PEG-variant biomaterials as selectively adhesive protein templates: model surfaces for controlled cell adhesion and migration

Evangelos Tziampazis\textsuperscript{a}, Joachim Kohn\textsuperscript{b}, Prabhas V. Moghe\textsuperscript{a,*}

\textsuperscript{a}Department of Chemical and Biochemical Engineering, Rutgers University, 98 Brett Rd., Piscataway, NJ 08854-8058, USA
\textsuperscript{b}Department of Chemistry, and New Jersey Center for Biomaterials, Rutgers University, Piscataway, NJ 08854, USA

Received 13 August 1999; accepted 30 September 1999

Abstract

Our study focused on the role of poly(ethylene glycol) (PEG) in actively regulating the biological responsiveness of protein-adsorbed biomaterials. To this end, we designed PEG-variant biomaterials from a family of tyrosine/PEG-derived polycarbonates to present surfaces ranging from low to intermediate levels of PEG concentration, below the PEG level requisite for complete abolition of protein adsorption. We analyzed the effect of PEG concentration on the amount, conformation and bioactivity of an adsorbed model protein, fibronectin, and on the attachment, adhesion strength and motility of L929 fibroblasts. Our results demonstrate that low levels of PEG can regulate not only the extent but also the conformation and specific bioactivity of adsorbed fibronectin. As the PEG concentration was increased from 0 to 6 mol\%, the amount of adsorbed fibronectin decreased linearly yet the fibronectin conformation was altered such that the overall bioactivity of adsorbed fibronectin was uncompromised. We report that the degree of cell attachment varied with PEG concentration in a manner similar to the dependence of fibronectin bioactivity on PEG. In contrast, the nature of cell adhesion strength dependence on PEG paralleled the pattern observed for fibronectin surface concentration. Our studies also indicated that the rate of cell migration was inversely correlated with PEG concentration over a narrow range of PEG concentration. Overall, these results highlight the striking ability of PEG-variant biomaterials to systematically regulate the behavior of adsorbed cell adhesion proteins and, consequently, effect cell functions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Poly(ethylene glycol); Tyrosine-derived polycarbonates; Biomaterials; Fibronectin; Cell adhesion; Cell migration

1. Introduction

Recent advances in surface and bulk chemistry have focused on synthesizing improved biomaterials with carefully tailored compositions that promote biocompatibility and bioactivity in well-controlled environments. However, after implantation, the ensuing material–protein interactions can frequently limit the ultimate performance of such biomaterials. Thus, there is a need to design biomaterial surfaces that are systematically responsive to protein adsorption in a precise, tissue-specific manner.

The biomaterial surface properties can control not only the nature and extent of the proteins that predominantly adsorb onto the biomaterial, but can also regulate conformational changes in the adsorbed protein and secondary phenomena such as cell-mediated protein turnover. However, a framework to predict and control the highly complex interactions between biomaterials and adsorbed proteins has not yet been forthcoming [1]. As a result, to date, biomaterial development efforts have not quite led to materials capable of fine-tuning protein adsorption events to promote bioactivity. The current state-of-the-art in biomaterials involves the development of substrates that completely block non-specific protein adsorption but may be additionally modified to present isolated peptide moieties to induce bioactivity [2,3]. Poly(ethylene glycol), or its high molecular weight equivalent, poly(ethylene oxide), is the moiety most commonly used to develop protein-repelling surfaces. Typically, PEG has been incorporated onto biomaterial surfaces through grafting [4–6], via simple surface treatments based on primary adsorption [7–9]; or secondary adsorption [10]; and through bulk incorporation via cross-linking [11] or block copolymerization [12,13].
Ever since the recognition of the antithrombogenic action of PEG [14], the design of most PEG-derivatized surfaces has sought to eliminate cell and protein adhesion using high PEG surface concentration [5,7,12]. A number of studies have illustrated the strong effects of PEG on the activity and conformation of proteins in solution [15–17]. However, the role of lower-range PEG interactions that may allow protein adsorption and cell adhesion on biomaterials has been largely overlooked. Thus, the biological ‘regulatory’ behavior of PEG-bearing surfaces is not clearly understood [18], especially at low and intermediate levels of PEG surface concentration, where conformational changes in adsorbed proteins may sensitively regulate cell adhesion processes.

In this work we have investigated the role of low and intermediate levels of surface PEG concentration in regulating the extent and bioactivity of cell adhesion proteins adsorbed on polymer surfaces. The attendant goal of the study was to characterize the cell adhesive and migratory response to the protein adsorbed PEG-variant biomaterials.

Our model PEG-variant surfaces were based on a family of tyrosine-derived biomaterials, which have been well characterized both in vitro and in vivo, and have shown great promise for use in tissue engineering applications [13,19–22]. We used a series of copolymers of PEG with DTR, a derivative of tyrosine. The chemical structure of the copolymers (depicted in Fig. 1), in general referred to as poly(DTR-co-f%PEG carbonate)s, supports the partial selection of material properties through the variation of three independent structure parameters: the percent mole fraction of PEG (f), the average molecular weight of the PEG blocks (M_w), and the pendant alkyl group (R) present in each tyrosine-derived diphenol [13]. In this work we have focused on the variation in PEG concentration alone by "fixing" the other two parameters to M_w = 1000 and R = E (ethyl). Thus, the members of the polymer family used in this work were poly(DTE-co-f%PEG carbonate)s. In this study the PEG concentration was varied in fine steps from f = 0, i.e., poly(DTE carbonate), to f = 10 mol% (due to the difference in the M_w of DTE and PEG, 1 mol% PEG is equivalent to 2.6 wt%).

The model protein of this study was the widely characterized cell adhesion protein, fibronectin (abbreviated as FN hereon). Fibronectin was an ideal candidate protein since its conformation is highly responsive to subtle variations in substrate properties, given its large size and flexibility [23–25]. This study reports for the first time that PEG, when present on a biomaterial surface in small, controlled amounts, can modify, in a systematic manner, the attachment of proteins and, in turn, the adhesion and migration of cells.

2. Materials and methods

2.1. Preparation and characterization of PEG-variant biomaterial surfaces

2.1.1. PEG-variant polymers

The tyrosine-PEG-derived copolymers, poly(DTE-co-f%PEG carbonate)s, were synthesized as described previously [13]. Briefly, the new polymers were created by introducing, through copolymerization, PEG segments into the backbone of tyrosine-derived polycarbonates developed earlier [26,27]. Polymer purity and chemical structure were evaluated by FT-IR, ^1H-NMR and ^13C-NMR as described earlier [13].

2.1.2. Preparation and surface analysis of films

The biomaterials were evaluated in the form of thin, transparent films on glass. Films were solvent-cast from dilute polymer solutions (1.25% w/v) in methylene chloride. It has been shown previously that for relatively low levels of PEG and short periods of hydration, experimental conditions used in this study, poly(DTE-co-f%PEG carbonate)s are largely insoluble [13]. Previous studies using X-ray photoelectron spectroscopy (XPS) also indicate that the PEG concentration in the bulk and surface.

![Fig. 1. Chemical structure of poly(DTE-co-PEG carbonate). In this work, the alkyl group, R, on all polymers was ethyl (E), and the M_w of PEG blocks was 1000. The molar fraction of PEG units in the copolymer was varied from 10 down to 0 mol% PEG.](image1)

![Fig. 2. Effect of PEG concentration on the hydrophobicity of PEG-variant biomaterials. Lower receding contact angles indicate lower hydrophobicity (increased hydrophilicity).](image2)
on the surface of these materials is equivalent (Phuvanart-nuruks and Kohn, unpublished results).

The hydrophobic/hydrophilic character of the polymer films was evaluated in terms of air–water contact angle measurements. For this purpose, water droplets were deposited from a syringe onto smooth, dry polymer films. The contact angles resulting from the interaction of the three phases (polymer, water, and air) were measured with the help of a Ramé-hart goniometer. Quasi-dynamic measurements, in addition to static ones, were performed by continuously varying the droplet size. Advancing (receding) contact angles were obtained by increasing (reducing) the droplet size at a constant rate [6]. Air–water contact angle measurements illustrated that increasing the concentration of PEG gradually to 10 mol% yielded progressively more hydrophilic surfaces (Fig. 2).

2.2. Analysis of fibronectin adsorption

Human plasma fibronectin (hpFN) was obtained from Sigma (St. Louis, MO) and was reconstituted to a final concentration of 20 μg/ml in PBS prior to use. This level is similar to the typical concentration of FN in complete cell culture media [28]. Fibronectin was adsorbed on biomaterial-coated glass films for 1 h at 37°C, and the excess protein was washed off by rinsing with PBS. Care was taken to prevent drying of the FN-coated disks prior to further analysis, since exposure to air can markedly alter the surface conformation of adsorbed protein [29].

2.2.1. Measurement of amount of fibronectin adsorbed

The amount of FN adsorbed on poly(DTE-co-PEG carbonate) surfaces was measured using radiolabeled FN as tracer. Briefly, human plasma fibronectin labeled with 125Iodine (ICN Biomedicals, Irvine, CA) was mixed with unlabeled hpFN and the resulting solution (20 μg/ml total FN in PBS) was used to incubate polymer-coated glass disks. To improve experimental accuracy, the disks were coated on both sides. After protein adsorption was allowed to occur (1 h, 37°C), the disks were thoroughly washed with PBS to remove any unadsorbed protein, and were then transferred into 20 ml scintillation vials. Following the addition of 10 ml of scintillation cocktail (CytoScint, ICN Biomedicals), the radioactivity was counted on a scintillation counter (Beckman, Fullerton, CA). Standard curves of radioactivity for 125I-labeled FN amount and the known ratio of tracer to unlabeled FN were then used to calculate the overall amount of fibronectin adsorbed to the polymer surfaces. The measurements obtained using radiolabeled FN were further verified using XPS analysis of fibronectin-adsorbed biomaterial surfaces (data not shown).

2.2.2. Bioactivity of adsorbed fibronectin

The bioactivity of adsorbed FN was assessed in situ using ELISA (Enzyme-Linked Immuno Sorbent Assay), as described by Kubo et al. [30]. Briefly, after FN adsorption, the available surface sites were blocked via incubation (1 h, 4°C) with a solution of 3% BSA and 5% normal rabbit serum in PBS. Next, the adsorbed FN was incubated (1 h, 37°C), first with rabbit anti-human fibronectin and then with goat anti-Rabbit IgG conjugated to horseradish peroxidase (both antisera from Biomedical Technologies Inc., Stoughton, MA). The peroxidase activity was detected using hydrogen peroxide and o-phenylenediamine dihydrochloride (Sigma, St. Louis, MO) as substrates. Finally, 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).

2.2.3. Visualization of adsorbed fibronectin

The bulk conformation of fibronectin adsorbed on PEG-variant biomaterials was investigated through imaging by atomic force microscopy (AFM) using silicon nitride probes. A MultiMode AFM (Digital Instruments, Santa Barbara, CA) was used to analyze the polymer surface before and after FN adsorption, under fluid contact mode as follows. First, the polymer surface was imaged in PBS; next PBS was replaced with a 20 μg/ml FN solution in PBS and, after FN was allowed to adsorb, the same region was imaged with AFM.

2.3. Cell responses to protein conditioned PEG-variant biomaterials

The L929 cell line was used as a model cell line to evaluate the effect of PEG-variant biomaterials on cell adhesion. Stock cultures of L929 cells (American Type Culture Collection, ATTC, Manassas, VA) were maintained in EMEM media supplemented with 10% FBS, in a humidified incubator at 37°C and 5% CO2. To better approximate the complex physiological environment in vivo, the same complete culture medium, supplemented with 100 U/ml of penicillin and streptomycin, was also used in the cell adhesion and migration experiments.

2.3.1. Cell attachment studies

Glass disks coated with poly(DTE-co-PEG carbonate) were allowed to hydrate by incubating in PBS for 1 h. The PBS was then replaced by a suspension of trypsinized L929 cells. After overnight incubation the culture medium was removed and the disks were washed carefully with PBS to remove any unattached cells. A colorimetric, MTS-based cell proliferation assay (CellTiter 96, Promega, Madison, WI) was used to enumerate the number of cells attached on poly(ether carbonates) with varying amounts of PEG.

2.3.2. Studies on cell adhesion strength

To quantify the strength of cell adhesion, L929 cells were plated onto the biomaterial surfaces as described
above. After overnight incubation, cell adhesion strength was evaluated using a fluid shear assay. Briefly, the polymer-coated glass slides were placed onto a parallel plate flow chamber designed for laminar fluid flow [31]. The shear level to which attached cells were exposed was controlled by varying with time the rate of PBS flow through the chamber, resulting in the following relationship between shear (τ, dyn/cm²) and time (t, min): τ = 18 + 10t. The flow chamber was placed onto an inverted microscope (IMT-2, Olympus, Melville, NY) and the cell population was monitored with a computerized image analysis system comprised of video-based acquisition (DEI-750, Optronics Engineering, Goleta, CA) and image processing software (Image-Pro® Plus, Media Cybernetics, Silver Spring, MD) subsystems. Acquired images were processed to automatically yield the number of cells per field, thus generating, for each image, plots of the cell number vs. shear. The level of shear at which 50% of the cell population remained attached, τ₅₀, was used to quantify cell adhesion strength.

2.3.3. Cell migration

Cells were plated onto polymer-coated glass disks as described for cell attachment. After cells were allowed to attach for 1 h, the disks were washed to remove unattached cells and were imaged under a laser scanning confocal microscope (LSM410, Carl Zeiss Inc., Thornwood, NY) equipped with a humidified CO₂ stage incubator. The movement of cells was tracked overnight by acquiring images every 3 min. The cell migration speed was determined as the time-averaged rate of displacement over increments that yielded a major change in cell direction, using a commercial image analysis software (Image-Pro® Plus, Media Cybernetics, Silver Spring, MD).

3. Results

3.1. Effect of PEG on the extent and bioactivity of adsorbed fibronectin

The incorporation of PEG in the copolymer had strong effects on protein adsorption. Using radiolabeled fibronectin as tracer, we quantified the extent of fibronectin adsorption on poly(DTE-co-PEG carbonate)s. As shown in Fig. 3, the amount of FN adsorbed on the polymer surface declined monotonically in a linear fashion with PEG concentration. These trends were also confirmed using X-ray photoelectron spectroscopy (results not shown).

We have used hpFN ELISA as a means of assessing the total bioactivity of FN adsorbed on poly(DTE-co-PEG carbonate)s. The ELISA results, given in Fig. 4a, reveal that the bioactivity of adsorbed FN was not considerably affected by the incorporation of low to moderate levels of PEG (2–6 mol%). However, when

![Fig. 3](image-url)  
**Fig. 3.** PEG-mediated regulation of the amount of fibronectin adsorption on PEG-variant biomaterials. As described in Materials and Methods, adsorbed hpFN was quantified using radiolabeled FN as a tracer.

![Fig. 4](image-url)  
**Fig. 4.** Bioactivity of FN adsorbed on PEG-variant biomaterials. (a) Cumulative surface bioactivity was evaluated using fibronectin ELISA. Asterisks denote values that are statistically different (P < 0.05) from the bioactivity value in the absence of PEG. (b) Specific FN bioactivity index was computed by normalizing FN bioactivity to adsorbed amount and expressing the values relative to the specific bioactivity of FN adsorbed on poly(DTE carbonate). The index error values were calculated by propagating the independent errors measured for the amount and overall bioactivity of adsorbed FN.
PEG concentration increased beyond a critical threshold (between 6 and 8 mol%), the FN bioactivity was severely compromised. The specific FN bioactivity (computed as the ratio of cumulative FN biological activity to the amount of FN adsorbed) exhibited a biphasic dependence on PEG concentration, first increasing until it reached a maximum at 6 mol% PEG and dropping sharply at higher PEG levels (Fig. 4b).

3.2. Effect of PEG on surface distribution of fibronectin

Atomic force micrographs of the topography of polymer surfaces before and after fibronectin adsorption are shown in Fig. 5. The results obtained via AFM analysis were also quantified to yield the average surface roughness (Fig. 6). It is evident that while a moderate concentration of PEG (up to 6 mol%) did not have a strong effect on the topography of the native polymer, it did exert strong influence over the net conformation of adsorbed fibronectin. The topography of adsorbed protein became progressively rougher as the PEG concentration increased to 4 mol%. However, a further increase of PEG concentration reduced surface roughness.

3.3. Effect of PEG on cell adhesion and migration

Our static adhesion assay showed that the number of cells attached to poly(DTE-co-PEG carbonate) was
sensitively modulated by PEG concentration. Cell attachment remained statistically invariant as PEG concentration was increased from 0 to 4 mol%; however, higher levels of PEG (6–10 mol%) monotonically suppressed cell attachment (Fig. 7a).

Additionally, our adhesion studies showed that the force required to detach adherent cells from surfaces conditioned using complete culture media, decreased progressively in linear manner, with increased PEG mole fraction (Fig. 7b). By 8 mol% PEG, the detachment forces declined to levels that were undetectable with our flow assay.

The migration behavior of L929 cells on PEG-variant surfaces was characterized in terms of mean cell speed. Cell speed was found to increase with increased PEG mole fraction, till an intermediate level of PEG concentration, but this trend was not sustained at higher PEG levels (Fig. 8). Cell migration levels were not computed at higher levels of PEG (8–10 mol%), since typical levels of cell attachment in this regime were very low.

4. Discussion

In this work we have studied the regulation by poly(ethylene glycol) (PEG) of fibronectin (FN) adsorption and the effect of subtle changes in PEG concentration on cell adhesion and migration on biomaterials, using a new family of copolymers, poly(DTE-co-PEG carbonate) [13,22], as a model system.

In this family of copolymers, increasing amounts of PEG blocks were incorporated randomly within the hydrophobic backbone of the parent polymer, poly(DTE carbonate). The actual PEG/DTE ratios in the copolymers were calculated through integration of 1H-NMR spectra; it was found that the actual PEG concentration was within 10% of its nominal value and within
the error of the NMR [13]. As expected, the incorporation of hydrophilic PEG blocks into the hydrophobic DTE backbone resulted in a reduction of the overall hydrophobicity of the copolymer (Fig. 2). When PEG concentration was increased to 10 mol%, the receding contact angle decreased to values similar to those reported for self-assembled monolayers of PEG [32]. This suggests that even at these relatively low mole fractions, there is significant PEG coverage on the wetted polymer surface, thus effectively masking the DTE backbone. However, at lower levels of PEG (2–8 mol%), the contact angle measurements were indicative of only partial coverage of the hydrophilic surface of poly(DTE carbonate).

The micro-scale distribution of the hydrophobic and hydrophilic domains on such copolymers may be central to the surface interactions with proteins [33–35]. While the exact distribution of PEG domains in poly(DTE-co-PEG carbonate)s has not yet been studied in detail, the copolymers do not exhibit any major phase separation over the PEG concentration range used in this study (based on high-resolution transmission electron microscopy, Matthew Libera, pers. comm.). Since poly(DTE-co-PEG carbonate)s are random block copolymers [13], it is reasonable to assume that at lower PEG levels, PEG may have been distributed in relative uniform clusters at the copolymer surface. Consistent with these observations, our atomic force micrographs of native copolymer surfaces showed comparable levels of surface roughness in polymers with up to 6 mol% PEG; and only a slight increase in roughness beyond 6 mol% PEG, which may likely be the result of minor phase separation and/or hydration and swelling.

4.1. Variation in PEG concentration differentially regulates the amount and bioactivity of adsorbed fibronectin

The progressive PEG-induced reduction of FN adsorption at the biomaterial surface (Fig. 3) could be reconciled using both macroscopic and molecular considerations. Increased PEG concentration reduces the number of possible hydrophobic–hydrophobic interactions likely between protein domains and the polymer backbone, thus shifting the thermodynamic balance between protein in solution and adsorbed protein [36]. From a mechanistic standpoint, PEG-induced alterations in the amount of fibronectin adsorbed are governed by the steric interactions between PEG and the protein [4]. The very low levels of fibronectin adsorbed (<0.1 ng/cm²) on poly(DTE-co-PEG carbonate) with more than 8 mol% PEG (21 wt%) suggests that even at this relatively low PEG concentration, most of the polymer surface was inaccessible to the relatively large FN molecules [23]. This is plausible, since each PEG chain may be heavily hydrated with two, or even three, water molecules per monomeric unit (i.e., ethylene glycol) in PEG [37], further increasing the surface coverage by PEG. In addition, the linear dependence of FN adsorption on PEG concentration suggests that below 8% PEG surface coverage by PEG was not saturated and any conformational changes in PEG potentiated due to PEG crowding, such as those reported previously for surfaces with grafted PEG chains [4], are likely minor.

Notably, PEG-mediated regulation of the overall biological activity of adsorbed FN had a binary (on–off) characteristic: fibronectin bioactivity was uniformly high when PEG concentration was at or below 6 mol%, but it plummeted near or beyond 8 mol% PEG (Fig. 4a). Taken together with the stoichiometric regulation of fibronectin adsorbed (Fig. 3), and based on our visual AFM observations of PEG-regulated fibronectin surface topography (Figs. 5 and 6), these studies provide indirect evidence that PEG concentration regulates adsorbed FN conformation as well.

In the region of 0–6 mol% PEG, where overall FN bioactivity remains uniformly high, the specific bioactivity increased monotonically with PEG concentration (Fig. 4b), whereas the surface topography (as revealed by AFM) exhibited a biphasic dependence on PEG concentration. One of the possible mechanistic explanations for our observations may be that the conformation of adsorbed FN changes from a tight configuration (e.g. coil bound to the substrate at multiple sites) on poly(DTE carbonate) surfaces, to a looser configuration (e.g. a partially expanded coil bound at fewer sites) on copolymer surfaces with 2 and 4 mol% of PEG, to a weakly tethered configuration (e.g. linear chain bound with the substrate at only very few sites) on 6 mol% PEG. Such a scheme (depicted as a cartoon model in Fig. 9) would be compatible with the reduction in the number of FN adsorption sites as PEG concentration increases. A high level of fibronectin activity requires fully extended FN fibrils where bioactive RGD-based adhesion sites may be exposed [23]. We suggest that the adsorption on 6 mol% PEG copolymer of a relatively small number (Fig. 3) of fully extended FN molecules would not considerably alter the overall surface topography of the polymer (Fig. 6) but still could result in high specific bioactivity (Fig. 4b).

In contrast, for the high PEG regime (above 8 mol% PEG), FN adsorption elicited little change in surface topography, indicative of the low levels of adsorption. Furthermore, the few adsorbed FN molecules may have reduced the overall surface roughness slightly by serving as ‘cavity fillers’ in the sparse DTE areas bridging PEG domains (Fig. 6). In addition, since PEG acts as a stabilizer of the globular form of proteins [47], at higher PEG concentration, adsorbed FN may be present in the form of more compact, globular conformations with a large degree of RGD encryption, which may explain the lower specific protein bioactivity (Fig. 4b).
It is unknown to what degree the observed regulatory effect of PEG on fibronectin adsorption is representative of its influence on the behavior of other adsorbed cell adhesion proteins. This, together with the effect of PEG on proteins adsorbed from complex solutions containing serum, is an area requiring additional study. Nevertheless, one of the most significant findings of our study is that the presence of PEG on a polymer surface can serve as a powerful regulatory determinant for protein adsorption. This recognition goes beyond the traditionally emphasized role of PEG to non-discriminately abolish protein adsorption. Instead, there is a relatively low range of PEG surface densities, at which protein adsorption is still possible, but the conformation of the adsorbed protein is affected by the presence of PEG. From a practical perspective, this observation offers the possibility to use low PEG surface densities to regulate the bioactivity of adsorbed proteins, and consequently, to regulate certain cellular responses.

4.2. Regulation of cell adhesion and migration on PEG-variant biomaterials

Cell attachment exhibited a sigmoidal dependency on PEG concentration, which was analogous to the dependence observed for the overall FN bioactivity (cf. Fig. 7a and 4a), suggesting that L929 attachment on poly(DTE-co-PEG carbonate) may be limited by the availability of cell binding epitopes on adsorbed ligands (such as fibronectin). This scenario assumes that the cell adhesion-specific bioactivity of adhesion proteins adsorbed from complete culture media can still be regulated by PEG in a manner similar to that observed with fibronectin adsorbed from a simple monocomponent solution (Fig. 4a).

In contrast to the sigmoidal dependence of cell attachment on copolymer PEG concentration, the cell adhesion strength values decreased linearly with PEG concentration. It is noteworthy that a similar nature of PEG dependence was observed for the total amount of fibronectin adsorbed (cf. Figs. 3 and 7b). A likely explanation for these trends is that after cells attach to adsorbed surface ligands, they may be able to rearrange both membrane receptors and surface ligands and in the process facilitate further binding [38]. If, as a result of such a rearrangement all local ligand binding sites become available, then adhesion strength, and possibly other long-term adhesion processes such as spreading, could be limited primarily by the stoichiometric ligand concentration, and less so by the ligand’s instantaneous, local steric conformation. Indeed, such a linear dependence on ligand concentration has been shown experimentally for osteoblast adhesion strength [39] and fibroblast spreading [28]. The degree of cooperativity that established adhesion sites exhibit in “recruiting” additional neighboring adhesion events is likely dependent both on the conformation and surface concentration of ligands and on the number of initial adhesive interactions. The mechanism by which PEG modulates this cooperative behavior is not well understood currently and this is an area warranting further study.

It is also possible that the observed effect of PEG concentration on cell adhesion (Fig. 7) may be the result of PEG levels affecting differentially (a) ligand conformation, and hence the binding affinity between the adsorbed ligand and its receptor, effectively altering the strength of the adhesive bond [40], or (b) the strength by which the
ligand adsorbs onto the polymer [41]. The latter mechanism may be triggered by the presence of hydrophilic PEG, causing a net reduction in the degree of hydrophobic-hydrophobic interactions between the biomaterial and adsorbed cell adhesion proteins. Naturally, all these mechanisms may act in parallel to regulate the cell adhesion behavior observed on PEG-variant biomaterials. Further experiments are currently under way in our laboratory to determine the key mechanism through which PEG controls cell adhesion strength on these polymers.

Regardless of the exact nature of the mechanisms underlying the action of PEG on cell adhesion, our studies show that the concentration of PEG in polymeric surfaces can also be used to regulate cell migration. The observed inverse relationship between the rate of cell migration and the extent of adhesion strength is in agreement with results from previous studies [42–45]. However, while those studies focused on controlling adhesion strength through variation of ligand concentration on simple substrates in a well-defined environment, our work extends the correlation in a more complex, physiologically relevant environment.

5. Conclusions

In this study, we designed small increments in the PEG concentration of copolymers to explore the effect of PEG on the biological responsiveness of biomaterials. In a narrow PEG concentration range (indicative of the transition of the polymer surface from being highly protein adsorbing to being essentially protein repellant), we report that PEG concentration had a strong regulatory effect on key cellular responses. We report that the specific bioactivity of adsorbed protein had a biphasic dependence on PEG concentration and that the cumulative protein bioactivity exhibited a binary, on/off type dependence on PEG concentration. Additionally, our studies highlight the role of protein–PEG interfaces with graded adhesion strength in controlling the rate of cell migration. Overall, our work offers a new paradigm whereby, the presence of low surface concentration of PEG on biomaterial surfaces may regulate the onset and dynamics of cell adhesion phenomena. These observations could have important implications in tissue engineering, particularly for the design of substrates that elicit a high degree of cell attachment while optimizing the rate of cell migration. Furthermore, since the conformation of adsorbed cell adhesion proteins can play a crucial role in determining whether cells proliferate or differentiate [46], the presence of small amounts of PEG on biomaterial surfaces may also prove useful to switch the balance between cell growth and differentiation.

Acknowledgements

This study was partially supported by a Johnson & Johnson Discovery Award and ConvaTec Young Professor Award to PVM, and support to JK from the NIH (grant GM 39455). Financial support by ConvaTec, a Bristol–Myers–Squibb company, Skillman, NJ and by the NJ Commission for Science and Technology, is gratefully acknowledged. The authors are also grateful to Dr. Daniel Dabbs, director of the AFM facility at Princeton University, and Drs. Das Bolikal, Elsie Effah, Christelle Lhommeau and Rose Gonzales, as well as Mr. Jason Cassaday, for technical assistance.

References


[42] Chon J, Netzel R, Rock B, Chaikoff E. $\alpha_x\beta_1$ and $\alpha_x\beta_5$ control cell migration on fibronectin by differentially regulating cell speed and motile cell phenotype. Ann Biomed Eng 1998;26:1091–101.


