralization of the bone matrix [7,9]. Another important criteria of cell–matrix interaction is the cytoskeletal organization of cells onto seeded surfaces. Studies have shown that the actin cytoskeletal element plays an important role in cell attachment and stabilization. Actin bundles coupled with adhesion plaques can transmit forces to the substrate and help to maintain cell shape and facilitate cell adhesion [7,17].

This study focuses on ECM protein production and cytoskeletal organization by primary human osteoblastic cells on biodegradable matrices such as polylactic-*co*-glycolic acid 50:50 (PLAGA) and polylactic acid (PLA), currently utilized in our laboratory and by others as scaffolds for skeletal tissue regeneration. We hypothesize that the osteocompatible, biodegradable polymer surfaces promote the expression of ECM proteins and that the levels of expression are dependent on polymer composition.

2. Methods and materials

2.1. Tissue engineered matrices

Tissue engineered matrices were prepared using a method developed in our laboratory. Briefly, poly(lactide-*co*-glycolide) (PLAGA, 50:50, Mw =50,000; American Cyanamid Co. Sunnyvale, CA, USA) and poly-L-(lactide) (PLA, Mw =60,000; Purac, Netherlands) polymers were dissolved in methylene chloride (Aldrich, 0.1 g/ml), and stirred at a constant speed for 3 h. The dissolved solution was then solvent-casted into a Teflon®-coated dish and placed under a vacuum hood for 2 h. The matrix was allowed to settle slowly overnight at −20°C. Once dried, the matrix was bored into disks 1.4 cm in diameter and 0.4 cm in height and further dried (Labconco 12, Kansas City, USA) for at least 24 h, in order to remove residual solvent. The matrices were stored at −20°C under argon until use.

2.2. Degradation studies

Degradation studies were performed on PLAGA and PLA matrices over 10 weeks. Degradation studies were performed in 0.1 M phosphate buffer solution (PBS, pH = 7.4). Polymeric matrices with a diameter of 1.4 cm were obtained and placed in 10 ml scintillation vials containing PBS at 37°C. At appropriate time points, matrices were removed, lyophilized and weighed to determine the percent mass weight lost over time. Samples were also prepared to study surface changes using a Rame-hart goniometer. Briefly, samples were prepared as described earlier and surface contact angle was measured in the presence of a hydrophilic solution (distilled water) and a hydrophobic solution (ethylene glycol).

2.3. Scanning electron microscopy

Matrices were fixed using 4% paraformaldehyde solution (Polysciences, Warrington, PA, USA) for 24 h, and washed three times in 0.1 M PBS (pH 7.4). Once the samples were thoroughly washed, they were dried using a graded series of ethanol dilutions (50–100%) at various intervals. Once drying was completed, the samples were coated with gold using a Denton Desk-1 Sputter Coater and visualized using an Amray 1830-D4 scanning electron microscope equipped with a Tungsten electron gun.

2.4. Human osteoblast cell culture

An approved method for isolation of primary human osteoblast cells from trabecular bone from the femoral head of patients undergoing hip arthroplasty was performed using a modified protocol [9]. Under sterile

conditions, trabecular bone chips were dissected from the femoral head, placed in vials containing calcium-free DMEM/F12K medium (Specialty Media, Lavallette, USA), and minced into small bone chips. The bone chips were then washed several times in fresh medium. To remove the fibrous matrix surrounding the bone chips, the bone chips were placed in a collagenase P enzyme media (Sigma, St. Louis, MO, USA) under a closed environment, while stirring constantly at 37°C in 5% CO_2 for 3 h. Digested chips were then washed repeatedly in sterile sodium chloride solution, vortexed between washes at a low constant speed and placed in a 150 mm cell culture flask (COSTAR, Cambridge, MA, USA) containing calcium-free DMEM/F12 medium, supplemented with 10% FBS, 2 mM L-glutamine, 50 μg/ml ascorbate and 50 μg/ml penicillin/streptomycin at 37°C in 5% CO_2. Within 2 weeks, cell migration from the bone chips occurred and the media was then supplemented with 110 mM $CaCl_2$. Cells consistently exhibited osteoblast morphology, osteocalcin and alkaline phosphatase production.

2.5. Cell adhesion studies

For adhesion studies, human osteoblastic cells were plated on PLAGA, PLA and the control tissue culture polystyrene (TCPS) over a 12-h period. Confluent human osteoblastic cells were removed using 0.5% trypsin (Sigma Chemical, St. Louis, MO, USA), counted using a hemocytometer, and plated at a density of 2.0×10^4 cells/disc on matrices and control TCPS, and then incubated at 37°C in 5% CO_2.

At 3, 6, and 12 h after plating, cell adhesion was measured using a fluorimetric dye 2′7′-bis(2-carboxyethyl) 5-carboxyfluorescein acetyloxymethylester (BCECF-AM, Molecular Probes, Junction City, OR, USA) which was internalized within the membrane of living cells and releases a fluorescent component upon lysing of the membrane [11]. Thirty minutes prior to harvesting, cells were washed in 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 an emission wavelength of 535 nm. Cell number was determined by comparison to the dye intensities released from known cell numbers [9].

2.6. Cell morphology studies

Studies were performed to examine the morphology of human osteoblast cell culture on PLAGA and PLA matrices under a phase contrast light microscope (Hitech Instruments, PA, USA) at 3, 6 and 12 h. Human osteoblastic cells (1.0×10^4) were plated on PLAGA and PLA surfaces, washed in PBS (pH 7.4), 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. In addition, parallel cultures were examined using scanning electron microscopy.

2.7. ECM protein production

To quantify the amount of extracellular matrix (ECM) protein production by primary human osteoblasts on various matrices, a modified enzyme-linked immunoassay was developed (Molecular Probes, Boston, MA). Primary human osteoblastic cells were plated at 2×10^5 cells/disc. At 24 h, the samples were fixed in a 4% paraformaldehyde solution for 1 h. The samples were washed several times in PBS and blocked in a 1% BSA-goat serum in PBS. The primary monoclonal antibodies specific for type I CO, FN, LM, VN, (Sigma Chemical, St. Louis, MO) OP and ON (a gift from Dr. Tuan's laboratory) were added at the dilution (1:1000) specified by the manufacturer and previous studies in the lab [9] at 37°C for 45 min. The samples were then washed in PBS several times, blocked in a solution of 1% BSA-goat serum in PBS and the secondary antibody of FITC (Sigma) was added. The amount of fluorescence intensity was determined using a SpectraFluor plus instrument (Tecan, Triangle Park, NC, USA) with an excitation of 485 nm and an emission of 535 nm. Sample fluorescence was compared to a linear standard curve based on concentrations of the FITC antibody.

2.8. Staining of ECM and cytoskeletal elements

Primary human osteoblast cells were plated on various matrices at a plating density of 1.0×10^4 cells/cm^2 for 12 h. The cells were fixed using a 4% paraformaldeyhde solution for 10 min and permeabilized in Hepes-Triton buffer for 15 min. The samples were then washed in PBS and blocked using a 3% BSA-serum at 25°C for 30 min. The samples were washed again in PBS and the specific primary antibodies for CO, FB, OP, ON, VN and LM (dilution 1:100, CO, FB, VN, LM monoclonal antibodies obtained from Sigma and OP,ON monoclonal antibodies were a gift from Dr. Tuan's lab) in 1% BSA-PBS were added for 1 h. The samples were washed with PBS twice and blocked in 1% BSA-goat serum in PBS. A fluorescein labeled secondary antibody (FITC, Sigma) was diluted (1:250) in 1% BSA-PBS for 30 min. The samples were imaged using an epifluorescence microscope (Zimmer) and by confocal laser scanning microscopy (CLSM; Noran Instruments Inc, Middleton, USA).

2.9. Cytoskeletal organization

The cytoskeletal organization of an individual osteoblast was examined on the matrices. The distribution of actin, a key protein in cellular structure, was observed over time. Human osteoblastic cells were plated on various matrices at a plating density of 1.0×10^4 cells/cm^2. At 3, 6, and 12 h, matrices were washed in PBS, fixed and stained with a rhodamine-phalloidin antibody (Molecular Probes) which was specific for actin. Samples were visualized by confocal laser microscopy (Noran Instruments) at an excitation of 488 nm. The location and assembly of actin were examined and correlations between the cytoskeletal organization and morphology of the cell were evaluated. Cellular actin organization was described as either Type I, Type II, or Type III osteoblasts. In general, Type I osteoblasts are round cells and represent a preliminary stage of actin assembly. Type II are larger with formation of actin fibers and increased cellular diameter. Type III cells are elongated osteoblast with organized actin fiber network, demonstrated spreading and cellular movement [9].

2.10. Statistical evaluation

Statistical evaluation was performed using the Student's *t*-test for cell adhesion studies and ANOVA for ECM studies. All studies were repeated and $n = 3$–6. Significance was determined at $p < 0.05$ for all groups studied.

3. Results

3.1. Polymeric matrices

Polymers of PLAGA and PLA were synthesized and fabricated into matrices, with a smooth and even surface distribution. PLAGA was found to be more hydrophilic than PLA, likely due to the presence of glycolide in the co-polymer [18]. Fig. 1 demonstrates by 6 weeks that PLAGA was found to have degraded at a higher rate than PLA, in agreement with the literature [18]. This trend was continued through the 10-week time point.

3.2. Cell adhesion morphology

The appearance of isolated human osteoblastic cells was examined. We found cell morphology on the polymer to be consistent with what has been reported in the literature [17]. During harvesting of the primary culture, it was observed that immediately following isolation, there was limited cellular migration from the trabecular bone chips at day 1. By day 7, there were few cells present with the majority of the flask unoccupied by osteoblasts. After day 14, cellular migration was

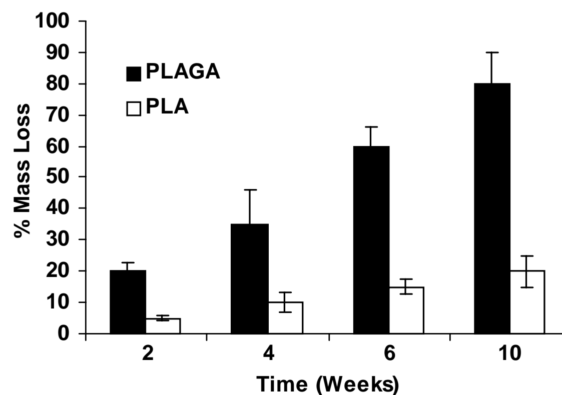

Fig. 1. Degradation profile of PLAGA, PLA. (a) degradation study demonstrated percent mass loss over time and (b) surface charges of PLAGA and PLA over 72 h.

noted along with increased ECM accumulation around the bone chips. The cellular morphology was elongated as well as circular for some osteoblastic cells. On examination of the cells at day 21, there was a substantial increase in the number of cells that had migrated from the bone chips, increasingly further away from the bone chips. The cellular pattern observed was circular, with several cells exemplifying a star-shaped elliptical pattern.

3.3. Cell adhesion on polymeric matrices

To assess adhesion of osteoblastic cells on tissue engineered matrices, studies were performed to evaluate adhesion kinetics over time. Fig. 2 shows the adhesion kinetics of human osteoblastic cells plated on PLAGA, PLA, and tissue culture polystyrene (TCPS) as a control at 3, 6 and 12 h. At 3 h, cells adhered at a faster rate to PLAGA relative to PLA and the control. At 6 h, cells adhesion levels remained higher on PLAGA than on PLA and were consistent with the control TCPS. Cell adhesion on PLA was significantly less than on both TCPS and PLAGA. By 12 h, the growth kinetics for cells plated on PLAGA was comparable to the control TCPS and significantly higher than on PLA matrices. However, more cells adhered by 12 h on the PLA than observed at earlier time points.

3.4. ECM protein production

Production of the various ECM components was evaluated on PLAGA, PLA and TCPS. The expression of CO, FN, LM, ON, OP and VN was analyzed and the results are presented in Fig. 3. Quantification by ELISA revealed that all of the above ECM components were present on both types of polymeric matrices, but with significantly higher levels of expression on PLAGA. Cells grown on PLAGA, demonstrated high levels of OP

and ON, followed by CO and FB. Both LM and VN were expressed at lower levels, but still detectable on all surfaces studied. Overall, ECM production by human osteoblasts was initiated on both substrates, and the levels of expression were found to be significantly higher on PLAGA than on PLA surfaces, even when controlled for cell number.

3.5. ECM localization and cytoskeletal organization

To determine ECM localization, immunofluorescence was performed on substrates of PLAGA and PLA over a 24-h period. Fig. 4 demonstrates the presence of the ECM molecules of OP, ON and FB on PLAGA. For PLA matrices, production and distribution of ECM molecules were minimal and barely above background (data not shown). For PLAGA matrices, we observed a difference in the location of ECM protein production on the matrix (Fig. 4). Immunofluorescence staining localized FB distribution on the surface of the polymer. OP and ON were distributed within the cell and were also found on the surface of the polymer.

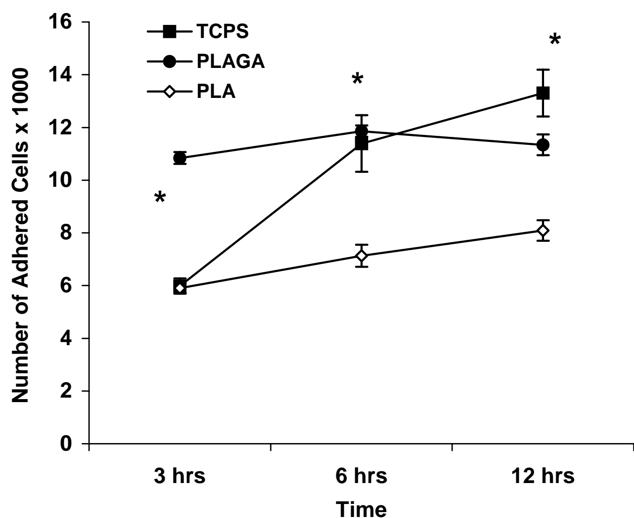

Fig. 2. Human osteoblastic cell adhesion kinetics on different substrates, PLAGA, PLA and tissue culture polystyrene (TCPS) over 12 h. $N = 5$ for all points. * denotes $p<0.05$ for comparison between PLAGA and PLA using Student *t*-test.

In addition, cytoskeletal distribution was examined. The cytoskeletal network was more extensive for osteoblasts cultured on PLAGA than on PLA. Fig. 5 shows cells grown on PLAGA formed Type I cells at a faster rate than on PLA at 3 h, indicating a circular organization of the cytoskeletal actin proteins. For cytoskeletal organization at 6 h, the most abundant cell type observed was Type II, which was again, seen at higher levels on PLAGA relative to PLA. By 12 h, the majority of the cells on PLAGA were of Type III, indicating a clear organization of the osteoblast actin fibers on cells grown on PLAGA compared to PLA. Overall, cells grown on PLAGA demonstrated a more extensive actin network organization than on PLA, indicating enhanced cell spreading, movement and signaling events.

4. Discussion

The objective of this study was to gain a better understanding of the mechanism responsible for cellular adhesion to tissue engineered materials based on biodegradable polymers, composites and other biomaterials. We believe that in order to sustain the current growth and discovery in tissue engineering, a thorough understanding of the nature of interactions between biological tissues and biomaterials must occur. In this

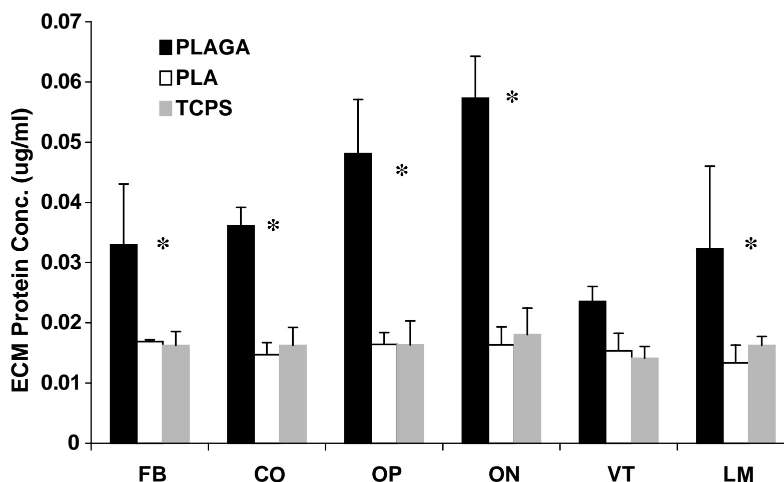

Fig. 3. Total ECM production of human osteoblastic cells grown on PLAGA, PLA and tissue culture polystyrene TCPS at 12 h. FB: Fibronectin; CO: Collagen; OP: Osteopontin; ON: Osteonectin; VT: Vitronectin; LM: Laminin.

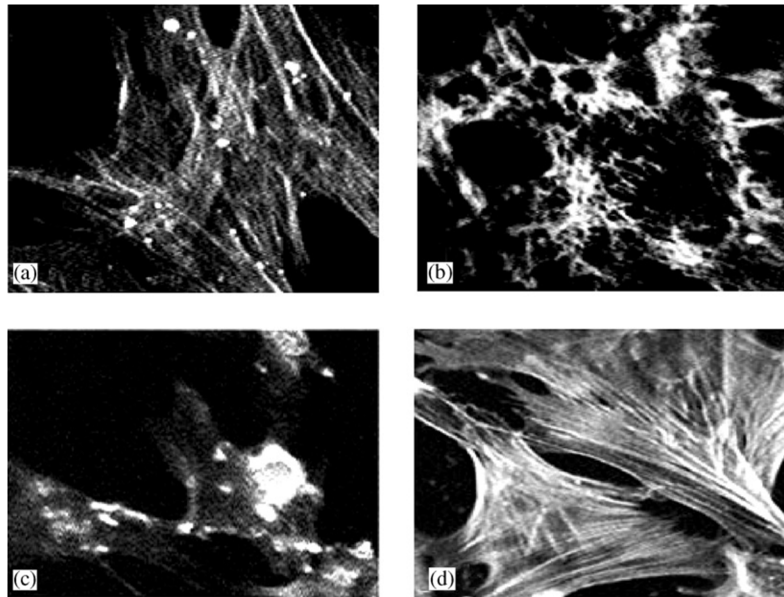

Fig. 4. Immunoflourescence staining of ECM and cytoskeletal component (actin) produced by human osteoblastic cells grown on PLAGA: Osteonectin (a), Fibronectin (b), Osteopontin (c) and Actin (d).

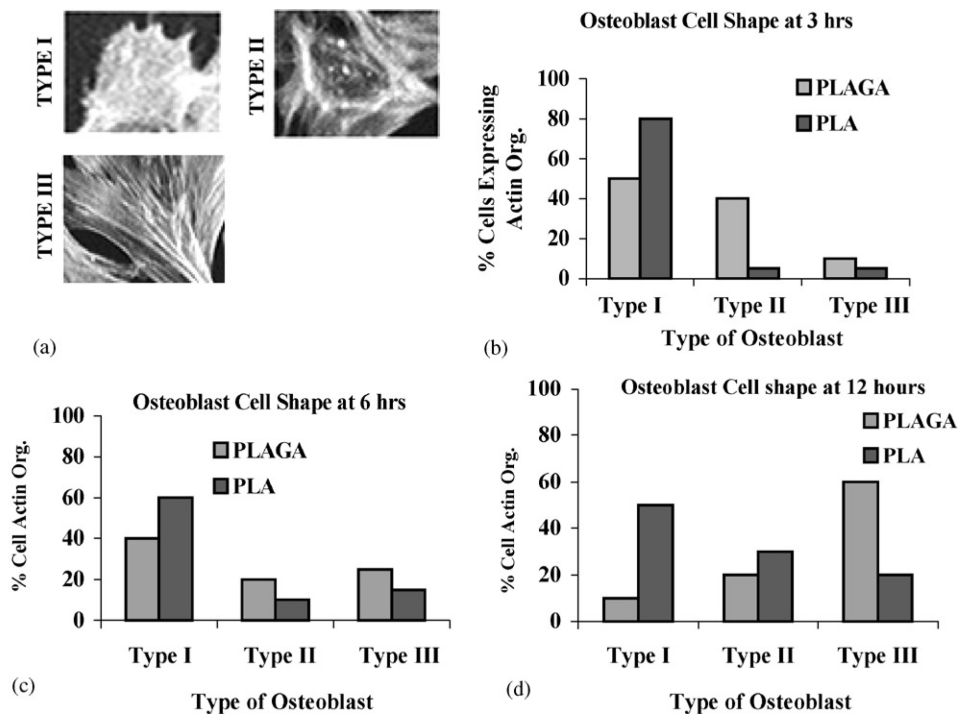

Fig. 5. Cytoskeletal organization of human osteoblastic cells on bioerodible polymers over a 12-h period. (a) Cytoskeletal actin classification determined by filamentous actin organization over a 12-h period. Type I cells contain a small amount of actin organization and thus are round and exhibit few cellular processes. Type II cells have more filamentous actin organization than Type I and are spread out more demonstrating cytoskeletal protein organization. Type III cells demonstrate extensive actin organization and filament framework. The cells are completely extended. (b) Cytoskeletal organization of human osteoblastic cells at 3 h on PLAGA and PLA. A higher percentage of Types II and III cells were observed on PLAGA than on PLA at 3 h. (c) At 6 h, there was more extensive actin formation and organization observed on PLAGA than on PLA as described by the higher number of Type III cells observed. (d) By 12 h majority of the cells grown on PLAGA exhibited Type III characteristic apposed to less observed on PLA, indicating more of a organized cytoskeletal framework of the human osteoblastic cells adhered to the matrix.

study, we have examined the molecular interactions and determined the expression of proteins that are involved when osteoblasts initially adhere to a surface. Previous studies in our laboratory have revealed that the composition of material or matrix of interest can modulate the adhesion kinetics of human osteoblasts

to material surfaces by altering the expression level of its adhesion receptor, the integrins [9,17,19].

In order to determine the mechanism responsible for cell adhesion to biodegradable polymers, studies on matrix characterization and cellular behavior were performed in vitro. When examining the surface of the tissue engineered matrices, our results revealed a smooth, even surface that was suitable for evaluation of molecular interactions of biomaterials, proteins, and cellular receptors. Schakenraad et al. [20] reported that changes in the materials surface alters the ability of the cell to adhere as well as to spread on the surface. Our results demonstrated that the degradation of the polymer was not a significant factor during the initial 24 h of adhesion. In addition, the PLAGA surface was more hydrophilic than PLA, which is consistent with what has been reported in the literature [19].

The primary human osteoblastic cells used in this study were isolated from human bone chips, and they adapted the typical morphology exhibited by osteoblast-like cells, while maintaining the expression of phenotypic markers. When we measured the adhesion kinetics of osteoblastic cells grown on PLAGA and PLA, it was found that human osteoblastic cell adhesion was not only time dependent, but also a function of polymer composition. For example, cellular adhesion to PLAGA was significantly higher than on PLA surfaces. As well this difference became less marked as incubation time increased from 3 to 12 h post-plating. This indicates that cellular adhesion may be dependent on either the chemical composition that differs between PLAGA and PLA, or the accumulation of the degradation products such as lactic acid [19]. However, the former may be more likely, since we found minimal differences in the degradation rates of the polymers and consequently, reducing the effect of degradation products on cellular adhesion during the first 24 h. However, future studies with longer time points need to be performed to explore the long-term effects of polymer degradation on cellular response.

ECM molecules play an important role in the cell's ability to adhere and proliferate [9–12,18,21–28]. Our objective was to characterize the production and distribution of ECM molecules by human osteoblastic cells adhered on biodegradable polymers, and to assess their importance on cell–polymer interactions. We found that cells grown on PLAGA produced higher levels of ECM molecules and cytoskeletal organization than seen on PLA or TCPS, the standard substrate for cell attachment and growth. Shah et al. demonstrated that ECM production varies depending on the test substrate that the cells are grown on. For example, cells grown on rough titanium surfaces yielded a different level of ECM protein production than on those of smooth titanium and cobalt chrome surfaces [9]. Within the ECM matrix, it has been reported that upon contact with a surface, human osteoblastic cells began to secrete a matrix that is composed of CO, FB, glycoproteins as well as other ECM components [7]. The secreted matrix is then used to support the adhesion of the osteoblast via adhesion receptors known as integrins [20]. Once the ECM is laid down, the integrin receptors have ligand binding domains that are specific for the amino acid sequence Arg–Gly–Asp (R–G–D) [29,30]. Once this binding occurs, several key events occur which affect cell signaling, nuclear organization and cytoskeletal formation [31–35]. Therefore, understanding the key steps in determining which ECM proteins are produced during initial cellular adhesion to biodegradable polymers may eventually reveal the mechanism responsible for controlling the subsequent behavior of the cell. When examining which of the ECM proteins were initially expressed by human osteoblastic cells on the polymers, we detected a difference in the production levels of these proteins. Expression of the key maturation proteins, OP and ON was found to be at higher levels along with the abundant ECM protein CO. To pinpoint the location of these proteins with respect to the cell, immunofluorescence imaging revealed that OP and ON were found both intracellularly and extracellularly, whereas the FB and CO was mostly extracellular.

The cytoskeletal organization of a cell is extremely important in modulating cell morphology, mobility and adhesion. The focal adhesion contact site, composed of the receptor and cytoskeletal protein talin and vinculin, which connect to actin, has been shown to be affected by the ability of the cell to form a proper cytoskeletal network [7,9,36–38]. Once a cell comes in contact with and binds to a surface, key signaling events occur that cause the focal contact elements to change and alter the binding capacity of the cell. The signal is then transferred to the f-actin filaments which affects the osteoblast's shape as well as the intracellular signaling process [7]. Our studies revealed that in observing the cytoskeletal actin network organization, cells grown on PLAGA formed the actin framework earlier than cells grown on PLA. This was evident by the abundance of type III cells that was observed on PLAGA than on PLA matrices. Immunoflouresence imaging also supported the accumulation of actin fibers along with revealing the typical morphological pattern observed by human osteoblastic cells.

The goal of this study was to further our understanding of the mechanism responsible for osteoblast adhesion to biodegradable polymers, along with developing a reliable approach to study cell–biomaterial interactions. The results of this study suggest that the biodegradable polymers, PLAGA and PLA, supported cellular adhesion and growth, and resulted in changes in the production of ECM proteins and the organization of the cytoskeleton. We believe that these changes in the

levels of ECM proteins are functionally translated into increased numbers of adhered cells observed on PLAGA. In addition, based on the results of this study, the composition of the polymer (PLAGA) may determine the level of ECM protein production. Future studies will seek to further evaluate and optimize ECM production on these tissue engineered matrices and to understand the mechanism responsible for determining which proteins are produced initially on a biomaterial surface.

Acknowledgements

This work was supported by NSF Grants BES9896282, EEC-9980298, INT011595, BES0115404, and BES0131341 (under CTL). Dr. Laurencin was the recipient of a Presidential Faculty Fellow Award.

References

[1] Middleton JC, Tipton AJ. Synthetic biodegradable polymers as orthopaedic devices. Biomaterials 2000;21:2335–46.
[2] Laurencin CT, ElAmin SF, Ibim SE, Willoughby DA, Attawia M, Allcock HR, Ambrosio AA. A highly porous 3-dimensional polyphosphazene polymer matrix for skeletal tissue regeneration. J Biomed Mater Res 1996;30:133–8.
[3] Attawia MA, Herbert KM, Uhrich KE, Langer R, Laurencin CT. Proliferation, morphology, and protein expression by osteoblasts cultured on poly(anhydride-co-imides). J Biomed Mater Res 1999;48:322–7.
[4] Attawia MA, Herbert KM, Laurencin CT. Osteoblast like cell adherence and migration through 3-dimensional porous polymer matrices. Biochem Biophys Res Commun 1995;213:639–44.
[5] Duneas N, Crooks J, Ripamount U. Transforming growth factor-b1: induction of bone morphogenetic protein genes expression during endochondral bone formation in the baboon and synergistic interaction with osteogenic protein-1(BMP-7). Growth Factors 1998;15:259–77.
[6] Deluca PP, Schrier JA. Recombinant human bone morphogenetic protein-2 binding and incorporation in PLGA microsphere delivery systems. Pharm Dev Technol 1999;4:611–21.
[7] Anselme K. Osteoblast adhesion on biomaterials. Biomaterials 2000;21:667–81.
[8] Hott M, Noel B, Bernache-Assolant D, Rey C, Marie PJ. Proliferation and differentiation of human trabecular osteoblastic cells on hydroxyapatite. J Biomed Mater Res 1997;37:508–16.
[9] Shah AK, Lazatin J, Sinha RK, Lennox T, Hickok NJ, Tuan RS. Mechanism of BMP-2 stimulated adhesion of osteoblastic cells to titanium alloy. Biol Cell 1999;91:131–42.
[10] Damsky CH. Extracellular matrix–integrin interactions in osteoblast function and tissue remodeling. Bone 1999;25:95–6.
[11] Ivaska J, Heino J. Adhesion receptors and cell invasion: mechanisms of integrin-guided degradation of extracellular matrix. Cell Mol Life Sci 2000;57:16–24.
[12] Guan JL, Chen HC. Signal transduction in cell–matrix interactions. Int Rev Cytol 1996;168:81–121.
[13] Hotchin NA, Hall A. The assembly of integrin adhesion complexes requires both extracellular matrix and intracellular rho/rac GTPases. J Cell Biol 1995;131:1857–65.
[14] Friedl P, Zanker KS, Brocker EB. Cell migration strategies in 3-D extracellular matrix: differences in morphology, cell matrix interactions, and integrin function. Microsc Res Tech 1998;43: 369–78.
[15] Labat-Robert J, Robert L. Interaction between cells and extracellular matrix: signaling by integrins and the elastin-laminin receptor [in process citation]. Prog Mol Subcell Biol 2000;25: 57–70.
[16] Garcia AJ, Boettiger D. Integrin-fibronectin interactions at the cell–material interface: initial integrin binding and signaling. Biomaterials 1999;20:2427–33.
[17] Sinha RK, Tuan RS. Regulation of human osteoblast integrin expression by orthopaedic implant materials. Bone 1996;18: 451–7.
[18] Freed LE, Vunjak-Novakovic G, Biron RJ, Eagles DB, Lesnoy DC, Barlow SK, Langer R. Biodegradable polymer scaffolds for tissue engineering. Biotechnology 1994;12:689–93.
[19] Gopferich A. Mechanism of polymer degradation and elimination. In: Domb AJ, Kost J, Wiseman DM, editors. Handbook of biodegradable polymers. Amsterdam: Hardwood Academic Publishers, 1997. p. 452–71.
[20] Schakenraad JM, Buscher HJ, Wildevuur CR, Arends J. The influence of substratum free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. J Biomed Mater Res 1986;20:773–84.
[21] Slivka MA, Chu CC. Fiber-matrix interface studies on bioabsorbable composite materials for internal fixation of bone fractures. II. A new method using laser scanning confocal microscopy. J Biomed Mater Res 1997;37:353–62.
[22] Attawia MA, Devin JE, Laurencin CT. Immunofluorescence and confocal laser scanning microscopy studies of osteoblast growth and phenotypic expression in three-dimensional degradable synthetic matrices. J Biomed Mater Res 1995;29:843–8.
[23] Attawia MA, Herbert KM, Uhrich KE, Langer R, Laurencin CT. Proliferation, morphology, and protein expression by osteoblasts cultured on poly(anhydride-co-imides). J Biomed Mater Res 1999; 48:322–7.
[24] Attawia MA, Uhrich KE, Botchwey E, Fan M, Langer R, Laurencin CT. Cytotoxicity testing of poly(anhydride-co-imides) for orthopedic applications. J Biomed Mater Res 1995;29: 1233–40.
[25] Laurencin CT, Attawia MA, Elgendy HE, Herbert KM. Tissue engineered bone-regeneration using degradable polymers: the formation of mineralized matrices. Bone 1996;19: 93S–9S.
[26] Norman ME, Elgendy HM, Shors EC, El-Amin SF, Laurencin CT. An in-vitro evaluation of coralline porous hydroxyapatite as a scaffold for osteoblast growth. Clin Mater 1994;17:85–91.
[27] Langer R. 1994 Whitaker lecture: polymers for drug delivery and tissue engineering. Ann Biomed Eng 1995;23:101–11.
[28] Mooney DJ, Mazzoni CL, Breuer C, McNamara K, Hern D, Vacanti JP, Langer R. Stabilized polyglycolic acid fibre-based tubes for tissue engineering. Biomaterials 1996;17:115–24.
[29] Grzesik WJ, Robey PG. Bone matrix RGD glycoproteins: immunilocalization and interaction with human primary osteoblastic cell in vitro. J Bone Miner Res 1994;9:487–96.
[30] Puelo DA, Bizios R. RGDS tetrapeptide binds to osteoblasts and inhibits fibronectin-mediated adhesion. Bone 1991;12:271–6.
[31] Delcommenne M, Streuli CH. Control of integrin expression by extracellular matrix. J Biol Chem 1995;270:26794–801.
[32] Grossener-Schreiber B, Tuan RS. Bone cell response to hydroxyapatite-coated surfaces in vitro. Sem Arthop 1991;2: 260–7.
[33] Nakamura H, Ozawa H. Cell–cell and cell–matrix interaction in bone remodeling. Nippon Seikeigekagakkai Gakkai Zasshi 1996; 70:740–50.

[34] Ruoslahti E, Engvall E. Integrins and vascular extracellular matrix assembly. J Clin Invest 1997;100:S53–6.
[35] McDonald JA. Receptors for extracellular matrix components. Am J Physiol 1989;257:L331–7.
[36] Danen EH, Lafrenie RM, Miyamoto S, Yamada KM. Integrin signaling: cytoskeletal complexes, MAP kinase activation, and regulation of gene expression. Cell Adhes Commun 1998;6: 217–24.
[37] Grzesik WJ. Integrins and bone—cell adhesion and beyond. Arch Immunol Ther Exp (Warszana) 1997;45:271–5.
[38] Zigmond SH. Signal transduction and actin filament organization. Curr Opin Cell Biol 1996;8:66–73.