The regulation of integrin-mediated osteoblast focal adhesion and focal adhesion kinase expression by nanoscale topography

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Abstract

An important consideration in developing physical biomimetic cell-stimulating cues is that the in vivo extracellular milieu includes nanoscale topographic interfaces. We investigated nanoscale topography regulation of cell functions using human fetal osteoblastic (hFOB) cell culture on poly(L-lactic acid) and polystyrene (50/50 w/w) demixed nanoscale pit textures (14, 29, and 45 nm deep pits). Secondary ion mass spectroscopy revealed that these nanotopographic surfaces had similar surface chemistries to that of pure PLLA because of PLLA component surface segregation during spin casting. We observed that 14 and 29 nm deep pit surfaces increased hFOB cell attachment, spreading, selective integrin subunit expression (e.g., \(\alpha_v\) relative to \(\alpha_5, \beta_1,\) or \(\beta_3\)), focal adhesive paxillin protein synthesis and paxillin colocalization with cytoskeletal actin stress fibers, and focal adhesion kinase (FAK) and phosphorylated FAK (pY397) expression to a greater degree than did 45 nm deep pits or flat PLLA surfaces. Considering the important role of integrin-mediated focal adhesion and intracellular signaling in anchorage-dependent cell function, our results suggest a mechanism by which nanostructured physical signals regulate cell function. Modulation of integrin-mediated focal adhesion and related cell signaling by altering nanoscale substrate topography will have powerful applications in biomaterials science and tissue engineering.

Keywords: Nanotopography; Osteoblast; Adhesion; Integrin; Focal adhesion kinase

1. Introduction

Extracellular signals regulate cell functions via specific biological interactions. For example, signals from Arg-Gly-Asp (RGD) within extracellular matrix (ECM) proteins regulate the expression of integrins [1]. Integrin-mediated intracellular signal transduction driven by signaling molecules, such as focal adhesion kinase (FAK), extracellular signal-regulated protein kinase (ERK), protein kinase A (PKA), Rho, etc., regulates a variety of cell functions including adhesion, migration, proliferation, differentiation, and apoptosis [1]. These processes are critical for anchorage-dependent cells as the quality of initial adhesion strongly affects the fate of these cells [2]. When cells form interfaces with biomaterials, such as the case with tissue engineering scaffolds or implants, biomaterial surfaces provide extracellular signals. Cells sense, communicate, and respond to signals from biomaterial surface chemistry, topography, charge, surface energy, and wettability [3–9]. Understanding the mechanisms by which cells sense and respond to chemical and physical signals from biomaterials.
will facilitate identification of novel biomaterial biomimetic properties that control cell behavior.

Exploiting biomimetic properties of biomaterials is an attractive strategy in developing novel cell-stimulating cues. An important consideration in choosing a biophysical property to mimic is the observation that cells in vivo contact with nanoscale topographic interfaces. For instance, basement membranes of various tissues are composed of a complex mixture of nm size (5–200 nm) pits, pores, protrusions, and fibers [10]. Bone is a composite structure filled with a number of nanostructured entities from collagen and hydroxyapatite. Type-I collagen, the most abundant ECM in bone, forms fibrillar bundles having ca. 5–10 nm deep striation patterns every 67 nm [11], and 50 × 25 × 4 nm³ sized hydroxyapatite crystals are embedded in collagen bundles [12]. Mimicking this nanotopographic physical milieu in vitro, without any biological surface modifications such as RGD peptide immobilization, may provide biomimetic conditions similar to what cells are exposed to in vivo.

That topography can affect cell behavior is suggested by studies demonstrating that cells align in the anisotropic direction of microscale ridges and grooves [10]. Rapid developments in nanofabrication techniques have facilitated the examination of cell function regulation via nanoscale topography [8–10,13]. Among these, self-organized nanotopography has been recently used to produce large area nanotopographic substrates for assessing cell response [8,9]. The polymer demixing technique can control not only the topographic pattern but also the scale of such topography within nanoscale (10–100 nm) [9,13].

We have developed thin film substrates with nanoscale topographies by using poly(t-lactic acid) (PLLA) and polystyrene (PS) demixing [9]. In this study, we produced films filled with randomly distributed nanoscale pits by adjusting PLLA/PS composition at 50/50 w/w by varying the spin-casting concentration. Nanotopographic scale was controlled such that the nanopit depth ranged between 10 and 50 nm. We examined the hypothesis that nanopit surfaces affect adhesion-related osteoblastic cell function to a different degree depending on the scale of nanotopography by assessing cell shape, adhesion, integrin expression, focal adhesion protein synthesis and colocalization with actin, and FAK and phosphorylated FAK (pY397) expression. Importantly, we examined the nanoscale topography effect under the similar surface chemistry of PLLA for all textured and flat films. This is an important consideration because differences in film surface chemistry may affect cell behavior and this will make the interpretation of topography effect difficult. We aimed to define a nanotopographic scale that is favorable for relatively short-term osteoblastic cell functions. By focusing on integrin-mediated focal adhesion structure and intracellular signaling molecules, we sought to examine the potential mechanism by which nanotopography regulates cell behavior.

2. Materials and methods

2.1. Self-organized nanotopography by polymer demixing

Nanoscale topographies were produced by using a polymer demixing technique. In PLLA (Mw = 50 × 10^3, Polysciences) and PS (Mw = 289 × 10^3, Aldrich Chemical Co.) demixing, PLLA/PS composition in the spin-casting solution was fixed at 50/50 w/w to produce pit-shaped topography based on our previous screening [9]. To produce pit textures with varying nanoscale depths, different total polymer concentrations of 0.5, 1, or 1.5% w/w in chloroform solution were used. Spin casting onto 25 mm glass cover slips was performed at 4000 rpm for 30 s. Single component PLLA films were also spin-cast from 1% w/w solution using the same spin-casting conditions. Spin-cast films were dried at room temperature with no further annealing.

2.2. Characteristics of film surface topography and chemistry

Topography of spin-cast films was assessed using atomic force microscopy (AFM, Nanoscope IIIa, Digital Instruments) in tapping mode under ambient conditions. At least three samples were observed each at three random spots. An average pit depth was measured by a cross-section analysis and root-mean-square roughness (Rq) and arithmetic mean roughness (Ra) were estimated by a roughness analysis, both by using software within the AFM apparatus. An average pit area and fractional pit coverage were quantified from 2-dimensional AFM height images by setting a threshold for pits using ImageJ image analysis software. Film surface chemistry was assessed by using X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectroscopy (SIMS), the details of which are reported in the ‘Supporting Information’. Under experimental conditions, we were able to assess film surface chemical composition to a depth of 90 Å and 20 Å beneath the air-film interface using XPS and SIMS, respectively.

2.3. Cell culture

Human fetal osteoblastic (hFOB 1.19, hFOB) cells were maintained using Dulbecco’s modified Eagle’s medium-Ham’s F-12 1:1 medium ( Gibco) supplemented with 10% v/v fetal bovine serum (HyClone) and 1% v/v penicillin-streptomycin (GIBCO). Cells were removed from tissue culture PS by applying a trypsin-ethylenediaminetetraacetic acid (EDTA) solution and cultured on test substrates at 1 × 10⁵ cells/cm² using a standard incubator. For sterilization, test substrata were exposed, prior to cell culture, to ultra violet light for 1 h.

2.4. Cell area and adhesion

Cells were cultured on test films for 3 h, washed with phosphate buffered saline (PBS), fixed for 20 min using a 4% w/v paraformaldehyde solution in PBS, and stained using a 0.5% w/v Coomassie blue solution. Using a Nikon Optiphot-2 microscope, seven random spot images (960 × 720 μm²) were taken from repeated cell culture experiments at 100 × magnification. Each image contained 20–50 cells for a total of at least 150 cells per condition. Area and circularity of cells were quantified based on the number of calibrated pixels covered by the cell using ImageJ software. When cells were clumped, the data were manually eliminated. For adhesion assay, cells cultured for 3 h were washed with PBS three times to remove nonadherent cells. Attached cell number was determined by trypaninization and hemacytometer counting. Adhesion assays were performed three times, each in triplicate.

2.5. Western immunoblotting

Immunoblotting of focal adhesion proteins was performed after 24 h of culture. Proteins were collected in distilled water supplemented with 0.1%
v/v Triton X-100, 1% v/v Tris-EDTA, 1% v/v protease inhibitors (Calbiochem), and 0.2 μM Na3VO4. The supernatant collected after centrifuging for 15 min at 13,000 min⁻¹ was used. Proteins were fractionated by electrophoresis and electrotransferred to polyvinylidene difluoride film. After blocking with 1% w/v bovine serum albumin (BSA) solution in TBS-T (10 mM Tris, 150 mM NaCl, 0.05% v/v Tween 20), the blots were exposed to mouse antibodies specific to integrin αv (BD Biosciences, 611012), integrin β3 (BD Biosciences, 611140), integrin β1 (Chemicon, MAB1965), paxillin (Sigma, P1093), vinculin (Chemicon, Fak100-90227), FAK (BD Biosciences, 610087), and phosphorylated FAK (pY397) (BD Biosciences, 611806) or rabbit antibody specific to integrin α5 (Chemicon, AB1949). After reacting with secondary antibody, immunoreactive bands were visualized using enhanced chemiluminescence detection and quantified using densitometry analysis with Quality One software (Bio-Rad). Blots were stripped and reprobed for the loading control of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Four immunoblots were carried out from different cell cultures. Mean and standard deviation of GAPDH-normalized intensities were calculated (n = 4) and are shown as a ratio to those of flat PLLA.

2.6. Immunofluorescence

After 24 h of culture, cells were fixed with a 4% w/v paraformaldehyde PBS solution, rinsed with a 0.05% v/v Tween-20 wash buffer, and permeabilized with a 0.1% v/v Triton X-100 solution. After blocking with a 1% w/v BSA solution in PBS, cells were incubated with anti-paxillin or anti-vinculin antibody solution (the same antibodies as immunoblotting). Cells were washed and reacted with fluorescein isothiocyanate (FITC)-conjugated secondary antibody. To detect actin simultaneously, tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin (Chemicon, Fak100-90228) was added in the secondary antibody solution. Double-stained cells were observed using a Nikon Optiphot-2 fluorescent microscope using filters appropriate for FITC or TRITC.

2.7. Statistics

For cell area, adhesion, and immunoblotting data, statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls post-hoc tests and presented as *: p < 0.05 and **: p < 0.01 when compared with flat PLLA control and #: p < 0.05 and ##: p < 0.01 when compared with 14 nm pit surfaces among nanotextured surfaces.

3. Results and discussion

3.1. Nanoscale pit textured topographies

PLLA/PS (50/50 w/w) demixed films displayed surfaces covered with randomly distributed circular or channel-like pits (Fig. 1). This is consistent with our previous screening of PLLA/PS composition effects in that PLLA/PS demixing, under given molecular weights, produces pit textures at a PLLA weight fraction (f) ≈ 0.5 or island texture at f ≈ 0.7 [9]. The scale of pits were controlled within nanoscale, e.g., 0.5%, 1%, and 1.5% w/w concentration produced 14, 29, and 45 nm deep pits, respectively (Table 1). With increasing pit depth, a pit area also increased. However, the fractional pit coverage per unit surface area was ca. 32% for all three textures. That the
fractional area coverage is maintained while individual topographic scale is varied is one advantage of these self-organized substrates. These films could thus provide model substrata for assessing cell response to nanotopographic scale. The increase in surface area was less than 1% for all three nanopits, e.g., 5 × 5 μm² AFM images showed average surface area of 25.15, 25.18, and 25.20 μm² for 14, 29, and 45 nm deep pits, respectively.

Pit-textured films displayed nanoscale roughnesses (4–16 nm) that were proportional to the pit depth (Table 1). \( R_q \) was larger than \( R_a \) by a factor of ca. 1.1, as is expected by their definitions [14]. Roughness parameters, a measure of distance from the average height, are sometimes misleading, e.g., two sinusoidal curves having the same amplitude but different wavelength result in the same roughness parameters. Thus, these parameters may not be effective for describing the fractional area coverage of topographic features. Based on the observations that the surface coverage of pits is similar and pit dimensions are proportional to the nanoscale pit depth, we used pit depth as a representative parameter and refer to these textures as ‘nanopit’ topographies. Pure PLLA films displayed flat surfaces (Fig. 1) with negligible roughness. Film thickness was ca. 40, 80, and 120 nm for 0.5, 1, and 1.5% w/w concentrations, respectively, as assessed by AFM after manual scratching.

### 3.2. Surface chemistry of nanopit films by XPS and SIMS

XPS and SIMS spectra of the three PLLA/PS (50/50 w/w) demixed films exhibited strong PLLA specific characteristics, and this trend was more pronounced in SIMS than in XPS (see ‘Supporting Information’). In Fig. 2, the film surface PLLA fraction obtained from XPS and SIMS, in comparison with a bulk fractional amount of PLLA repeating unit in the blend solution (dotted line at 0.59), is summarized. The PLLA dose of 0.59 was calculated by dividing the weight fraction by the repeating unit molecular weights, e.g., PLLA/PS 50/50 w/w leads to (50/(71.05):(50/(104.15)) = 0.59:0.41. All the XPS and SIMS data show compositions above 0.59, indicating that PLLA tends to segregate to the film surface. The PLLA film surface segregation was attributed in our previous study to the molecular weight-driven entropy effect overwhelming the surface energy effect [9]. That is, relatively lower molecular weight PLLA segregates to the air–film interface, despite its relatively higher surface energy, to reduce the entropic penalty that would occur if relatively high molecular weight PS is exposed to the surface. In the present study, PLLA enrichment at the film surface was less when the polymer-demixing concentration was lower producing thinner films, e.g., the PLLA composition assessed down to 90 Å depth (XPS) was less when spin-cast from a 0.5% w/w solution relative to that from a 1.5% w/w solution. In thinner films, the film structure may be frozen-in before PLLA film surface segregation is fully achieved, probably because solvent would evaporate relatively faster. When focusing on the topmost film surface down to 20 Å depth (SIMS), however, variation in PLLA composition among nanotopographies was small (0.93–0.96).

Cells interact with only the topmost surface chemistry of these films. SIMS revealed that differences in surface chemical composition were 4–7% between demixed and pure PLLA films and less than 3% among nanotopographies. The precise spatial distributions of such chemical compositions (chemical maps) could not be defined under the current SIMS experimental set-up. However, considering that the SIMS data in Fig. 2 represent the average chemical compositions at the air-film interface, our data
indicate that the nanopit textures have topmost film surfaces filled primarily with PLLA (>93%). Therefore, the SIMS data suggest that topography effects on cells could be assessed exclusively while surface chemistry remains mostly unchanged (though exact spatial distribution is not known).

3.3. Systematic effects of nanopit scale on cell area and adhesion

PLLA/PS-demixed nanopit textures systematically affected adherent cell area and number. hFOB cells cultured on 14 and 29 nm deep pit surfaces were well spread, while cells on 45 nm deep pits and flat PLLA were less spread out (Fig. 3). Cell area was significantly greater on 14 and 29 nm pits than on PLLA flat (p<0.01 and 0.05, respectively).

Circularity of cells, defined as \(4\pi \times \frac{\text{area}}{\text{perimeter}^2}\), was higher when cell spreading was suppressed (45 nm pits) indicating that a smaller population of cells had an elongated polygon shape. Fig. 4 shows that cell attachment was altered in a fashion similar to cell area. Shallower nanopit surfaces (14 and 29 nm) induced significantly greater cell attachment than did flat PLLA surfaces. Among nanopit textures, cell adhesion increased with decreasing pit depth (14 > 29 > 45 nm).

Our results on nanoscale pits are similar to those on nanoscale island textures previously reported by us [8] and others [15–17]. In these studies, fibroblasts and osteoblasts cultured on PS/poly(4-bromostyrene) (PS/PBrS)-demixed island topographies displayed greater adhesion on 11–13 nm high islands than on 85–95 nm high islands. The greater hFOB cell adhesion on 14 nm deep nanopits

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Fig. 3. (Upper) Optical microscope images of Coomassie blue stained hFOB cells cultured for 3 h on PLLA/PS (50/50 w/w)-demixed nanopit-textured films and flat PLLA films. (Lower) Cell area and circularity quantified by ImageJ software after culturing for 3 h. Statistical significance assessed by ANOVA followed by Student-Newman-Keuls post-hoc tests is shown as *: \(p<0.05\) and **: \(p<0.01\) when compared with flat PLLA control and #: \(p<0.05\) and ##: \(p<0.01\) when compared with 14 nm deep pit surfaces among nanotextures.
and the attenuation with pit depth up to 45 nm, together with the previous results on nanoislands, suggest that nanotopography induces greater cell adhesion when topographic features have ca. 10–20 nm scale z-axis dimension (height or depth), regardless of topographic shapes (island or pit). Also, this effect deteriorates up to a z-axis topographic scale of ca. 100 nm for both shapes. Taken together, these data suggest that nanoscale topography can be exploited as a novel tool for controlling cell adhesiveness.

3.4. Differential integrin expression on nanoscale pit textures

Nanopit surfaces affected the integrin expression in hFOB cells but only for specific subunits. Immunoblotting revealed that αv integrin displayed the most dramatic variations with respect to nanopit scale during the 24 h culture period (Fig. 5). Level of integrin αv expression was significantly higher in hFOB cells cultured on 14 and 29 nm culture period (Fig. 5). Level of integrin αv expression was significantly higher in hFOB cells cultured on 14 and 29 nm pits relative to those on 45 nm pits or flat PLLA (p < 0.05). Integrin β3, which forms the vitronectin receptor with αv [1], however, did not exhibit significant variation with nanotopographies. Integrin α5 and β1, components of the fibronectin receptor [1] did not change, as a function of nanotexture, either. Taken together, these data indicate that nanopit texture regulation of hFOB integrin expression occurs selectively on specific integrin subunits (αv relative to α5, β1, or β3). The variation of integrin αv is generally consistent with cell area and attachment (Figs. 3–5), suggesting that nanotopography effect on cells may at least partly be mediated by integrin αv expression.

It is not clear how nanotopography affects integrin expression. In the literature, there is controversy regarding topography-induced changes in protein adsorption and its mediation of topography effects on cells. For example, fibronectin adsorption from a single protein buffer increased on rougher titanium surfaces with microscale roughness [18]. However, this trend was reversed when protein adsorption data were corrected for surface area increase of textured surfaces [19]. Sharp discontinuities in topography (edges) may induce a local increase in surface energy that may affect protein adsorption and altered protein adsorption in edges would in turn influence cell behavior [20]. However, reliable measurement of the surface energy of edges is difficult complicating the interpretation of these results. Furthermore, cells display contact guided orientation on unsharp topographies that would not be expected to have altered surface energies or protein adsorption [21], suggesting that cells can respond to surface topography independent of local surface energy changes.

In some studies, topography modification accompanied large differences in surface chemistry [20,22], making it difficult to distinguish topography effects from surface chemistry effects. In a recent study using nanoscale titanium films having the same surface chemistry, no significant differences in albumin and fibrinogen adsorption were observed within a roughness scale of 2–21 nm [23], a scale very similar to that of our study. As reviewed by Wilson et al. [24], differential cell responses to different surface chemistry or wettability can largely be attributed to differences in the composition and bioactivity of adsorbed proteins. However, this may not be the case for cell response to topography. These authors concluded that topography effects on cells are largely independent of the mechanisms mediated by differences in protein adsorption and are more likely mediated by constraints on cell morphology or altered secretion of cell adhesive molecules (such as fibronectin) by cells [24]. This suggests that direct cell sensing and response to topography may play a more important role in topography effects on cells than indirect mediation via adsorbed proteins, as long as the surface chemistry is the same.

We found that nanotextured PLLA/PS demixed films with varying topographic scale have a similar surface chemistry (seen by SIMS) and very little difference in surface area (<1%). It is unlikely that surface chemistry resulted in changes in adsorption of serum proteins. However, we cannot completely rule out the possibility that differential serum protein adsorption on varying nanotopographies may play a role in nanotopography regulation of cell behavior. In summary as regards integrin expression, we demonstrated that nanoscale topographies can affect selective integrin expression after 24 h of culture under very similar surface chemistry, though the mechanism by which this occurs is yet not clear.

3.5. Paxillin and vinculin expression and colocalization with actin on nanopit textures

Focal adhesion protein development in hFOB cells after 24 h of culture was affected by nanoscale topographies as regards both relative abundance and colocalization with...
the cytoskeleton. Paxillin and vinculin act as anchors of actin cytoskeletons and form focal adhesion assembly by linking actins to transmembrane integrins. Western blotting revealed that paxillin expression after 24 h of culture was significantly greater in hFOB cells cultured on 14 and 29 nm pit surfaces relative to those on flat PLLA control ($p < 0.05$, Fig. 6). Vinculin did not display significantly altered expression with respect to nanopit textures.
Changes in paxillin and vinculin expression were positively linked with structural organization assessed by immunofluorescence (Fig. 7). hFOB cells cultured on 14 and 29 nm pits displayed well developed paxillin plaque-like structures (green). They also displayed highly tensioned actin stress fibers (red) the growing ends of which were anchored to paxillin. However, in cells on 45 nm pits or flat PLLA, neither distinct paxillin plaques nor stressed actin fibers were observed. Vinculin–actin staining displayed similar trends to some degree but not as strong as the case of paxillin–actin. Importantly, the variation in paxillin expression assessed by immunoblotting is very similar to that of integrin αv, suggesting the possibility that development of focal adhesion structures, e.g., integrin αv-paxillin-actin, may mediate the nanoscale topography effect on cells.

3.6. FAK and phosphorylated FAK (pY397) expression on nanopit textures

Nanoscale pit topographies also induced differences in expression of integrin-mediated cell signaling molecules (FAK) in osteoblastic cells. FAK is a tyrosine kinase that colocalizes with integrins at the C-terminal focal adhesion-targeting (FAT) domain via associations with integrin binding proteins, paxillin and talin [25]. FAK can be phosphorylated at tyrosine-397, tyrosine-576, tyrosine-577, tyrosine-861, and tyrosine-925. Tyrosine-397 is autophosphorylated when integrin-ligand binding is activated and phosphorylated FAK (pY397) creates binding sites for Src homology 2 (SH2) domains of various structural and intracellular signaling molecules such as Src-family protein tyrosine kinases (PTKs) [25,26]. Western blotting revealed that both autophosphorylated FAK (pY397) and site-nonspecific FAK expression in hFOB cells were affected by nanotopographies, i.e., significantly higher FAK (pY397) and FAK levels in cells on 14 and 29 nm nanopits after 24 h of culture relative to cells on 45 nm pits or flat PLLA ($p<0.05$, Fig. 8). This reveals the same trend as with integrin αv and paxillin, indicating that integrin αv may be involved in the triggering of tyrosine-397 autophosphorylation. This also suggests that variations in FAK (pY397) and FAK activations on nanotextured surfaces may at least partly originate from nanopit scale dependent changes in integrin αv and paxillin.

While emerging data demonstrate nanotopography effects on cell behavior [8–10,15–17], the mechanism by which this occurs is unclear. Recently, Dalby et al. [27,28] suggested a so called ‘self-induced mechanotransduction’ mechanism based on the hypothesis that surface topography alters nuclear morphology and chromosome position in adherent cells leading to changes in gene transcription. This self-induced mechanotransduction mechanism is correlated with the ‘direct mechanotransduction’ mechanism as hypothesized in the tensegrity model by Ingber [29,30] wherein forces encountered by cells during cell

Fig. 6. Western immunoblotting of focal adhesion paxillin and vinculin proteins in hFOB cells cultured for 24 h on PLLA/PS (50/50 w/w)-demixed nanopit-textured films and flat PLLA films. See Fig. 5 legend for band intensity analysis and Fig. 3 legend for statistics.
adhesion are directly transmitted to the nucleus via cytoskeletons and altered cytoskeletal tension then feeds back to induce local changes in focal adhesion assembly. Dalby et al. [28] argued that most of the effects observed in cells on topography originate from changes in the ability of cells to spread and that the direct mechanotransduction mechanism supports the theory of chromosome position change. Our results on cell spreading and focal adhesion-cytoskeleton formation (Figs. 3, 5–7) support the concept that nanotopography affects cell behavior via a direct mechanism.

In addition to direct or self-induced mechanisms described above, we propose that differential integrin regulation, which would further mediate focal adhesion assembly and intracellular signaling, may play an important role in nanotopography regulated effects on cells. Our study is the first demonstration that nanoscale topographies can induce differential expression in selective integrins, paxillin, FAK, and FAK (pY397). Upregulation of focal adhesion structure (paxillin–actin upregulation and colocalization) in cells on 14–29 nm deep nanopits correlates with integrin αv upregulation (relative to other integrins α5, β1, or β3) as does the activation of signaling molecules such as FAK and phosphorylated FAK (pY397) (Figs. 5–8). These results suggest that the cell stimulatory effect of nanoscale topographies may be achieved

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Fig. 7. Paxillin and vinculin (both green) immunofluorescent staining double-labeled with actin (red) for hFOB cells cultured for 24 h on PLLA/PS (50/50 w/w)-demixed nanopit-textured films and flat PLLA films.
by upregulating specific integrin expression (e.g., \( \alpha_v \)) that subsequently results in recruitment of paxillin to focal adhesion complexes as well as in activation of FAK and further downstream signaling events. FAK, due to its ability to bind to various structural and signaling proteins, has been implicated in a diverse array of cellular processes including migration, growth factor signaling, cell cycle progression, and cell survival [25,26]. Assessing events downstream of FAK signaling pathways in cells on different nanotopographies may elicit novel explanations for extracellular physical (e.g., nanotopography) regulation of cell function.

4. Conclusions

Nanoscale pit topographies were produced using a self-organizing technique to assess short-term osteoblastic cell behavior. The nanotopographic scale was controlled such that nanopit depth of PLLA/PS (50/50 w/w)-demixed films was altered between ca. 10 and 50 nm. These films had topmost surface chemistries very similar to those of pure PLLA films, as assessed by XPS and SIMS, indicating that topography effects on cells could be examined largely independent of surface chemistry effects. The shallower depth nanopits induced significantly greater hFOB cell spreading and attachment than did deeper nanopits or flat PLLA surfaces (14–29 > 45 nm deep pits \( \approx \) flat PLLA). Importantly, specific integrin (\( \alpha_v \)) mediated focal adhesion structure formation (paxillin–actin) and the activation of FAK and phosphorylated FAK (pY397) were significantly enhanced for hFOB cells on 14–29 nm deep pits relative to cells on 45 nm pits or flat control. These results support the direct mechanotransduction mechanism proposed for topographic control of cell behavior and emphasize the potential importance of nanotopography regulation of specific integrins. Our results also suggest that topography modification within the nanoscale may be exploited as novel biophysical signals for controlling cell functions.

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Appendix A. Supplementary Materials

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References


