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Antibacterial Property of a Polyethylene Glycol-Grafted Dental Material

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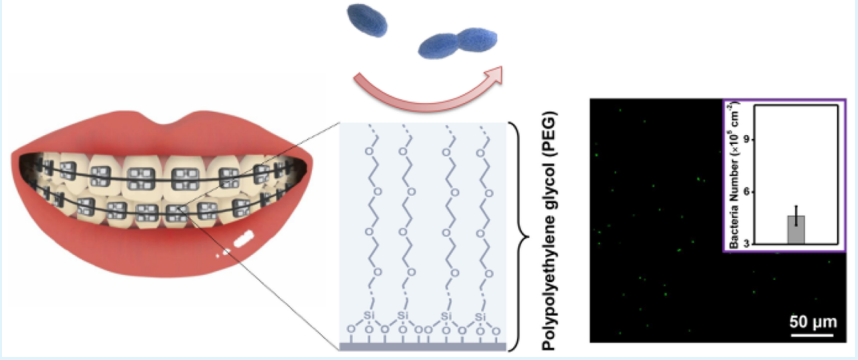
Supporting Information



ABSTRACT: Dental materials often cause bacterial adhesion and promote bacterial biofilm formation, which brings a series of long-standing and significant problems in oral health. However, the current development of antibacterial research in dental devices is limited by the lack of materials endowed with good antibacterial properties against oral bacteria. Here, we present a new strategy for reducing the initial adhesion of bacterial on dental biomaterials by chemically bonding long-chain polyethylene glycol. Our work represents an important step toward solving the problem of bacterial accumulation on dental devices.

KEYWORDS: *antibacterial, antiadhesion, dental materials, polyethylene glycol, bacterial*

In recent years, a series of emerging biomaterials endowed with both the functionality and aesthetics have been constantly developed and demonstrated to be effective and advantageous. The requirements that biomaterials need to cover are very broad, variously depending on the type of biomaterial application. One of them is of crucial importance to achieve, that is, the antibacterial property of the materials,[1] especially of the dental devices immersed in saliva containing bacteria in the mouth.[2] Most of these materials can achieve a good antibacterial performance against the two well-known leading etiological agents *Staphylococcus aureus*[3−5] and *Staphylococcus epidermidis*[6−8] or the typical Gram-negative bacteria like *Escherichia coli*.[3,8−10] However, the oral bacteria are often neglected. Dental materials can quite easily accumulate food debris, and dental plaque-retentive sites will be generated if the food debris cannot be removed in a timely fashion. These plaque-retentive sites can lead to a rapid shift in the bacterial composition and an increased number of bacteria that can cause the formation of a cariogenic or periodontopathogenic biofilm.[2] To solve this problem, researchers have developed some types of antibacterial agents. For example, physically coated inorganic nanostructured TiO_2 (titanium dioxide),[11] silver nanoparticles,[12] and a combination of TiO_2 and Ag[13] have been found to exhibit good antibacterial performance. Although current strategies mainly focus on inhibiting the proliferation of bacteria accumulated on the dental appliance by the introduction of bactericidal agents,[11−14] they ignore the fact that reducing bacterial adhesion in the first place would be more effective. Therefore, a new type of dental appliance with antiadhesion effects against bacteria should be developed.

Once microorganisms are attached to a substratum surface, a multistep process starts leading to the formation of a complex, adhering microbial community that is termed a "biofilm". Therefore, bacterial adhesion on medical devices in aqueous solutions cannot be easily prevented because microorganisms have a strong tendency to become associated with surfaces.[15]

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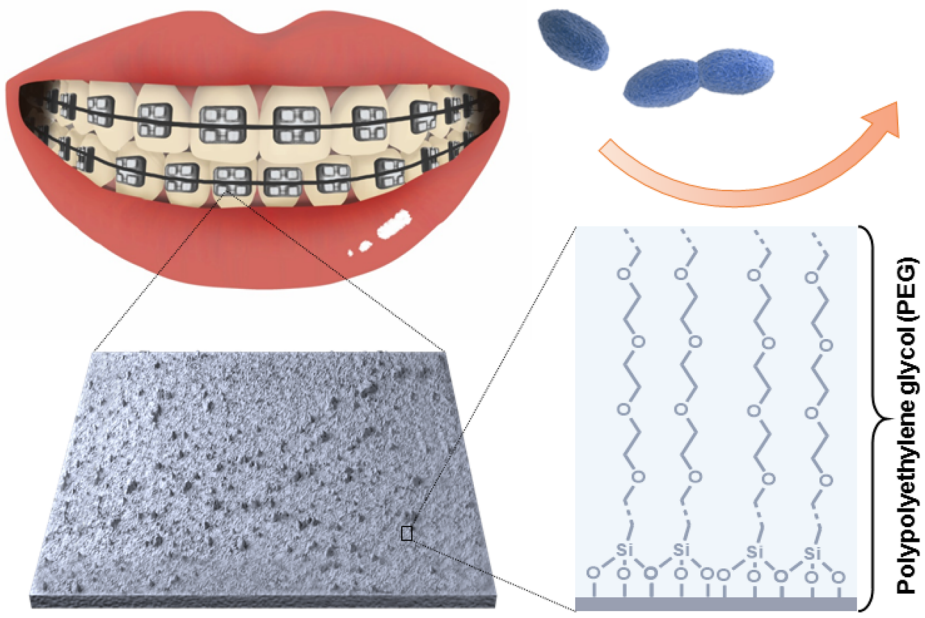

Figure 1. Design of an antiadhesive orthodontic appliance grafted with a long-chain PEG coating. The grafted PEG coating results in the formation of a thin water layer that endows the orthodontic appliance with excellent antiadhesive property against *S. mutans*.

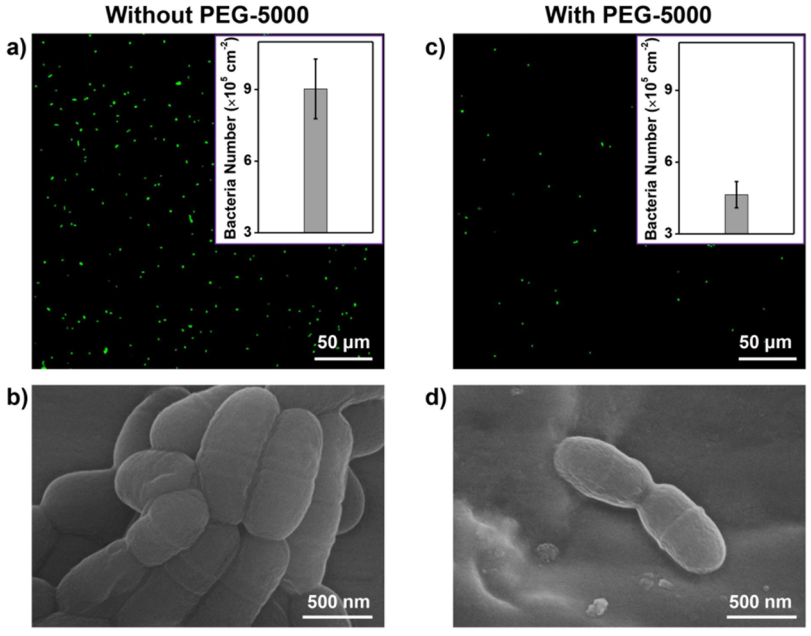

Figure 2. (a, b) For stainless steel archwires without a PEG coating, significant *S. mutans* adhesion can be observed. (c, d) For stainless steel archwires coated with PEG with a molecular weight of 5000 (PEG-5000), the number of adhered *S. mutans* is greatly reduced.

Based on the urgent need for antiadhesive materials, a series of biointerfaces have been developed and demonstrated to be effective in the fight mostly against protein and cell adhesion.[16−19] Polyethylene glycol (PEG) is well-known as an antibiofouling material[20] to provide a hydrophilic environment on a substrate surface. For instance, it was shown to reduce protein adsorption and platelet adhesion in a blood-material interface.[21] However, there is little research on the PEG-coated antiadhesion dental materials. Would the PEG modification represent an efficient strategy to solve the problems associated with bacterial accumulation on dental devices?

Herein, by grafting biocompatible long chain PEG coating on fixed dental appliance, i.e., stainless steel archwire, we have achieved effectively reduced adhesion of *Streptococcus mutans* (*S. mutans*), the most notably leading acidogenic oral bacteria, which can cause white spot lesions (WSLs) or even serious demineralization of the tooth enamel. The grafted PEG coating is capable of forming a stable thin water layer through hydrogen bonding with water molecules, which can effectively prevent bacterial adhesion and the subsequent formation of biofilm (Figure 1). Moreover, the PEG-coated stainless steel archwire shows excellent antiadhesion effects in a long period of time. We believe that this novel PEG-grafting strategy, which can significantly reduce initial acidogenic bacteria adhesion, could

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greatly reduce the risk of tooth enamel demineralization and could solve the problems associated with the accumulation of bacteria around the dental materials during practical treatment.

We prepared an antiadhesive tooth archwire using silane chemistry between the stainless steel archwire and silane-ended PEG molecules with varied polymer chain lengths. The existence of PEG coating on stainless steel archwire was indicated by the peak components of O 1s and C 1s in the X-ray photoelectron spectroscopy (XPS). As shown in Figure S1a, for stainless steel archwire without PEG, there is a peak at about 530 eV corresponding to metal oxides (Me-O), whereas for stainless steel archwire with PEG-coating, the peak at 530 eV disappears and a new peak at about 532 eV corresponding to the C═O/O−C═O and C−O−H/C−O−C species of the PEG units appears. Moreover, comparing with bare stainless steel archwire, after PEG modification, the ratio of carbon atom in the C−O bond (at about 286.4 eV) to total carbon element increases significantly, and the proportion of carbon atom in the C−C/C−H bond (at about 284.6 eV) decreases (Figure S1b) because of the introduction of PEG coating. These XPS results confirmed that the well-defined PEG-coated stainless steel archwires could be successfully fabricated.

As a proof-of-concept study, we compared the antiadhesive properties of stainless steel archwires without and with a PEG coating. Here, we used silane-ended PEG with molecular weight of 5000 (PEG-5000) to modify the stainless steel archwires. Three milliliters of *S. mutans UA159* cell suspension (1×10^7 cells mL^{-1}; prestained with SYBR Green fluorescent nucleic acid) was loaded and kept at 37 °C for 30 min (see the Methods section for more details). The adhered bacteria were then imaged and counted using a fluorescence microscope (Nikon, Ti-E). Significant *S. mutans UA159* adhesion was observed on the bare stainless steel archwire (without PEG-5000) (fluorescent image in Figure 2a); the bacterial density was 9.03×10^5 cm^{-2} (inset in Figure 2a). The scanning electron microscopy (SEM) image revealed that an obvious colony of bacteria developed on the bare stainless steel archwire (Figure 2b). Compared to the bare stainless steel archwire without PEG-5000, bacterial adhesion on the PEG-5000-modified stainless steel archwire was greatly inhibited (fluorescent image in Figure 2c), and the bacterial density was reduced to 4.64×10^5 cm^{-2} (inset in Figure 2c). The SEM image of this archwire revealed that only sporadic bacteria were present (Figure 2d). These results demonstrate that PEG coating is effective as an antiadhesive coating to dental material.

During our experiment, we found that the molecular weight of PEG grafted on the stainless steel archwire was critically important for achieving good antiadhesive properties. When increasing the molecular weight of PEG from 350 to 20000, the number of bacteria adhered on the PEG-coated stainless steel archwire decreased with increasing PEG molecular weight until reaching a minimum for PEG molecular weight of 5000 (Figure 3). However, it should be noted that further increasing the molecular weight would lead to increased number of bacterial, because there is generally an optimal molecular weight to achieve antiadhesive property.[22] In our case, stainless steel archwire modified with PEG-5000 exhibits the best anti-adhesion. To understand how the molecular weight of the PEG coating influences *S. mutans UA159* adhesion, we investigated the microscopic morphology using atomic force microscopy (AFM) and macroscopic wettability measurements obtained with an OCA20 system. The water contact angle (CA) on the bare stainless steel archwire was 56.2 ± 0.9° because of the

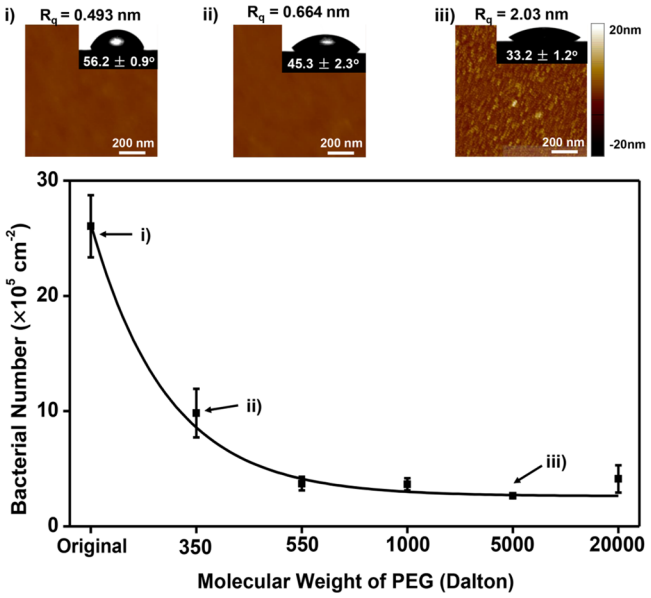

Figure 3. When increasing molecular weight of PEG from 350 to 20000, the antiadhesive property of the PEG-coated stainless steel archwires increased with the increasing molecular weight of the PEG chains, possibly because of the relative hydrophilicity of longer-chain PEG modified stainless steel archwires.

intrinsic hydrophilic property of stainless steel. The AFM image revealed that the stainless steel archwire was relatively smooth, with a surface roughness of approximately 0.493 nm (Figure 3i). When PEG with a molecular weight of 350 (PEG-350) was introduced onto the surface, the water CA decreased to 45.3 ± 2.3°. The AFM image indicated that PEG-350 did not induce significant morphology changes, and the PEG-350-modified stainless steel archwire was also very smooth, with a surface roughness of approximately 0.664 nm (Figure 3ii). The enhanced surface wettability is mainly attributable to the hydrophilic characteristic of the PEG chains. For PEG-5000, obvious nanoscale aggregates were observed, which led to an increased surface roughness of approximately 2.03 nm. The combination of the enhanced surface roughness and the hydrophilic property of PEG made the PEG-5000-coated stainless steel archwire substantially more hydrophilic. The water CA was 33.2 ± 1.2°, which is favorable for the formation of a thin water layer and the subsequent antiadhesion performance (Figure 3iii). The results of this experiment coincide with the fact that the protein resistance increases with higher chain length of the oligoethylene glycol (OEG) units.[23] Therefore, we chose PEG-5000 as the antiadhesion agent for stainless steel archwire modification to achieve an antiadhesive property.

To demonstrate the role of PEG-5000 in establishing the dynamic antiadhesive properties, we conducted similar bacteria-adhesion experiments on stainless steel archwires at 37 °C with and without PEG-5000 modification. Figure 4 summarizes the correlation between the incubation time and the number of *S. mutans UA159* adhered on the stainless steel archwires with and without PEG-5000. For the stainless steel archwire modified with PEG-5000, the number of bacteria is maximized after 15 min of incubation (Figure 4). By contrast, on the unmodified stainless steel archwire, the number of bacteria increases sharply with incubation time until 45 min, after which it remains constant at a value that corresponds to a 4-fold increase relative

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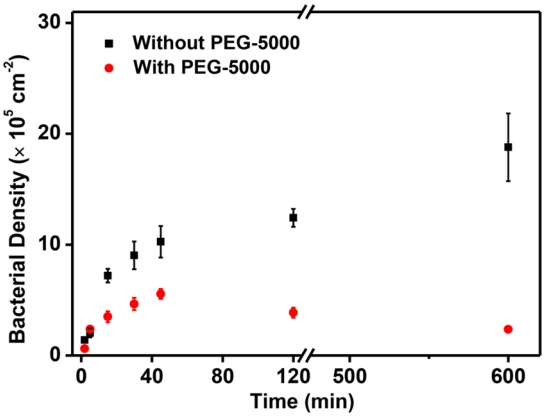

Figure 4. PEG modification endowed the stainless steel archwires with much better dynamic antiadhesive properties than those without PEG-5000, and a very low level of bacterial density was maintained, even after 10 h.

to that measured on the PEG-5000-modified archwire. Furthermore, the numbers of bacteria adhered on the PEG-5000-modified archwires remained very low, even after 10 h of incubation. Orthodontic patients are generally advised to brush their teeth three times a day to prevent the accumulation of food residues around fixed orthodontic appliances during treatment. Therefore, in a 10 h period, patients undergoing orthodontic treatment should brush their teeth at least once, indicating that PEG-5000 modification is suitable for practical application. These results demonstrate that PEG-5000 modification is an effective strategy for the reduction of *S. mutans UA159* adhesion on archwires.

In conclusion, we demonstrated that PEG-modified dental materials exhibit excellent antibacterial properties against initial acidogenic bacteria, and thus, PEG modification represents an efficient strategy to solve the problems associated with bacterial accumulation in dental treatment. The PEG with proper molecular weight can achieve good antiadhesive property and produce superior bacteria-resistive performance because of the enhanced hydrophilicity resulting from the surface energy and surface roughness. PEG modification also achieved excellent dynamic antibacterial performance during a long enough time that can match the daily tooth brushing time interval. Furthermore, PEG was approved by the U.S. Food and Drug Administration for internalization in the human body in 1992,[24,25] making this strategy more promising for practical clinical application in dental treatment.

■ ASSOCIATED CONTENT

Supporting Information

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Materials, detailed methods and characterization, bacterial cultivation, bacterial adhesion experiments, and analysis (PDF)

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B.H., L.P., S.W., and H.L. designed the experiments. L.P. and L.C. synthesized and characterized the PEG-bonded tooth archwires. L.P., B.H., H.L., and S.W. analyzed the data and wrote the manuscript. All authors discussed the results and commented on the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Campoccia, D.; Montanaro, L.; Arciola, C. R. A Review of the Biomaterials Technologies for Infection-Resistant Surfaces. *Biomaterials* **2013**, *34*, 8533−8554.
(2) Bos, R.; van der Mei, H. C.; Busscher, H. J. Physico-Chemistry of Initial Microbial Adhesive Interactions–Its Mechanisms and Methods for Study. *Fems Microbiol. Rev.* **1999**, *23*, 179−230.
(3) Lewis, A. L.; Cumming, Z. L.; Goreish, H. H.; Kirkwood, L. C.; Tolhurst, L. A.; Stratford, P.-W. Crosslinkable Coatings From Phosphorylcholine-Based Polymers. *Biomaterials* **2001**, *22*, 99−111.
(4) Feng, H.; Wang, G.; Jin, W.; Zhang, X.; Huang, Y.; Gao, A.; Wu, H.; Wu, G.; Chu, P. K. Systematic Study of Inherent Antibacterial Properties of Magnesium-Based Biomaterials. *ACS Appl. Mater. Interfaces* **2016**, *8*, 9662−9673.
(5) Wang, J.; Li, J.; Qian, S.; Guo, G.; Wang, Q.; Tang, J.; Shen, H.; Liu, X.; Zhang, X.; Chu, P. K. Antibacterial Surface Design of Titanium-Based Biomaterials for Enhanced Bacteria-Killing and Cell-Assisting Functions Against Periprosthetic Joint Infection. *ACS Appl. Mater. Interfaces* **2016**, *8*, 11162−11178.
(6) Wang, Q.; Uzunoglu, E.; Wu, Y.; Libera, M. Self-Assembled Poly(Ethylene Glycol)-Co-Acrylic Acid Microgels to Inhibit Bacterial Colonization of Synthetic Surfaces. *ACS Appl. Mater. Interfaces* **2012**, *4*, 2498−2506.
(7) Pavlukhina, S. V.; Kaplan, J. B.; Xu, L.; Chang, W.; Yu, X.; Madhyastha, S.; Yakandawala, N.; Mentbayeva, A.; Khan, B.; Sukhishvili, S. A. Noneluting Enzymatic Antibiofilm Coatings. *ACS Appl. Mater. Interfaces* **2012**, *4*, 4708.
(8) Zhao, J.; Ma, L.; Millians, W.; Wu, T.; Ming, W. Dual-Functional Antifogging/Antimicrobial Polymer Coating. *ACS Appl. Mater. Interfaces* **2016**, *8*, 8737−8742.
(9) Kiristi, M.; Singh, V. V.; Esteban-Fernandez de Avila, B.; Uygun, M.; Soto, F.; Aktas Uygun, D.; Wang, J. Lysozyme-Based Antibacterial Nanomotors. *ACS Nano* **2015**, *9*, 9252−9259.
(10) Cao, Z.; Mi, L.; Mendiola, J.; Ella-Menye, J. R.; Zhang, L.; Xue, H.; Jiang, S. Reversibly Switching the Function of a Surface Between Attacking and Defending Against Bacteria. *Angew. Chem., Int. Ed.* **2012**, *51*, 2602−2605.
(11) Welch, K.; Cai, Y.; Engqvist, H.; Stromme, M. Dental Adhesives with Bioactive and On-Demand Bactericidal Properties. *Dent. Mater.* **2010**, *26*, 491−499.
(12) Sharma, V. K.; Yngard, R. A.; Lin, Y.; Silver Nanoparticles. Green Synthesis and their Antimicrobial Activities. *Adv. Colloid Interface Sci.* **2009**, *145*, 83−96.
(13) Liu, S. X.; Qu, Z. P.; Han, X. W.; Sun, C. L. A Mechanism for Enhanced Photocatalytic Activity of Silver-Loaded Titanium Dioxide. *Catal. Today* **2004**, *93*−*95*, 877−884.
(14) Cao, B.; Wang, Y.; Li, N.; Liu, B.; Zhang, Y. Preparation of an Orthodontic Bracket Coated with an Nitrogen-Doped Tio(2-X)N(Y) Thin Film and Examination of its Antimicrobial Performance. *Dent. Mater. J.* **2013**, *32*, 311−316.

(15) Lappin-Scott, H. M.; Costerton, J. W. Bacterial Biofilms and Surface Fouling. *Biofouling* **1989**, *1*, 323−342.
(16) Stevens, M. M.; George, J. H. Exploring and Engineering the Cell Surface Interface. *Science* **2005**, *310*, 1135−1138.
(17) Zhou, J.; Fan, J. B.; Nie, Q.; Wang, S. Three-Dimensional Superhydrophobic Copper 7,7,8,8-Tetracyanoquinodimethane Bio-interfaces with the Capability of High Adhesion of Osteoblasts. *Nanoscale* **2016**, *8*, 3264−3267.
(18) Liu, H.; Liu, X.; Meng, J.; Zhang, P.; Yang, G.; Su, B.; Sun, K.; Chen, L.; Han, D.; Wang, S.; Jiang, L. Hydrophobic Interaction-Mediated Capture and Release of Cancer Cells On Thermoresponsive Nanostructured Surfaces. *Adv. Mater.* **2013**, *25*, 922−927.
(19) Meng, J.; Zhang, P.; Zhang, F.; Liu, H.; Fan, J.; Liu, X.; Yang, G.; Jiang, L.; Wang, S. A Self-Cleaning Tio2 Nanosisal-Like Coating Toward Disposing Nanobiochips of Cancer Detection. *ACS Nano* **2015**, *9*, 9284−9291.
(20) Boulmedais, F.; Frisch, B.; Etienne, O.; Lavalle, P.; Picart, C.; Ogier, J.; Voegel, J. C.; Schaaf, P.; Egles, C. Polyelectrolyte Multilayer Films with Pegylated Polypeptides as a New Type of Anti-Microbial Protection for Biomaterials. *Biomaterials* **2004**, *25*, 2003−2011.
(21) Park, K. D.; Kim, Y. S.; Han, D. K.; Kim, Y. H.; Lee, E. H.; Suh, H.; Choi, K. S. Bacterial Adhesion On Peg Modified Polyurethane Surfaces. *Biomaterials* **1998**, *19*, 851−859.
(22) Wu, C. How Does a Polymer Brush Repel Proteins? *Chin. J. Polym. Sci.* **2014**, *32*, 1575−1580.
(23) Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. Factors that Determine the Protein Resistance of Oligoether Self-Assembled Monolayers –Internal Hydrophilicity, Terminal Hydrophilicity, and Lateral Packing Density. *J. Am. Chem. Soc.* **2003**, *125*, 9359−9366.
(24) Veronese, F. M. Peptide and Protein Pegylation: A Review of Problems and Solutions. *Biomaterials* **2001**, *22*, 405−417.
(25) Fishburn, C. S. The Pharmacology of Pegylation: Balancing Pd with Pk to Generate Novel Therapeutics. *J. Pharm. Sci.* **2008**, *97*, 4167−4183.

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