Comparative response of epithelial cells and osteoblasts to microfabricated tapered pit topographies in vitro and in vivo

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Abstract

Microfabricated tapered pits in vivo can stimulate connective tissue and bone attachment to percutaneous devices, secondarily preventing epithelial migration, and promoting long-term implant survival. Epithelial cells, which form a seal with a dental implant, acting as a barrier, and osteoblasts, which form bone, can come into contact with the same implant topography. To investigate whether the phenotypic characteristics of each cell type influenced cell response to micro-topography, we compared the response of the two cell types to the same dimensions of tapered pits, in vitro, and in vivo. Increased spreading, mature FAs, and restricted migration characterized individual PLE cell response to tapered pits. In contrast, osteoblasts were highly migratory, formed smaller, punctate adhesions and mineralized. Epithelial sheets formed from high-density PLE cultures demonstrated that tapered pits did not inhibit migration of the PLE sheets in vitro, similar to in vivo observations. In vitro, PLE sheet migration correlated with increases in vinculin, tyrosine phosphorylation, cytokeratin and ERK 1/2 phosphorylation. The findings of this study show that tapered pits stimulate osteoblast mineral deposition in vitro and in vivo, but do not prevent epithelial sheet migration. In vitro results suggest that epithelial sheet migration could involve altered FA mediated signal transduction.

1. Introduction

Upon placement in the jaw, a dental implant must successfully interface with at least three distinct cell populations, osteoblasts (bone), fibroblasts (connective tissue), and epithelium [1,2], at different regions along the long axis of the implant. Each cell type is specialized to perform a specific physiological function. Osteoblasts secrete matrix and mineral to form bone, and fibroblasts produce collagen rich connective tissue, with both tissues helping to anchor the implant. Epithelial cells attach at the dorsal interface of the tissue and implant, ideally forming a tight seal between the tissue and the implant surface. Inappropriate, as well as inadequate, adhesion of these three cell populations has been implicated in poor implant survival [2–5]. Failure of bone and connective tissue to form a tight seal to the implant can result in epithelial downgrowth, which can lead to deep pocket formation as well as possible avulsion of the implant, whereas an inadequate epithelial seal can lead to infection [3,6–8]. Therefore, identifying the surface features that enhance bone and connective tissue attachment to the implant, whilst, discouraging epithelial downward migration would be advantageous for optimizing implant longevity [2,3,7,9].

Advances in implant design have often focused on altering the micro-topography and chemistry of the implant surface [2,10–12]. In particular, implant surface topography has been identified as a potent modulator of bone and connective tissue attachment in vivo, as well as showing success in inhibiting epithelial downgrowth [4,6,7]. Chehrouri et al. [4], identified that both grooved substrata and tapered pits (TPs) could promote connective tissue attachment in vivo, which in turn reduced the level of epithelial downgrowth [4]. However, not all groove depths formed an absolute barrier to epithelial downward migration, and inhibition of this downgrowth for times greater than 2 weeks seemed related more to the level of...
attachment of bone and connective tissue to the implant surface, than a direct effect of the topography on the epithelial cells themselves [4,13].

That different cell types demonstrate varying degrees of adhesion to different types of topographies opens the possibility of optimizing adhesion of defined cell populations on specific regions of the implant surface by modulating the surface topography along the length of the implant. However, in vitro investigations of cell behaviour have shown that it is not a simple task, because topographies have diverse effects on cell behaviour. For example, microfabricated discontinuous edged topographies significantly enhance osteoblast mineralization in vitro [14], but also promote the attachment, spreading and migration of both fibroblast and epithelial cells [15]. In contrast, rough surfaces produced through processes such as acid etching support excellent fibroblast and osteoblast attachment [16,17], but discourage epithelial attachment and spreading [18].

As osteoblasts and epithelial cells have markedly different cell migratory behaviours and both interface with dental implants [2], we investigated whether the behaviour of the two cell types in vitro and in vivo on TPs would be related to the innate phenotypic properties that the cells exhibit in tissues in vivo. TPs and inverted pyramids (IPs) within the range of 30–120 μm in depth were used due to their potential to promote tissue ingrowth, which is essential for implant integration [6,19]. For this study, the response of rat calvarial osteoblasts (RCOs) and porcine periodontal ligament epithelial (PLE) cells to TP provided the in vitro model, and a one or two stage rat percutaneous model described by Chehroudi et al. used for the in vivo model [4]. The results from this study demonstrate that TP topographies are osteoconductive in vitro and in vivo, but their influence on epithelial migration is limited.

2. Materials and methods

2.1. Surface fabrication

The TPs and IPs were etched on silicon substrates, using microfabrication techniques originally developed by Camporesi et al. [20]. The substrata were produced in the laboratories of the Center for Microelectronics, Department of Electrical Engineering, at University of British Columbia. The depth and spacing of the TPs and IPs are controlled by the time of etching and the crystalline orientation of the silicon wafer. The TPs and IP employed in the study are depicted diagrammatically in Fig. 1A–C, and the dimensions of the TPs and IP are shown in Table 1. The depth of surface ranged from 30 to 120 μm to observe a wide range of depth effect. The TP structures had a flat surface at the bottom of the pit for cell spreading and adhesion. The ridge was constant at 10 μm amongst all TP surfaces. The 30 μm IP contained a wide ridge and was designed specifically to ensure the same number of pits were present on all the surfaces, irrespective of pit size.

2.1.1. Fabrication and preparation of in vitro substratum replicas

For in vitro experiments, epoxy replicas were fabricated as previously described [15]. Briefly, epoxy replicas were fabricated from negative impressions of the silicon wafers, baked at 60 °C for 4 days and sputter-coated (Randex 3140 Sputtering System, Palo Alto, CA) with 50 nm of Ti. Prior to use, the replicas were cleaned by ultrasonication in 7X detergent [15], Argon-gas glow discharge treatment [21], performed prior to seeding with PLE or RCOs, completed cleaning and sterilization procedures.

2.1.2. Fabrication and preparation of in vivo implants

Fabrication, and preparation of the implants were performed as previously described by Chehroudi et al. [4]. In brief, implants were made from two components; a percutaneous and a subcutaneous component that had microfabricated and control smooth surfaces. The titanium coated implants were cleaned by ultrasonication in a detergent 7X (ICN Biomedicals, Inc., Costa Mesa, CA), Argon-gas glow discharge treatment [21], performed prior to seeding with PLE or RCOs, completed cleaning and sterilization procedures.

Table 1

<table>
<thead>
<tr>
<th>Surface</th>
<th>Depth (μm)</th>
<th>Pitch (μm)</th>
<th>Top of pit (μm)</th>
<th>Bottom of pit (μm)</th>
<th>Ridge (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30IP185S</td>
<td>30</td>
<td>185</td>
<td>45</td>
<td>N/A</td>
<td>140</td>
</tr>
<tr>
<td>30TPS05S</td>
<td>30</td>
<td>50</td>
<td>45</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>60TP105S</td>
<td>60</td>
<td>105</td>
<td>85</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>120TP185S</td>
<td>120</td>
<td>185</td>
<td>175</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>120TP280S</td>
<td>120</td>
<td>280</td>
<td>270</td>
<td>110</td>
<td>10</td>
</tr>
</tbody>
</table>
2.2. In vitro cell isolation and culture

Epithelial cells from porcine periodontal ligament, and osteoblast cells from newborn rat calvaria were isolated and cultured as previously described [22,23]. The two cell types were cultured on tissue-culture plastic in alpha minimal essential medium (Stem cell Technology, Vancouver, BC, Canada) supplemented with antibiotics (penicillin G, 100 mg/ml (Sigma, St. Louis, MO); gentamicin, 50 mg/ml (Sigma); Fungizone, 3 mg/ml (Gibco, Grand Island, NY)) and 15% fetal bovine serum (Flow, McLean, VA) at 37°C in a humidified atmosphere of 95% air 5% CO2. Both cell types were trypsinized (0.25% trypsin, 0.1% glucose, citrate-saline buffer pH 7.8) counted electronically (Cell Counter, Coulter Electronics Limited, Luton, England) prior to seeding on topographical cues. For low-density cultures, cells were seeded onto the substrata at 20,000 cells per cm², and for high-density cultures, 100,000 cells per cm². During experiments, osteoblast media was supplemented with 31.5 mg/ml Na-β-glycerolphosphate, 0.58 mg/ml L-ascorbic acid phosphate magnesium salt n-hydrate, and 5 μg/ml of Alizarin Complexone (Sigma), with media changes performed twice a week in experiments over 3 days in length to ensure sufficient nutrient supply.

2.3. In vivo implant procedure and specimen collection/processing

Male inbred Sprague Dawley rats, weighing between 350 and 600 g were intubated and halothane anesthesia performed according to the requirements of the Canadian Council of Animal Care, under University of British Columbia Animal Care certification. Implants were either placed as a one stage or two-stage procedure [4]. In one-stage procedure, the two sub and percutaneous components were attached together and secured with a titanium miniature pin at the parietal areas of the rats. One stage implants were harvested 3-weeks post-implantation. For two stage implants, the subcutaneous component was placed 8 weeks prior to the addition of the percutaneous component to allow connective tissue ingrowth. The whole implants were then harvested 3 weeks after placement of the percutaneous component. Animals were euthanized with an overdose of sodium pentobarbital, and whole body perfusion performed using 2.5% glutaraldehyde prior to implant removal. Specimens were fixed in 4% paraformaldehyde and polymerized In 0.5% osmium tetraoxide and viewed on a Zeiss light microscope (Zeiss Microscopes, Upstate Biotechnology, Charlottesville, VA), rabbit anti-mouse antibodies were verified to be cross-reactive with porcine and rat tissue, and a dilution series was performed for each antibody to select the optimal working concentration. For observing PY99, FA kinase, ERK 1/2, vinculin and pan cytokeratin, all samples were fixed in 4% paraformaldehyde, and cell membranes permeabilized with 0.5% Triton X-100 (Sigma) for 5 min. Blocking of non-specific binding was performed using 1% bovine serum albumin (BSA, Sigma) and the samples were incubated with the appropriate primary antibody, at a pre-determined concentration for 90 min. This was followed by three 10 min washes in 1% BSA in PBS. The secondary antibody was either a sheep—anti-rabbit (For ERK 1/2, FA Kinase) or a goat anti-mouse (For PY99, vinculin, pan cytokeratin), IgG conjugated to Alex Fluor 596 (Molecular Probes Inc., Oregon, WA) at a concentration of 1:200 for 1 h. All antibodies were diluted in PBS containing 0.1% BSA. Labeling was visualized on a Zeiss Axioscope 2 or a Nikon Eclipse C1 confocal microscope using up to 63 × objectives, under TRITC optics using a Princeton Pentamax CCD camera, and images captured using Nikon Eclipse software. Images were imported to Photoshop 7.0 for analysis and sizing.

2.7. Immunoblotting

Immunoblotting was performed as described previously [25]. In short, protein levels were quantified using the BCA protein assay kit (Pierce, Rockford, IL), and 30 μg of total protein was mixed in a 1:2 ratio with Laemelli buffer and 2-mercaptoethanol and boiled for 5 min to denature the protein samples. Samples were then loaded into either 10% or 14% SDS page gels and the samples run at 175 V for 45 min. Following this, the gels were blotted onto PVDF membranes at 100 V for 2 h. For the detection of the specific antigens, the PVDF membrane was incubated with 1:1000 dilution of the appropriate monoclonal antibody overnight at 4°C, and then washed with TBS washing buffer for 1 h. After application of the biotinylated goat anti-mouse IgG at a dilution of 1:5000 (Bio-Rad, Mississauga, Ont., Canada), the membrane was developed using enhanced chemiluminescent method. For quantification, densitometric analysis was performed using the Image J analysis software.

2.8. Statistics

Data were analysed using the software program SPSS (version 11.04 for Macintosh OS X; SPSS Inc., Chicago, IL). For western blots experiments, three replicate experiments were performed for each surface (smooth, 120TP280S) and each label (tensin, vinculin, ERK 1/2). Comparisons were made between smooth and the experimental surfaces by time (4 weeks) using paired t-tests (p<0.05).

3. Results

3.1. Epithelial and bone attachment in vivo

Three weeks post-implantation, epithelium had migrated over the 120TP280S topographies on one stage implants (Fig. 2A). The epithelium was observed to consist of stratified layers, which had migrated and replaced the connective tissue attachment on the TP surface of the implant. On both one stage and two stage implants with smooth control surfaces, epithelium had either partially or completely marsupialized the entire implant body (data not shown; for example see [4]). On two stage implants, connective tissue matured and grew into the TPs on the subcutaneous component at 8 weeks, and after the addition of the percutaneous component, the epithelium was only
able to migrate until it encountered the connective tissue/implant interface (Fig. 2B). The proximity of the subcutaneous part of the two-stage implant to the periosteum of the calvaria often resulted in bone formation on the textured surfaces, and this was evident in 120TP280S at after 8 weeks (Fig. 2C). Mineralized tissue was observed to completely fill the area of the 120TP280S pits (Fig. 2C, D), and mature osteocyte-like cells were present within the region of mineralized tissue (Fig. 2D).

3.2. In vitro migration of individual PLE and RCO cells

To assess the basic response of individual PLE cells and RCO to TPs and 30μm IP, time-lapse microscopy was employed. On the 60TP105S, 120TP185S 120TP280S, PLE...
cells were observed to migrate down onto the slopes of the pits from the ridges, but were noted to be unable to migrate back out, seemingly becoming trapped in the TPs (Fig. 3 and Movie 1). On 30TP50S and 30IP185S, where the space at the top was narrower, PLE cells had a tendency to cover over the top of the pits, and exhibited little migration, but in contrast, RCO cells were able to traverse between TPs irrespective of the depth, or spacing of the pits (Fig. 3 and Movie 2). Furthermore, RCOs were able to bridge across the top of the pits, and were observed to migrate down the ridges, across the base, and back up the slopes of the deepest pits, 120TP185S, and 120TP280S.

3.3. Cell morphology

Scanning electron microscopy was employed to observe the morphological response of PLE cells and RCOs to different dimensions of TPs and IP (Fig. 4). On smooth surfaces, epithelial cells were well spread with very close attachment to the surface, but they did not exhibit any preferred orientation. On 30IP185S, PLE cells were well spread on smooth areas, but were also observed to cover over the top section of the IP. On 60TP105S, PLE cells bridged from ridge to ridge covering over the top of the pits. On 120TP280S, PLE cells were spread, spanning over ridges, but were also found spread on the 55° slopes of the TPs. RCOs were able to bridge and spread on all IP and TPs tested, as was observed in time-lapse movies. The most common RCO morphology was an elongated, spindle shaped with a well-developed leading edge. RCOs were able to bridge from one pit across a ridge and down onto the slope of the neighbouring pit.

3.4. Focal adhesion formation

As PLE cells exhibited restricted migration, and were well spread, forming close attachments to the pits compared with RCOs, we hypothesized that the PLE cells may differ in the formation of focal adhesions (FA) on these surfaces, when compared with RCOs. PLE and RCOs were cultured on the various dimensions of TPs and 30 µm IP for 24h and then stained for vinculin, a FA protein. On smooth surfaces, PLE cells were observed to form large plaque FAs, and in comparison, RCOs exhibited much smaller punctate adhesions, although plaque adhesions were evident on leading lamellapodia (Fig. 5). When cultured on 30IP185S, both PLE cells and RCOs that bridged over the pits exhibited large plaque formation on the smooth areas up to the edge of the pits. Similarly, on 60TP105S, PLE cells covered the entire pits, forming FAs on all ridges at the top of the TPs. On 120TP185S and 120TP280S, the largest area of FA formation was localized to the smooth areas adjacent to the top of the pits, with fewer focal contacts formed in the area of the cells towards the base of the TPs. On both 60TP105S and 30IP1755S, RCO cells that bridged between
ridges, or between ridges and floors, formed FAs at each area of the cell in contact with the surface. RCO cells also spread on the slopes of the TPs and IP, with prominent vinculin containing leading lamellae.

3.5. Influence of TPs on high-density cultures

When seeded as cultures of individual cells, both PLE cells and RCOs were found to exhibit distinct patterns of behaviour. To more accurately mimic the in vivo situation, high-density cultures of both cell types were seeded on smooth controls, 120TP185S, and 120TP280S to assess how population density influenced the response of both PLE cells and RCOs. 120TP185S was selected as the two cell types exhibited different migratory responses to this substratum in vitro, and 120TP280S was employed as it had stimulated different responses from the two tissue types in vivo.

3.5.1. RCO production of mineralized matrix

The formation of mineralized matrix by RCOs, was assessed through incorporation of alizarin complexone to the newly formed mineral deposits [14]. On smooth surfaces at 2 and 4 weeks, mineral deposits were small and localized to regions above the cells, with nodule formation rarely observed at 4 weeks (Fig. 6A, C). At 2 weeks post-seeding, mineral incorporation on 120TP280S was significantly more intense than that seen on smooth surfaces, with nodules extending up from the base of the pit (Fig. 6B). Mineralization took the form of variable sized globular deposits. By 4 weeks, large mineral deposits were evident, that in some cases filled entire pits and extended over the ridges between neighbouring TPs (Fig. 6D).

Fig. 4. Scanning electron microscope images of the morphology of PLE cells and RCOs on varying dimensions of microfabricated, tapered pits, and smooth control surfaces. In (A) smooth surfaces, (B) 30IP185S, (C) 60TP105S, and (D) 120TP280S.
3.5.2. PLE cell sheets

In vivo, covering and lining epithelium exist as sheets of continuing cells with little extracellular matrix [2]. To mimic the in vivo condition, PLE cells were seeded at high density on the smooth region adjacent to the area of the substratum containing either the 120TP185S or 120TP280S substratum, where they formed sheets (Fig. 7A). Observations from time-lapse microscopy revealed that PLE cells at the edge of the sheet migrated onto the ridges of the pits and separated from the sheet (Fig. 7B and Movie 3). The PLE cells subsequently became trapped within the pit. Visualization of the cells using F-actin labeling demonstrated that the PLE cells had partially filled the first row of pits and moved into the subsequent row of pits as early as 24-h post-seeding (Fig. 7C). At 4-week post-seeding, PLE cells completely filled all pits adjacent to the smooth portion of the substratum.
Fig. 6. Mineralization of osteoblasts at 2 and 4 weeks post-seeding. On (A, C) flat surfaces, the staining localizes at the cellular level at both time-points, with little evidence of nodule formation evident, even at 4 weeks. On 120TP280S, nodule formation and globular mineral deposits are evident at (B) 2 weeks, and by (D) 4 weeks the pits are completely filled with mineral. In all images, mineralization was assessed through incorporation and visualization of AC under light ($\lambda_{\text{max}} = 543$ nm) on an epifluorescence microscope using a low light-intensity CCD camera.

3.6. Influence of substratum topography on adhesion associated proteins in migrating PLE sheet cultures

Cells can form two distinct types of adhesions, focal and/or fibrillar adhesions [26]. FAs are characterized by the presence of vinculin, while fibrillar adhesions, which are considered more mature adhesion sites, are rich in tensin. Western blot analysis revealed a significant increase in the amount of vinculin present in PLE cells migrating on 120TP280S than on smooth surfaces ($p < 0.05$) (Fig. 8A), but no significant difference was found in the amount of tensin (Fig. 8B).

3.7. Influence of substratum topography on cell signaling in migrating PLE sheet cultures

FAs have been identified as being a main cellular site of tyrosine phosphorylation, but in contrast, fibrillar adhesions are not associated with such phosphorylation [25,26]. As PLE cells cultured on 120TP280S contained significantly more vinculin than cells on smooth surfaces, western blotting for general tyrosine phosphorylation was performed. Overall, PLE cells cultured on 120TP280S showed an increase in the molecular weight of the phosphorylated proteins in comparison with cells grown on smooth, although no significant increase in the number of bands was detected (Fig. 9A). As we have previously shown that ERK 1/2 increased in RCOs grown on pits compared to smooth surfaces [25], PLE cells were probed for phosphorylation of these MAP kinases. Both ERK 1 and ERK 2 were phosphorylated in PLE cells on 120TP280S, but in comparison, PLE cells on smooth had a significantly lower level of phosphorylation of ERK 1 and 2 ($p < 0.05$) (Fig. 9B). Localization of the ERK 1 and 2 signals through immunocytochemistry in PLE cells grown on 120TP280S, demonstrated that the majority of the signal was present in the nucleus, but on smooth surface was distributed through the cytoplasm (Fig. 9C, D).

3.8. Influence of substratum topography on differentiation in migrating PLE sheet cultures

As multiple layers of epithelium which can be indicative of a differentiating epithelium [27], were observed in vivo and in vitro in PLE sheets growing over pits, immunocytochemistry was employed to assess qualitatively the levels of pan
cytokeratins (AE1:AE3) expressed by the cells in vitro. Increased staining intensity for pan cytokeratins was observed in PLE sheets grown on 120TP280S for 4 weeks compared to PLE sheets on smooth surfaces (Fig. 10).

4. Discussion

4.1. In vivo, tapered pits enhance bone formation, but do not inhibit long-term epithelial downward migration

Previous observations with microfabricated topographies suggested that long-term prevention of epithelial migration down percutaneous implants was related more to the attachment of connective tissue to the implant, than the direct influence of the implant topography itself on epithelial migration, which was effective only in the short term [4,6,7,28]. Similar results were obtained in vivo in this study, with epithelial downward migration only impeded by the presence of mature connective tissue formed in the TPs on the subcutaneous component of two stage implants prior to the addition of the percutaneous component. Bone ingrowth to the TPs was marked at 8 weeks on the subcutaneous component of two stage implants, with osteocyte like cells present within the mineralized tissue. These in vivo observations suggest that osteoblasts maybe more responsive to the TP topographies or the micro-environment surrounding them, but the migration of the stratified epithelial sheets seems largely unaffected by TP topographies. In vivo, osteoblasts and epithelial cells perform physiologically distinct functions, with the former creating bone and the latter migrating to cover and line other tissues. Thus the possibility arises that these innate cell type specific functions influence cellular response to topography. As these innate behaviours can be observed in vitro, we examined the response of the two cell types to TPs and IP in vitro.

4.2. In vitro individual RCOs and PLE cells exhibit differences in their migration, adhesion formation, and spreading on TPs, IP, and smooth surfaces

Individual PLE cells and RCOs were observed to have different morphologies, as well as exhibit different migratory behaviour when cultured on TPs and IP. The comparative differences in migration and morphology between individual PLE cells and RCOs indicated potential
differences in cell adhesion mechanisms, a hypothesis confirmed by immunolabelling for the FA protein, vinculin. The control of FA formation and cellular migration is an important area of research focus [28–30], particularly in the area of cell engineering and biomaterials [18,31,32]. It is interesting that individual epithelial cells show a reduced migratory ability and tendency to spread forming numerous large FAs. This would suggest that the in vivo function of PLE, the covering and lining of tissues could be a primary determinant of their response to TPs and IP in vitro, given their tendency to spread over these types of topographies. If properly controlled, this close adhesion to the topographies could allow for a tight seal at the top of the implant, preventing infection.

4.3. PLE sheet migration on TPs in vitro, is characterized by altered tyrosine phosphorylation, multilayering, cytokeratin expression and increased ERK 1/2 phosphorylation

In vivo, epithelial cells exist primarily in sheets, not as individual cells, and cell–cell contacts would be expected to lessen flexibility. Using high-density cultures seeded on the
edge of the region of TPs, it was observed that the topographies did not prevent the migration of the sheet, with multilayering evident, most likely due to proliferation of the PLE cells within the TPs. In addition, the staining intensity of pan cytokeratins increased compared with smooth. It is possible that as the PLE cells multilayer within the pits, the cells begin to terminally differentiate; the PLE cells switch from low molecular weight to high molecular weight cytokeratins. However, full analysis of the cytokeratin expression will need to be analysed by western blot to confirm this.

PLE sheets were observed to migrate up to 1000 µm across the TPs from the initial seeding zone over a 4-week period. The average downward migration by epithelium in vivo after 3 weeks in the study by Chehroudi et al. [4], was 475 ± 100 µm on smooth surfaces, and 265 ± 111 µm on 120TP280S. The most likely explanation for the differences between the 2D sheets in vitro migration and the 3D in vivo migration is the presence of connective tissue, platelets, inflammatory cells, cytokines, and fibrin in vivo, which would likely strongly influence the ability of the epithelium to migrate across the implant [4]. Also to be considered is the influence of cell–cell contacts in vivo where multiple cell layers exist, which may make the sheet less flexible, and therefore tend to bridge over the topography and essentially by-pass it. However, species variation (rat for in vivo, porcine for in vitro) and the source of epithelial cells (dermal for in vivo, epithelial cell rests of Malassez for in vitro) must also be considered in the interpretation of the results. Nevertheless, it is evident the epithelial sheets are not impeded by the presence of TPs, either in vivo or in vitro.

We have previously shown that mineral formation by RCOs grown on microfabricated TPs is associated with an increase in the levels of general tyrosine phosphorylation and the MAP kinases, ERK 1/2 [25]. Cellular tyrosine phosphorylation is often associated with vinculin rich FAs [26], and we identified a near 3-fold increase in the amount of vinculin in PLE sheets migrating on 120TP280S compared with smooth. One possible explanation for this observation is that as the PLE cells multilayer in the TPs, they begin to form adhesions on both the dorsal and ventral side, which could require an increase in vinculin content to sustain the increased number of cell–cell adhesions. An increase in the molecular weight of phosphorylated proteins, observed in PLE sheets grown on 120TP280S, could be related to the increase in vinculin containing FAs. Interestingly, there was no significant increase in the fibrillar adhesion protein, tensin, between smooth and 120TP280S. Fibrillar adhesions are not associated with increases in tyrosine phosphorylation [25,26], so it is likely that any alterations in signal transduction would be related to the additional vinculin rich FAs. FA mediated signal transduction is an area of increasing importance in the biomaterials field [33], mainly because of new “bio-active” materials being designed to enhance cell response to implanted biomaterials [34].

To further investigate signal transduction in PLE sheets, we qualitatively measured the phosphorylation of the MAP kinases ERK 1 and 2, which are sensitive to alterations in surface geometry [25], and has been shown to play an important role in early adhesion mediated intracellular signaling [35]. A quantitative increase in both phosphorylated ERK 1 and 2 was observed in PLE sheets grown on 120TP280S, with the signal localizing to the nucleus. Activated ERK 1 and 2 have to translocate to the nucleus to exert many of their effects, although the continued accumulation of phospho-ERK 1 and 2 in the nucleus requires continued adhesion and integrin activation [36]. Both ERK 1 and 2 also interact with paxillin and FA kinase both molecules associated with formation of focal contacts [37]. We have previously shown that a reduction of FA kinase activity mediated by PP2 inhibition of src reduces the level of ERK 1 and 2 phosphorylation in RCOs grown on microfabricated grooves, but does not affect migration [24]. It is therefore possible that increased adhesions formed by the PLE sheets on 120TP280S is responsible for the increase in ERK 1 and 2, although
analysis of integrins will be required to confirm this hypothesis. The observed increases in ERK 1 and 2 phosphorylation, in both PLE cells and RCO cells grown on microfabricated substratum topographies, infers that this molecule maybe of particular importance in adhesion mediated signal transduction regulation of cell response to substratum topography.

5. Conclusions

Using TP topographies of precisely defined geometry produced by microfabrication, we have demonstrated that epithelium and osteoblasts respond differently to the same topographies. Furthermore, individual PLE cells do not respond in the same manner as PLE sheets. The comparative differences are related to adhesion mechanisms, morphology and intracellular signaling. We suggest that the inherent functional properties of epithelial cells and osteoblasts strongly influence how they respond to surface topography.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biomaterials.2007.01.026.

References


