J|A|C|S COMMUNICATIONS

Published on Web 12/04/2009

Noncovalent Cell Surface Engineering with Cationic Graft Copolymers

John T. Wilson, Venkata R. Krishnamurthy, Wanxing Cui, Zheng Qu, and Elliot L. Chaikof*

Departments of Biomedical Engineering and Surgery, Georgia Institute of Technology and Emory University, 101 Woodruff Circle, Suite 5105 WMRB, Atlanta, Georgia 30322

Received October 18, 2009; E-mail: echaiko@emory.edu

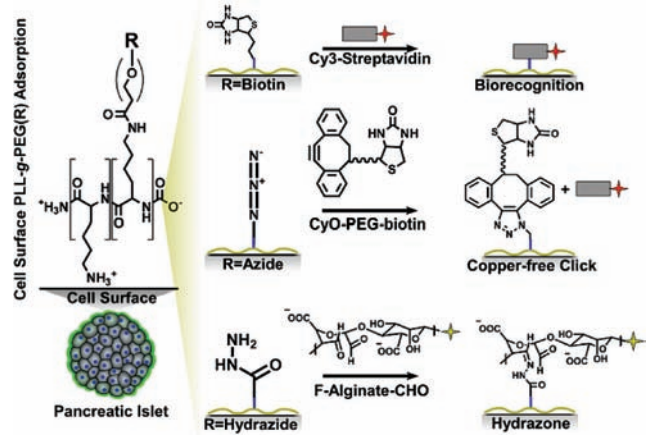
Chemical approaches to cell surface engineering have emerged as powerful tools for resurfacing the molecular landscape of cells and tissues.[1] Introduction of exogenously derived molecules alongside native cell surface constituents affords opportunities to control biochemical and cellular responses, with important implications for drug delivery, cell-based therapy, and tissue engineering. Most cell surface modification strategies utilize covalent chemistries that target native cell surface constituents (e.g., lysine residues) or noncanonical reactive moieties introduced by metabolic or genetic engineering approaches.[1,2] However, the perturbation of cellular physiology inherent to such strategies can interfere with important cellular functions governed by cell surface molecules.[1a,2a,3] Hence, a need exists for noncovalent cell surface modification strategies.

Here we report a versatile and facile noncovalent approach to cell surface engineering achieved through electrostatic adsorption of poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) copolymers to cellular interfaces. Isolated pancreatic islets were used as a model system in these investigations owing to the widespread use of cell surface modification in improving the outcome of islet transplantation, a promising treatment for diabetes.[4] PLL-*g*-PEG copolymers were rendered cytocompatible through appropriate control of the grafting ratio, and used as 'cell surface active' polymeric carriers for ligands and reactive groups. Copolymers bearing terminally functionalized PEG grafts were used to display biotin, hydrazide, and azide moieties, which selectively captured streptavidin-, aldehyde-, and cyclooctyne-labeled probes, respectively, on the islet surface (Scheme 1). Additionally, coadsorption of polymers enabled simultaneous display of multiple functional groups on cell surfaces.

The cytotoxicity inherent to most polycations poses a significant molecular hurdle to the assembly of electrostatically stabilized cell surface-supported monolayers.[5] Indeed, incubation of islets with poly(L-lysine) (PLL) significantly reduced islet viability (Figure 1a). Polycation toxicity is dependent, in part, on charge density,[5] and we therefore postulated that PLL cytotoxicity could be attenuated by grafting methoxy terminated tetra(ethylene glycol) chains ($PEG_4(OCH_3)$) to a critical number of lysine residues. PLL-*g*-$PEG_4(OCH_3)$ copolymers with different extents of backbone modification were synthesized via active ester coupling between *N*-hydroxysuccinimide (NHS)-functionalized PEG and ε-amino groups of PLL and copolymer toxicity was assessed. As shown in Figure 1a, polymer cytotoxicity decreased as the extent of PEG grafting increased, with modification of ~40% of lysine residues resulting in a copolymer that did not adversely influence islet viability (referred to herein as PP-OCH_3). Hence, a critical threshold for PEG grafting exists, below which polycation cytotoxicity is considerable.

While the adsorption of PLL-*g*-PEG polymers to abiotic surfaces has been extensively studied,[6] the behavior of this class of copolymers at viable cellular interfaces has not been rigorously investigated. Many polycations induce the formation of pores in the plasma membrane, a phenomenon that mediates cell death and enables transport of

Scheme 1. Cell Surface Engineering with PLL-*g*-PEG Copolymers



molecules, including the polycation itself, across the cell membrane.[5] Indeed, as shown in Figure 1b, FITC-labeled PLL in contact with islets translocated across cell membranes and into the cytoplasm of individual cells. Conversely, AlexaFluor488-labeled PP-OCH_3 was distributed in a pattern consistent with the extracellular architecture of isolated pancreatic islets (Figure 1c), indicating maintenance of cell membrane integrity upon adsorption of the copolymer to cell surfaces. An unexpected finding was that a PEG-free analogue of PP-OCH_3, synthesized by acetylating ~40% of lysine monomers, exerted significant toxicity and localized intracellularly (see Supporting Information (SI)). Collectively, these results suggest synergism between decreased charge density and PEG grafting in attenuating PLL cytotoxicity, likely through inhibiting membrane pore formation. Therefore, only PLL-*g*-PEG copolymers with a unique balance of grafted PEG chains and free lysine monomers adsorb to cell surfaces without compromising cell viability.

Based on these findings, structurally comparable copolymers were synthesized with PEG_4(biotin), PEG_4(hydrazide), or PEG_{12}(azide) grafts

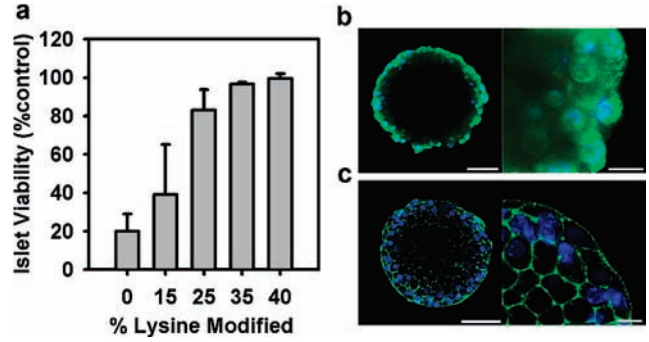


Figure 1. (a) Islet viability after exposure to PLL and PLL-*g*-$PEG_4(OCH_3)$ copolymers with variable degrees of backbone PEGylation. Confocal micrographs of islets after incubation with FITC-labeled PLL (b) and AF488-labeled PP-OCH_3 (c). Scale bars: 50 μm (left), 10 μm (right).

10.1021/ja908887v

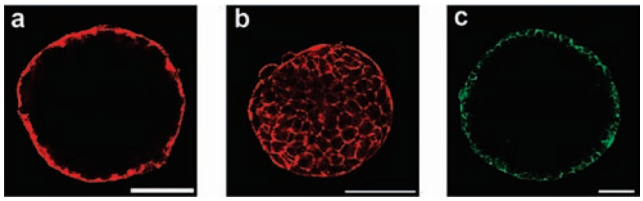


Figure 2. Representative confocal micrographs of islets incubated with PLL-*g*-PEG(R) copolymers bearing R = biotin (a), azide (b), and hydrazide (c) groups followed by incubation with appropriate bio/chemo-orthogonal probes (Scheme 1). All probes were localized on the surface of peripheral cells, as represented in (a), as well as cells within the core of the islet, as depicted in (b). Negligible binding of probes to islets treated with PP-OCH_3 was observed (see SI). Scale bar: 50 μm.

(PP-biotin, PP-hydrazide, and PP-N_3, respectively). Functionalized variants also did not exert cytotoxicity (see SI) and, hence, were explored as vehicles for carrying functional groups to the islet surface. Islets were incubated with functionalized copolymers for 40 min, rinsed to remove nonadsorbed copolymer, and incubated with fluorescently labeled bio/chemo-orthogonal probes detectable by confocal microscopy (Scheme 1). Specifically, biotin groups were probed using Cy3-labeled streptavidin (Cy3-SA), and the presence and reactivity of cell surface hydrazide and azide groups were assessed using fluorescein-labeled alginate-aldehyde (F-Alg(CHO)) and a cyclooctyne(CyO)-PEG-biotin conjugate (followed by biotin detection with Cy3-SA), respectively. As demonstrated in Figure 2, islets incubated with PP-biotin specifically bound Cy3-SA, islets exposed to PP-hydrazide selectively immobilized F-Alg(CHO) via formation of N-acyl hydrazones, and cell surface adsorbed PP-N_3 captured cyclooctyne-PEG-biotin through strain-promoted cycloaddition. Significantly, to our knowledge, this is the first report to describe generation of cell surface hydrazides, a particularly useful handle for immobilizing oligosaccharides and glycoconjugates.[1a,7] Moreover, cell surface azide groups have only been generated through metabolic engineering approaches, a process which may require several days and is not amenable to all cell types.[1a,2b,3] By contrast, PP-N_3 adsorption facilitates presentation of cell surface azido groups within minutes, providing a facile and rapid alternative for chemically targeting cell surfaces via copper-free click chemistry.

Coadsorption of differentially functionalized PLL-*g*-PEG copolymers offers opportunities for simultaneous display of multiple groups. To demonstrate this concept, islets were incubated in a solution containing equal concentrations PP-biotin and PP-hydrazide, followed by Cy3-SA, F-Alg(CHO), or a mixture of the two probes. Islets incubated with a mixture of biotin- and hydrazide-functionalized copolymers were capable of capturing individual probes (see SI), as well as both probes in combination (Figure 3). In principle, a library of copolymers bearing a diverse array of small molecules (e.g., peptides, oligosaccharides, other reactive groups) could be used combinatorially to achieve exquisite control over the composition of cell surfaces in a single step.

Cell surface engineering using PLL-*g*-PEG copolymers has a number of advantages over conventional covalent approaches, particularly NHS-ester coupling previously employed for islet modification.[4] In contrast to NHS-ester conjugates, PLL-*g*-PEG copolymers allow cells to be functionalized in aqueous solvents containing primary amines (e.g., cell culture media) and may be dissolved well in advance of application without the hydrolysis associated with NHS-esters. Using PP-biotin as a model, adsorbed PLL-*g*-PEG copolymers generated surface densities of functional groups similar to that obtained by treatment with NHS- PEG_4(biotin) (0.26 ± 0.01 vs 0.23 ± 0.03 fmol streptavidin/islet, $p > 0.05$). However, islets treated with the NHS-ester reagent adopted an altered morphology, characterized by more

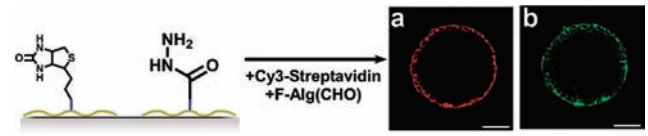


Figure 3. Simultaneous display of cell surface biotin and hydrazide groups through coadsorption of PLL-*g*-PEG. Representative confocal micrograph of an islet incubated in a mixture of biotin- and hydrazide-functionalized copolymers followed by treatment with a solution containing both (a) Cy3-SA and (b) F-Alg(CHO). Scale bar: 50 μm.

frequent protrusions, whereas the characteristic smooth border of cultured murine islets was maintained upon exposure to PP-biotin (see SI). While such morphological changes are not well understood, it is reasonable to suspect that adhesive interactions essential for maintenance of islet integrity may be compromised by covalent modification of surface proteins.[8]

In summary, we report a new approach to chemically modify cell and tissue surfaces through adsorption of appropriately designed cationic graft copolymers. A novel finding is that grafting short PEG chains to PLL abrogates cytotoxicity in a grafting ratio- and PEG-dependent manner, ultimately yielding cytocompatible polycations that present functional groups upon adsorption to cell surfaces. This strategy opens new opportunities in cell surface engineering, including generation of unique cell surface motifs, rapid and combinatorial surface modification, and use of biologically complex solvents. Hence, tailored PLL-*g*-PEG copolymers offer a promising and enabling tool for bio/chemically remodeling cells and tissues with broad potential in biomedical and biotechnological applications.

Acknowledgment. This work was supported by grants from the National Institutes of Health (DK069275) and the Juvenile Diabetes Research Foundation. J.T.W. acknowledges the Whitaker Foundation for generous fellowship support.

Supporting Information Available: Detailed methods of islet isolation, culture, and surface modification. Synthesis and characterization of PLL-*g*-PEG copolymers and reactive probes. Supplemental data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13–21. (b) Chen, I.; Howarth, M.; Lin, W.; Ting, A. Y. *Nat. Methods* **2005**, *2*, 99–104. (c) Boonyarattanakalin, S.; Martin, S. E.; Sun, Q.; Peterson, B. R. *J. Am. Chem. Soc.* **2006**, *128*, 11463–11470.
(2) (a) Kellam, B.; De Bank, P. A.; Shakesheff, K. M. *Chem. Soc. Rev.* **2003**, *32*, 327–337. (b) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007–2010. (c) Lin, C. W.; Ting, A. Y. *J. Am. Chem. Soc.* **2006**, *128*, 4542–4543. (d) Liu, W.; Brock, A.; Chen, S.; Chen, S.; Schultz, P. G. *Nat. Methods* **2007**, *4*, 239–244.
(3) Rabuka, D.; Forstner, M. B.; Groves, J. T.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2008**, *130*, 5947–5953.
(4) (a) Wilson, J. T.; Chaikof, E. L. *Adv. Drug Delivery Rev.* **2008**, *60*, 124–145. (b) Stabler, C. L.; Sun, X. L.; Cui, W.; Wilson, J. T.; Haller, C. A.; Chaikof, E. L. *Bioconjugate Chem.* **2007**, *18*, 1713–1715. (c) Xie, D.; Smyth, C. A.; Eckstein, C.; Bilbao, G.; Mays, J.; Eckhoff, D. E.; Contreras, J. L. *Biomaterials* **2005**, *26*, 403–412. (d) Cabric, S.; Sanchez, J.; Lundgren, T.; Foss, A.; Felldin, M.; Kallen, R.; Salmela, K.; Tibll, A.; Tufveson, G.; Larsson, R.; Korsgren, O.; Nilsson, B. *Diabetes* **2007**, *56*, 2008–2015.
(5) (a) Hong, S.; Leroueil, P. R.; Janus, E. K.; Peters, J. L.; Kober, M. M.; Islam, M. T.; Orr, B. G.; Baker, J. R., Jr.; Banaszak Holl, M. M. *Bioconjugate Chem.* **2006**, *17*, 728–734. (b) Menger, F. M.; Seredyuk, V. A.; Kitaeva, M. V.; Yaroslavov, A. A.; Melik-Nubarov, N. S. *J. Am. Chem. Soc.* **2003**, *125*, 2846–2847. (c) Fischer, D.; Li, Y. X.; Ahlemeyer, B.; Krieglstein, J.; Kissel, T. *Biomaterials* **2003**, *24*, 1121–1131.
(6) (a) Ruiz-Taylor, L. A.; Martin, T. L.; Zaugg, F. G.; Witte, K.; Indermuhle, P.; Nock, S.; Wagner, P. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 852–857. (b) Kenausis, G. L.; Voros, J.; Elbert, D. L.; Huang, N. P.; Hofer, R.; Ruiz-Taylor, L.; Textor, M.; Hubbell, J. A.; Spencer, N. D. *J. Phys. Chem. B* **2000**, *104*, 3298–3309.
(7) (a) Zhi, Z. L.; Powell, A. K.; Turnbull, J. E. *Anal. Chem.* **2006**, *78*, 4786–4793. (b) Park, S.; Lee, M. R.; Shin, I. *Bioconjugate Chem.* **2009**, *20*, 155–162.
(8) (a) Ilieva, A.; Yuan, S.; Wang, R.; Duguid, W. P.; Rosenberg, L. *Pancreas* **1999**, *19*, 297–303. (b) Wang, R. N.; Rosenberg, L. *J. Endocrinol.* **1999**, *163*, 181–190.

JA908887V