Poly(ethylene glycol) enhances cell motility on protein-based poly(ethylene glycol)–polycarbonate substrates: A mechanism for cell-guided ligand remodeling

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Abstract: The regulation of cell motility on ligand-adSORbed poly(ethylene glycol) (PEG)-based polymeric biomaterials is governed by variables that are not well characterized. In this report, we examined keratinocyte migratory responsiveness to PEG-variant tyrosine-derived polycarbonates adsorbed with equivalent levels of the cell adhesion ligand, fibronectin. The equivalently adsorbed ligand adopted differential distributions, confirmed via atomic force microscopy, and the total number of exposed cell-binding domains (CBD), quantified through immunosorbent fluorometry, varied as a function of PEG concentration. Specifically, the CBD exposure was maximized at 4 mol % PEG and diminished at 8 mol % PEG, suggesting, based on our previous work (Tziampazis et al., Biomaterials 2000;21:511–520), that activation of cell adhesion and motility could be potentially promoted through increased CBD exposure at intermediate levels of PEG. This was confirmed through cell migration studies wherein cell speed values increased from 11 to 22 μm/h as the PEG concentration was increased from 0 to 4 mol %. Unexpectedly, however, high cell motility rates were sustained at 8 mol % PEG despite diminished levels of initial CBD exposure beyond 4 mol % PEG, suggesting that factors other than the initial CBD exposure may additionally have a role in activating cell migration at higher levels of PEG. Through studies of direct ligand mobility, cell-ligand-polymer interactions via atomic force microscopy, and CBD variation and integrin receptor roles in ligand remodeling, we offer evidence that cell motility is enhanced by a new mechanism for the regimen of higher PEG concentration: upon cell attachment and spreading, the ligand exhibits greater “slippage” at the polymer interface, and undergoes cell-engendered remodeling, which further activates cell motility, likely through enhanced exposure of hitherto encrypted sites for cell binding and signaling. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 69A: 114 –123, 2004

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INTRODUCTION

Biomaterial interfaces have been engineered to systematically induce cell activation by modulating the spatiotemporal aspect of cell binding to biofunctionalized materials.1–5 Biomaterial substrates and scaffolds that can display appropriate levels of strategic bioactivating molecules have been reported to significantly enhance cell motility and tissue infiltration.6,7

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Biomaterials inductive for cell motility can be candidates for graft-supportive or wound-reparative materials during reepithelialization, which is currently a major challenge for serious skin burns, diabetic foot ulcers, and venous leg ulcers. In vivo, during tissue repair, motile epidermal cells frequently migrate by rapidly clearing and remodeling the ligand matrix from the substrate. Therefore, tissue-engineered constructs that permit a more physiological behavior of seeded cells would be ideal for clinical applications. As the first step in examining the potential of PEGylated polycarbonates to this end, we are currently investigating the role of PEGylated materials in modulating the adhesive and migratory responses of human primary skin epidermal cells, keratinocytes.

Previously, the primary role of polymer-based ligands on cell migratory behavior has been well documented. In these reports, ligand concentration was varied, showing that the rate of cell migration is governed by interactions between cell-based integrin receptors and extracellular ligands adsorbed on tissue culture substrates. In our previous studies, the role of substrate PEG concentration on fibronectin-mediated alterations in cell adhesion and motility was examined, but the mechanistic effects of PEG could not be readily decoupled from those of the ligand. Beyond the well-established and frequently used PEG-mediated repression of protein or cell adhesion, the role of incorporating lower, protein and cell-permissive levels of PEG within the ligand-presenting polymeric substrate has not been controllably exploited. We hypothesize that PEG is instrumental in eliciting differential ligand conformations as well as mobilizing ligands adhered to cells, which can markedly alter the resultant cell migratory responses.

To that end, we have examined the role of the ligand–substrate interface in regulating keratinocyte migration, using whole length fibronectin, as a model cell adhesion ligand. Previously, we have shown that at a constant bulk ligand concentration, increasing PEG concentration monotonically lowered the amount of ligand adsorbed. In that study, the migration rate of L929 fibroblast cells was increased with PEG concentration, which was correlated to the increase in cell binding activity for fibronectin when a net lower level of ligand was adsorbed. To further decouple the role of variable ligand concentration from ligand conformation, here we have studied cell migratory responsiveness to equal levels of ligand adsorbed to the PEG-variant substrates. We found that progressive incorporation of PEG into the copolymer substrate enhanced keratinocyte migration on fibronectin. Two intriguing mechanisms are proposed: at lowest PEG levels, this increase may be primarily supported by increased exposure of cell-binding domains (CBDs) of the ligand, driven by ligand–PEG interactions; at intermediate PEG levels, the increase appears to be contingent on secondary cell–ligand interactions effected by PEG.

METHODOLOGY

Cell culture

Human keratinocytes were isolated via enzyme digestion from neonatal foreskin as previously described and cultured in serum-free keratinocyte growth medium (Clonetics, San Diego, CA) containing 0.1 ng/mL epidermal growth factor, 5 μg/mL insulin, 0.5 μg/mL hydrocortisone, 50 μg/mL gentamicin, 50 ng/mL amphotericin-B, 0.15 mM calcium, and 30 μg/mL bovine pituitary extract. Cell passages 2 to 3 were used for all experimental work.

Substrate preparation

PEG-variant copolymers were synthesized by condensation copolymerization with phosphogene as described by Yu and Kohn and examined in thin-film form on 15-mm glass coverslips. The coverslips were cleaned by sonication twice in sodium hydroxide, hydrochloric acid, 2% Sparkleen solution and ethanol, before being dried at room temperature. Thin polymer films were obtained by spin coating 1.25% (w/v) solution of the copolymer in methylene chloride onto cleaned coverslips. Equivalent amounts of human plasma fibronectin (Sigma, St. Louis, MO) were adsorbed onto all copolymer surfaces. Previously established isotherms were used to determine the bulk ligand concentration required to maintain equivalent ligand concentrations on all surfaces. Briefly, fibronectin labeled with 125Iodine (ICN Biomedicals, Irvine, CA) was mixed with unlabeled fibronectin. The resulting solutions were used to incubate polymer-coated glass disks (1 h, 37°C). Afterward, the disks were thoroughly washed with phosphate-buffered saline (PBS) to remove any unadsorbed protein and were transferred into 20-mL scintillation vials. After the addition of 10 mL of scintillation mixture (CytoScint; ICN Biomedicals), the radioactivity was counted on a scintillation counter (Beckman, Fullerton, CA). Standard curves of radioactivity for 125Iodine-labeled fibronectin amount and the known ratio of tracer to unlabeled fibronectin were then used to calculate the overall amount of fibronectin adsorbed to the polymer surfaces. Ligand concentrations of 5, 10, 20, 40 and 80 μg/mL were layered onto 0, 2, 4, 6, and 8 mol % PEG copolymers, respectively, to establish constant surface-ligand concentration. Protein solutions were incubated (1 h, 37°C) and then washed three times in PBS to remove any unbound ligand from the surface.

Visualization of ligand-adsorbed copolymer nanotopography

PEG copolymer topography was visualized with atomic force microscopy (AFM) (Nanoscope IIIa; Digital Instru-
ments, Santa Barbara, CA). One-micrometer scan areas were acquired under fluid phase tapping mode with silicon nitride probes. Initially, copolymers were incubated in PBS (1 h, 37°C) and imaged. Then the copolymers were layered with the appropriate amount of fibronectin and imaged 1 h after adsorption. The x, y, and z coordinates from the AFM were used to generate a topographic image of the sample surface. The z coordinate was used to calculate the root mean square roughness as the standard deviation of the z values within a given area, given by

$$
\sigma = \sqrt{\frac{\sum(Z_i - Z_{ave})^2}{N}}.
$$

**Cell migration studies**

The copolymer-coated coverslips were immobilized in 24-well plates and seeded with human keratinocytes in Keratinocyte growth medium at 2800 cell/cm² on the protein-adsorbed PEG copolymers. Experiments were also performed in which cells were supplemented with 10 μg/mL mitomycin C to rule out cell proliferation as an artifact of the cell migration experiment.20 Briefly, the mean-squared displacement, given by \( <d^2(t = n\Delta t)> = \frac{1}{(N-n+1)} \sum_{i=0}^{N-n} [(x((n + i)\Delta t) - x(i\Delta t))^2 + (y((n + i)\Delta t) - y(i\Delta t))^2] \), is a function of time, with two major single cell motility parameters, root mean squared cell speed, \( S \), and directional persistence time, \( P \). The mean squared displacement for the entire time interval is given by:

**Quantitation of force curves between ligand and PEG-variant substrate**

AFM in fluid immersion mode was used to quantitate the force between the ligand and substrate. Silicon nitride cantilevers were dip-coated in various PEG copolymer solutions to coat the tips. Ligand solutions at concentrations of 5, 20, and 80 μg/mL were incubated onto clean glass slides (1 h, 37°C). The coverslips were then rinsed three times in PBS to remove unbound ligand. The appropriate PEG copolymer derivatized cantilever tip with a spring constant of 0.32 N/m was engaged in fluid-phase contact mode. As the tip oscillated, the cantilever-deflection from the photodiode is monitored, generating a force curve between the ligand-adsorbed glass slide and PEG-derivatized cantilever tip. The force curve is a plot of the cantilever deflection signal as a function of the voltage applied to the piezo tube. The force was calculated by \( F = -kx \) where \( k \) is the force constant of the spring and \( x \) is the distance from the control point in nanometers.

**Ligand remodeling on PEG-variant copolymer**

To examine the degree of extracellular ligand mobility in the presence of cells on the PEG-variant substrate, 0.7-μm assay (ELISA). Briefly, after adsorption of equivalent amounts of fibronectin to the copolymer surfaces, the available binding sites were blocked (1 h, 4°C) with a solution of 3% bovine serum albumin and 5% normal rabbit serum. Next, mouse anti-human fibronectin monoclonal antibody specific for the CBD (Clone 3E3; Chemicon International, Temecula, CA) was incubated (1 h, 37°C) followed by goat anti-mouse immunoglobulin (IgG) conjugated to horseradish peroxidase (1 h, 37°C) (Sigma). Peroxidase activity was detected using hydrogen peroxide and o-phenylenediamine dihydrochloride (Sigma) as substrates. After 30 min incubation in the dark at room temperature, the absorbance was read at 450 nm on a 96-well plate reader (MR650; Dynatech Laboratories, Chantilly, VA).

Subsequently, the antibody reactivity of adsorbed fibronectin exposed to cells was assessed. After protein adsorption, keratinocytes were seeded at a density of 2800 cells/cm² and incubated (3 h, 37°C). A procedure modified from a recent study by Garcia and coworkers32 was followed. Briefly, cells were washed three times with PBS and incubated (15 min, 4°C) in sulfo-BSOCEES (Pierce Chemical, Rockford, IL) cross-linker. This cross-links integrins attached to the matrix. After quenching unreacted cross-linker with 50 mM Tris, cells were lysed (30 min, 4°C) with a solution of 350 μg/mL Phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, and 10 μg/mL aprotinin (Sigma). Wells were then treated with 0.1% Sodium dodecyl Sulfate (15 min, 37°C) to extract the lysed cells, leaving behind the matrix and bound integrins. The cross-linker of the integrin was reversed with 50 mM NaHCO₃ and 0.1% Sodium dodecyl sulfate (2 h, 37°C). Subsequently, ELISAs were conducted in situ as described above to infer the number of available CBD sites in terms of the CBD-specific fluorescence.
fluorescently labeled microspheres (Polysciences, Inc., Warrington, PA) conjugated with ligand were adsorbed onto PEGylated substrates. Briefly, ligand at concentrations of 5, 20, and 80 μg/mL were incubated with the microspheres at a concentration of 2.5% (w/v) (1 h, 37°C). Next, the ligand-microcarriers were adsorbed at 3.9 × 10⁹ microspheres/well onto PEG-variant copolymers (1 h, 37°C). After cell incubation (4 h, 37°C), images were acquired as described previously. As a control, microspheres were adsorbed on the copolymer surfaces in the absence of any ligand to ascertain whether cell migration was driven by the presence of microspheres. Microsphere translocation was quantified using time-lapse imaging and two-dimensional motion analysis via Image Pro Plus. For each field, 15 microspheres were analyzed per condition at 10-min increments for 10 h. The speed of microsphere translocation was determined using the same equations as those for cell migration.

Molecular mechanisms facilitating cell attachment and migration, and ligand rearrangement

To isolate the role of key integrins involved in keratinocyte adhesion to the fibronectin ligand on PEG-variant substrates, cell-attachment studies were subjected to inhibition due to adhesion-blocking integrin-specific antibodies. Briefly, keratinocytes were preincubated (4°C, 45 min) with monoclonal antibody to αvβ3 or αvβ5 at a concentration of 10 μg/mL. Cells were then seeded at 2800 cells/cm² and incubated (37°C, 1 h) on copolymers that had been preadsorbed with equivalent levels of fibronectin. The media with unattached cells was removed and centrifuged for 10 min at 14,000 rpm. The supernatant was aspirated and the cells were resuspended in 100 μL of media. Subsequently, cell counts were performed on the hemacytometer. Control experiments were performed in which cells were seeded with control preimmune IgG.

To identify the molecular nature of integrins possibly involved in keratinocyte migration, cell-migration studies subjected to integrin-specific blocking antibodies were conducted. Here, cells were incubated (4°C, 45 min) with either monoclonal antibody to αvβ3 (clone P1D6; Chemicon)³³ or monoclonal antibody to αvβ5 (clone P1B5; Chemicon)³⁴ at a concentration of 10 μg/mL. Cells were then seeded at 2800 cells/cm², their cell migration was monitored and analyzed as previously described. Control experiments were performed with mouse IgG (Sigma).

Experiments were further conducted to determine the role of integrins in possibly mediating the cell-based rearrangement of fibronectin adsorbed on PEG-variant copolymer substrates. Antibody reactivity assays for the CBD domain were performed, as described above, after cells preincubated with antibodies to αvβ3 or αvβ5 (4°C, 45 min) were cultured for 3 h on the PEG-variant substrates preadsorbed with equivalent levels of fibronectin. Subsequently, ELISAs were conducted in situ as described above to infer the relative number of available CBDs on the adsorbed fibronectin ligand for each PEG concentration. Antibody controls were performed with mouse IgG, and substrate controls were the 0% PEG case.

Quantitation of force curves between cells and ligand/PEG-variant substrate

The atomic force microscope was used in fluid immersion mode to quantitate the interaction force between the keratinocytes and fibronectin adsorbed to PEG-derivatized silicon nitride cantilevers. Silicon nitride cantilevers were dip-coated in various PEG copolymer solutions to coat the tips. PEGylated cantilevers were incubated (1 h, 37°C) with the appropriate ligand solutions at concentrations of 20 and 80 μg/mL for the 4 and 8 mol% PEG, respectively, so as to generate equivalent adsorbed concentrations of fibronectin for different levels of PEG. Keratinocytes were seeded at a density of 2800 cells/cm² onto clean glass slides (1 h, 37°C). The ligand-adsorbed cantilever tip was allowed to carefully approach the surface of individual keratinocytes. As the tip oscillated, a force curve between the ligand-adsorbed PEGylated cantilever and the cell was generated.

RESULTS

Surface topography

Atomic force micrographs using tapping mode AFM were acquired to visualize the topography and analyze surface roughness of various PEG copolymers with equal amounts of ligand (see Fig. 1). Although equal amounts of ligand were adsorbed on all surfaces, it is evident that the concentration of PEG in the copolymer had a strong influence over the net distribution of the adsorbed fibronectin. The topography gradually became less rough as the PEG concentration was increased, decreasing from 1.17 ± 0.08 to 0.75 ± 0.03 nm.

Effect of PEG on cell migration

The migration behavior of keratinocytes on PEG-variant copolymers pretreated with equivalent amounts of fibronectin was examined. As Figure 2 illustrates, cell speed increased linearly as PEG concentration in the copolymer increased, with a lowest value of 11 μm/h, reaching a maximum value of 22 μm/h at 4% PEG that was sustained at higher concentrations of PEG. Experiments with mitomycin C indicate that preventing cell proliferation did not affect migration in this system (data not shown).

CBD-specific antibody reactivity of ligand-adsorbed polymers

We assessed the antibody reactivity of the PEG copolymer-adsorbed protein with an ELISA specific for
the CBD (Fig. 2). The ligand adsorbed on PEG copolymer surfaces showed a biphasic trend for the relative extent of CBDs, with a maximum at 4% PEG. Notably, in the presence of cells, the trend increased linearly and reached a plateau at 4% PEG, indicating an equivalent number of sites. Our studies were focused on the cell responses to 0, 4, and 8% PEG from this point onward: 0% representing no PEG, 4% the first point of maximum migration, and 8% PEG as the highest level of PEG permissive for ligand adhesion. The correlations between cell migration speed and the CBD exposure were graphed for ligand distributions obtained before cell seeding [Fig. 2(a)], and for the ligand distributions after 4 h of cellular incubation, allowing for any cellular remodeling of the ligand [Fig. 2(b)]. Both curves were qualitatively similar, however, for 8 mol % PEG polymers, a discrepancy was noted between the trends for cell speed data, and the CBD exposure quantified in the absence of cell remodeling. The discrepancy was ameliorated when cell-remodeling conditions were considered.

Ligand distribution and remodeling on PEG-variant polymers: role of cell integrins

To identify the receptor-mediated roles in the cell attachment and remodeling on the ligand on the PEG copolymers, integrin-inhibition studies were conducted. The inhibition of $\alpha_5\beta_1$ allowed for nearly 100% of the cells to attach to the matrix. The inhibition of $\alpha_5\beta_1$ lowered the cell migration rate on all copolymer surfaces by an average of 50% of the original value (Fig. 3). However, the migration rate at 4% and 8% was not statistically different ($p > 0.05$); subsequent
studies to quantitate the number of CBD after inhibition of \( \alpha_3 \beta_1 \) showed that an equivalent number of CBD were exposed at 4 and 8% PEG (Fig. 4).

On the contrary, inhibition of \( \alpha_3 \beta_1 \) allowed for approximately 77% attachment of keratinocytes to the matrices composed of lower concentrations of PEG whereas higher concentrations of PEG had attachment just below 50%. Interestingly, migration studies with this integrin inhibited showed a significant decrease in the cell migration rate between 4 and 8% PEG (Fig. 3). Quantitation of CBD sites after incubation of cells with \( \alpha_3 \beta_1 \) inhibitors showed fewer sites exposed at 8% PEG than at 4% PEG (Fig. 4).

**Modulation of ligand mobility caused by PEG and cell remodeling**

To determine the translocation of ligand-adsorbed spheres deposited extracellularly on the PEG copolymers, we tracked the microspheres in the vicinity of motile keratinocytes. We hypothesized that if the ligand was remodeled and sequestered by the cells, the ligand-adsorbed microspheres would exhibit significant translocation. We found that at higher concentrations of PEG (8 mol %), the microspheres translocated at the rate of \( 9.19 \pm 1.4 \, \mu\text{m/h} \). At lower levels of PEG (mol % <4), the microsphere mobility was close to zero and not detectable.

To characterize the electrostatic interactions between the substrate and ligand, force curves were generated on the atomic force microscope in the absence of cells. A large repulsive force, 0.07 nN, was exerted at higher levels of PEG, whereas at lower PEG levels, the force was greatly reduced [Fig. 5(a)].

Force curves were next generated with the AFM to characterize interactions between ligand-adsorbed polymers (at higher or lower PEG concentration) and adherent cells. As illustrated in Figure 5(b), for low PEG substrates, a snap-off is observed during retraction of the ligand-adsorbed PEGylated tip, whereas prolonged cell-associated retention of the ligand was observed during the retraction of 8 mol % PEGylated tips [Fig. 5(c)].

**DISCUSSION**

This study examined the role of PEG in regulating cell migration on PEGylated substrates adsorbed with...
equivalent levels of a cell adhesion and signaling ligand, fibronectin. Herein, we report that the progressive incorporation of PEG in the substrate establishes two regimens that support enhanced levels of cell migration. In the first, wherein the concentration of PEG is low (4%), the number of exposed CBD sites seem to have an important role on the induction of cell motility. In the second regimen, wherein the PEG concentration is higher (8%), cell-induced ligand mobility and remodeling becomes dominant, ultimately sustaining cell migration rate, even with a net lower initial exposure of CBD sites.

Increasing amounts of PEG blocks were randomly incorporated within the back bone of the parent polymer, poly(DTE carbonate), resulting in a reduction of the overall hydrophobicity of the copolymer. We varied the bulk ligand concentration to generate PEG-variant copolymers with equivalent levels of fibronectin. Examination of the nanotopography of these ligand-adsorbed copolymers via AFM revealed unique protein distributions on the various copolymer surfaces. This implies that even at relatively low mole fractions of PEG (<8 mol %), there was sufficient PEG coverage to effect protein nanoscale distribution on the surface. Because the ligand distributed differently on the various copolymer surfaces, it was pertinent to examine the protein conformation on the various substrates, especially with regard to the accessibility of cells for the CBDs on the adsorbed ligand. The number of CBDs exposed was quantified, revealing that more sites were exposed at 4% PEG than at higher or lower concentrations of PEG in the substrate. Although lower concentrations of PEG allow for some degree of protein adsorption, it seems that at these lower concentrations, protein binding activity can be compromised. Previous studies have reported that keratino-
cyte migration levels can be sensitively governed by the availability of CBDs. Our migration studies showed that, when equivalent net levels of ligand are presented on differentially PEGylated copolymers, the cell migration rate increased with increasing PEG content. Interestingly, the same level of migration is achieved at intermediate and higher concentrations of PEG, whereas at higher concentrations, steric repulsion facilitates remodeling of the matrix by $\alpha_\beta$. Over time keratinocytes traverse the matrix with a fixed number of CBD sites on substrates with lower PEG concentrations, whereas on substrates of higher PEG concentrations, the effect of ligand mobility becomes noticeable with an increase in the number of CBD sites exposed, ultimately facilitating migration.

Figure 6. Proposed mechanism for PEG-variant copolymer regulation of keratinocyte migration on ligand-adsorbed substrates. Initially, (a) at lower PEG concentrations, the number of CBD sites exposed is greater than on substrates with (b) higher concentrations of PEG. At earlier time points on substrates with (c) lower PEG concentrations, the number of sites exposed is fixed, whereas at (d) higher concentrations, steric repulsion facilitates remodeling of the matrix by $\alpha_\beta$. Over time keratinocytes traverse the matrix with a fixed number of CBD sites on substrates with lower PEG concentrations (e), whereas on substrates of (f) higher PEG concentrations, the effect of ligand mobility becomes noticeable with an increase in the number of CBD sites exposed, ultimately facilitating migration.

centration were in part caused by rapid reorganization of the ligand by the keratinocytes, particularly on substrates with higher PEG concentrations. Previous unpublished studies indicate that fluorescently labeled fibronectin could be sequestered by L929 cells over time at higher levels of PEG (Tziampazis, private communication). In the present work, ligand-adsorbed microspheres were shown to be mobile at 8% PEG whereas spheres at lower levels of PEG or without ligand were relatively passive, indicating that at higher substrate PEG concentrations, ligand slippage can potentially be promoted. Mobility observations
were made only in the presence of cells, pointing to an active cell-based ligand remodeling process.

The presence of PEG on the polymer substrates such as the ones used in this study can make the surfaces progressively more hydrophilic, and consequently introduce more charge.\(^3^7\) To quantitatively elucidate the underlying mechanism driving ligand reorganization, force curves were generated with the AFM and studied in the absence or presence of cells. The repulsive interactions between the ligand and PEG copolymer were significantly enhanced at 8 mol % PEG.\(^3^8\) In contrast to 4 mol % PEG, cell retraction from ligand/PEG interfaces does not yield a cell-ligand “snap-off” seen using atomic force microcurves at 8 mol % PEG, indicating ligand sequestration by detaching cells. These data, along with: 1. microsphere mobility studies that show cell motility at 8 mol % PEG, but not at 4 mol % PEG, results in significant matrix (ligand) reorganization, and 2. quantitation of cell-specific CBD site exposure that indicate on 8 mol % PEG the number of sites exposed is initially lower but is significantly increased to levels comparable on 4 mol % PEG when cells are allowed to attach and remodel the ligand substrate, indeed support our “cell remodeling” hypothesis.

We focused our molecular rearrangement studies to two fibronectin-specific integrin receptors in keratinocytes: \(\alpha_5\beta_1\), which mediates binding to the CBD,\(^3^9\) and \(\alpha_3\beta_1\) integrin.\(^4^0\) Based on our data, the \(\alpha_5\beta_1\) integrin is likely to be mediating initial attachment as well as subsequent ligand reorganization. In our studies at higher concentrations of PEG, we theorize that the steric repulsion facilitates \(\alpha_5\beta_1\)-mediated sequestration of the ligand and further rearrangement of the CBD adhesion and signaling sites that may be important for the sustenance of cell migration levels, whereas \(\alpha_3\beta_1\) does not seem to have a primary role in mediating migration.

Overall, our results suggest that PEGylation of the polymeric substrate may alter mechanisms of ligand-governed keratinocyte motility. When PEG is incorporated into the substrate, two regimens can be established, diagrammed in a cartoon in Figure 6. At low PEG concentrations, cell migratory responsiveness may be driven via structural changes in ligand distribution leading to altered CBD exposure allowing for increased cell access to binding sites and enhanced cell migration rate. At higher PEG concentrations, CBD exposure is effected through ligand slippage from the polymeric substrate (which is engendered because of cell-ligand adhesion) and consequent changes in the ligand conformation engendered by cellular rearrangement dynamics. This allows for higher levels of cell migration to be sustained on polymer substrates with higher concentrations of PEG. Because this regime permits the active remodeling of the matrix ligand and cell motility, we speculate that these polymers could be potential candidates for in vivo studies to examine acceleration of reepithelialization. Overall, our work suggests a promising strategy to optimize ligand-permissive levels of PEG in the substrate to promote cell motility based on active cell-based ligand remodeling mechanisms.

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