Surface Characteristics of Poly(γ-alkyl α L-glutamate)s with Different Alkyl Groups

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ABSTRACT: A series of poly(γ-alkyl α L-glutamate)s with different alkyl groups were synthesized by the ring opening polymerization of corresponding α-amino acid *N*-carboxyanhydrides. The characteristics of these polyglutamate surfaces were evaluated by attenuated total reflectance Fourier transform infrared spectroscopy spectra, water contact angle, water absorption, protein adsorption, and platelet adhesion measurements. Changing the length of the alkyl side chain provides a unique opportunity to study the influence of carbon number in the alkyl group on the surface properties of the polyglutamates. Water contact angle and water absorption data show that the hydrophilicity of these polyglutamate surfaces decreases with the increasing of methylene in the alkyl group. Protein adsorption on these polyglutamate surfaces increases with the enhancing of surface hydrophobicity. However, the changes in platelets adhesion could be attributed to the hydrophilicity/hydrophobicity of the polyglutamates and the specific effect of alkyl group. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 1679–1684, 2011

Key words: surface; biocompatibility; polyglutamate; synthetic polypeptide; water contact angle; protein adsorption; platelet adhesion

INTRODUCTION

Synthetic polypeptides have played an important role as models of natural polypeptides and proteins.[1,2] Because of its excellent biodegradability and biocompatibility, the synthesis and application of synthetic polypeptides have become the focus of research today.[3–5] This kind of biomimetic polymer can be obtained by two approaches, by the condensation of either activated amino acids or oligopolypeptides, or more conveniently, by the ring opening polymerization of α-amino acid *N*-carboxyanhydrides (NCAs). Compared with natural proteins, synthetic polypeptides offer more advantages in stability and processbility. By designing and synthesis of polypeptide with various kinds of components, it becomes possible to tune the structure and properties of obtained polypeptide, which has potential application in various scientific fields such as tissue engineering, pharmaceutical chemistry, and biomedical science.[6–10] When polypeptide are utilized as substrates in these fields, more attention should be paid to the surface properties, because first contact with body fluid or environments usually takes place at the surface of substrates.

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Among various synthetic polypeptides, poly(γ-alkyl α L-glutamate)s (PALG) are well known to both biochemists and polymer chemists. They are normally in the form of stiff α-helix conformation known as hairy-rod polymer with the helical backbone as the rod and the alkyl chain as the hair. Through ring opening polymerization of NCAs with different alkyl chain,[11–14] several PALGs are readily synthesized with "hair" of different lengths. Because of the unique structure and ease of synthesis, PALGs are appealing models to evaluate the influence of alkyl chains on the crystallization or self-assembling process of these polymers. The conformation, molecular dynamics, and spatial heterogeneities studies of stiff macromolecules bearing flexible side chains have revealed a layered structure of the rod-like polymer backbone with phase separated hydrocarbon side chains, which can form either crystalline or amorphous domain depending on the nature of the main polymer chains and their organization.[15] However, studies concerning surface properties brought by these polypeptides are rare. Polyglutamates with ethyl or stearyl side groups were immobilized on the surface of polypropylene microporous membrane. Platelets adhesion was greatly suppressed by the incorporation of polyglutamates compared with nascent polypropylene microporous membrane,[16–17] although hydrophobility of these surfaces was comparable. Hence, it is necessary to generate surfaces derived from pure polypeptide as model to illustrate their properties in

details and to elucidate their effects on protein and platelet adhesion.

In previous work, we have synthesized poly(γ-stearyl α L-glutamate)s (PSLG) by ring opening polymerization of γ-stearyl α L-glutamate NCA with multifunctional porphyrin[18] or rare earth metal complexes[19] as the initiators. Superhydrophobility could be obtained by PSLG when electrospun into microfibrous mat, which was mainly induced by the stearyl groups.[20] In this work, a series of PALGs with different alkyl groups were synthesized and the influence of alkyl groups on the surface characteristics is described.

EXPERIMENTAL

Materials

All chemicals were of analytical grade. Poly(γ-ethyl α L-glutamate) (PELG), poly(γ-octyl α L-glutamate) (POLG), poly(γ-dodecyl α L-glutamate) (PDLG), and PSLG were synthesized according to the reported procedure.[21–24] Bovine serum albumin (BSA, purity $> 98\%$) was purchased from Sino-American Biotechnology, and Bovine fibrinogen (BFg, Type I-S, 65–85%) was obtained from Sigma-Aldrich.

Fabrication of films from the synthesized polyglutamates

Polyglutamate was dissolved in trifluoroacetic acid-chloroform (10 vol.% of trifluoroacetic acid) mixture solvent to a concentration of 10 wt%. Then, the solution was cast onto a clean polytetrafluoroethylene substrate. After placed in atmosphere at room temperature for 12 h, the film was dried under reduced pressure at room temperature for 2 h.

Characterization

Intrinsic viscosity [η] for each polyglutamate was measured in THF at 30 ± 0.5°C using an Ubbelchde viscometer. Fourier transform infrared spectroscopy (FT/IR) (Nicolet, Nexus-470, Vernon Hills, IL) with the accessories of attenuated total reflectance (ATR) was also used to characterize the polyglutamates with different alkyl groups.

Water contact angle and water absorption measurements

Surface hydrophilicity/hydrophobicity of the polyglutamates was checked on the basis of water contact angle and water sbsorption measurement. Water contact angles of polypeptide films were determined using a contact angle goniometer (Ningbo Xungao Intelligent Technology, CTS-200, China) equipped with digital video camera. Using a typical sessile method, a water droplet (≈5 μL) was added onto a dry film in air. An image was recorded and static contact water angle was then calculated from the image with software. Five measurements at different positions of the same sample were averaged to give a reliable value. To determine the capacity of water absorption, a dry polyglutamate film (1×1 cm^2) was immersed in de-ionized water at 37 ± 0.5°C for 3 days. Water absorption was defined as $(w_2 - w_1)/w_1$, where w_1 and w_2 represent the weight of the dry film and the film adsorbed with water, respectively.

Protein adsorption

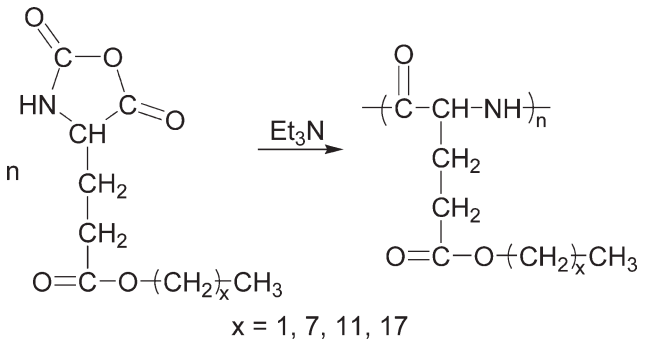
Protein adsorption onto the polyglutamate surface was evaluated by surface plasma resonance (SPR) technique. SPR pure gold sensor chip was cleaned by dipping in ethanol for 10 min, then in freshly made piranha solution (concentrated H_2SO_4 and 30% H_2O_2 in proportion of 3 : 1) for another 1 min, followed by extensively rinsing with Milli Q water (18.2 $M\Omega$-cm). After being dried in nitrogen gas, the cleaned sensors were spin-coated with polypeptides from 1 wt% solutions. According to the ratio of albumin and fibrinogen in blood plasma,[25] BSA and BFg were diluted in a phosphate buffered saline solution (PBS, pH = 7.5, 5.618 g Na_2HPO_4 and 0.403 g KH_2PO_4 dissolved in l L of deionized water) to a concentration of 250 μg/mL and 25 μg/mL, respectively. Protein solution was injected into the Reichert SR7100 DC instrument (Reichert, Depew, NY) and flowed over sensor surface at a rate of 25 μL/min. PBS was the buffer solution during the whole testing process. Testing temperature was rigorously controlled at 25.0 ± 0.1°C throughout the experiment. The amount of adsorbed protein was calculated from the following equation:

$$\Delta m = C_{\mathrm{spr}} \Delta RU$$

where Δm is the mass of adsorbed protein, ΔRU is the response unit from SPR and C_{spr} is a proportionality constant, which has been calibrated to ~ 0.066 ng/cm^2 for protein adsorption.[26,27]

Platelet adhesion

Fresh platelet-enriched plasma (PRP) was purchased from the Second Affiliated Hospital of Zhejiang University. First, a polyglutamate film (1×1 cm^2) was placed onto a piece of glass slide. Then, a sample of 40 μL of PRP was carefully dropped on the film surface. After incubation for 30 min at room temperature (~ 27°C), the film surface was carefully rinsed three times in PBS (pH = 7.5) to remove the unadhered platelets. Platelets adhered on the film surface were preserved with 2.5% glutaraldehyde/PBS solution



Scheme 1 Schematic representation for the synthesis of polyglutamates with different alkyl groups.

for 30 min, followed by a dehydration procedure using a series of ethanol-water mixtures (40, 50, 60, 70, 80, 90, 100 vol% of ethanol) for 20 min and then air dried. Finally, the film surface was characterized by Field Emission Scanning Electron Microscopy (Sirion-100, FEI, USA) after gold sputtering.

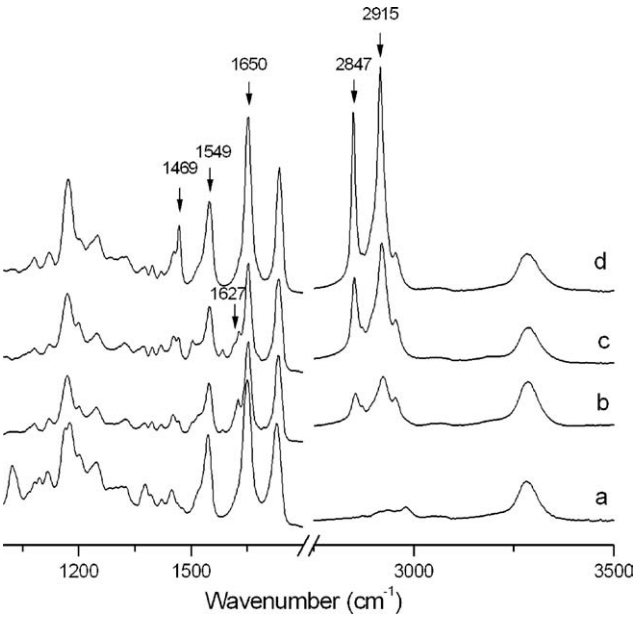


Figure 1 FT-IR/ATR spectra of the polyglutamate films. (a) PELG; (b) POLG; (c) PDLG; and (d) PSLG.

RESULTS AND DISCUSSION

As shown in Scheme 1, PELG, POLG, PDLG, and PSLG were synthesized by the ring-opening polymerization of corresponding NCAs, which is the most economical and expedient process to get synthetic polyglutamates with high molecular weight.[21–24,28] These polyglutamates differ only in the side alkyl group (carbon number equals to 2, 8, 12, or 18). It is appealing to study the influence of the alkyl group on the surface characteristics of polymer with these polyglutamates as the models. Table I shows typical results for the synthesis of these polyglutamates.

FT-IR/ATR spectra of the polyglutamate films are shown in Figure 1. With the increasing of methylene in the alkyl group, the intensity of absorption peaks ascribed to methylene (ν_{as} = 2915 cm^{-1}, ν_s = 2847 cm^{-1} for stretching vibration, δ_s = 1469 cm^{-1} for bending vibration) increases, which is similar to the results reported by Jeon et al.[29] Each spectrum has same absorbance peaks at 1650 cm^{-1} and 1549 cm^{-1}, indicating the existence of α-helix conformation in these polygultamtes films. However, the weak peak at 1627cm^{-1} in the spectra of POLG and PDLG demonstrates that there are a few β-sheet polyglutamates for these two samples.

TABLE I
Typical Results for the Synthesis of Polyglutamates

Polyglutamate	Monomer/initiator (mol/mol)	Yield (wt %)	Viscosity (dL/g)
PELG	100 : 1	95.6	0.308
POLG	100 : 1	92.3	0.225
PDLG	100 : 1	94.8	0.521
PSLG	100 : 1	80.3	0.230

Water contact angle and swelling behavior have been commonly used to characterize the relative surface wettability. Figure 2(a) shows the static water contact angles of the polyglutamate films. It can be observed that the hydrophilicity was contrary to the length of the alkyl group. Jeon et al.[29] have speculated that PSLG formed in α-helix conformation with the main chain as backbone and the long stearyl groups stretched outside. This molecular model is also suitable for the polyglutamates studied in our cases, which is helfpul to explain the dependence of hydrophilicity on the alkyl group of polyglutamates. As the length of the alkyl group increases, the impact of hydrophilic polyglutamate backbone on the surface wettability of the film decreases, while the influence of hydrophobic alkyl group increases. It seems that the decisive factor for the surface wettability of PELG is the polyglutamate backbone, while in the cases of other three polyglutamates, the long hydrophobic alkyl groups screen the polyglutamate backbone and become the main factor. Therefore, PELG is hydrophilic while the other three polyglutamates are relatively hydrophobic. As can be seen from Figure 2(b), dynamic measurements within 250 s also show that the water contact angle of the PELG surface decreases more obviously than those of the POLG, PDLG, and PSLG surfaces because water droplets penetrate into the hydrophilic polyglutamate more easily during the measurement.

Water absorption of the polyglutamate films was also measured. Results listed in Figure 3 are consistent with those from water contact angle measurements. It can be speculated that the short alkyl chain makes the water molecules closer to the ester and

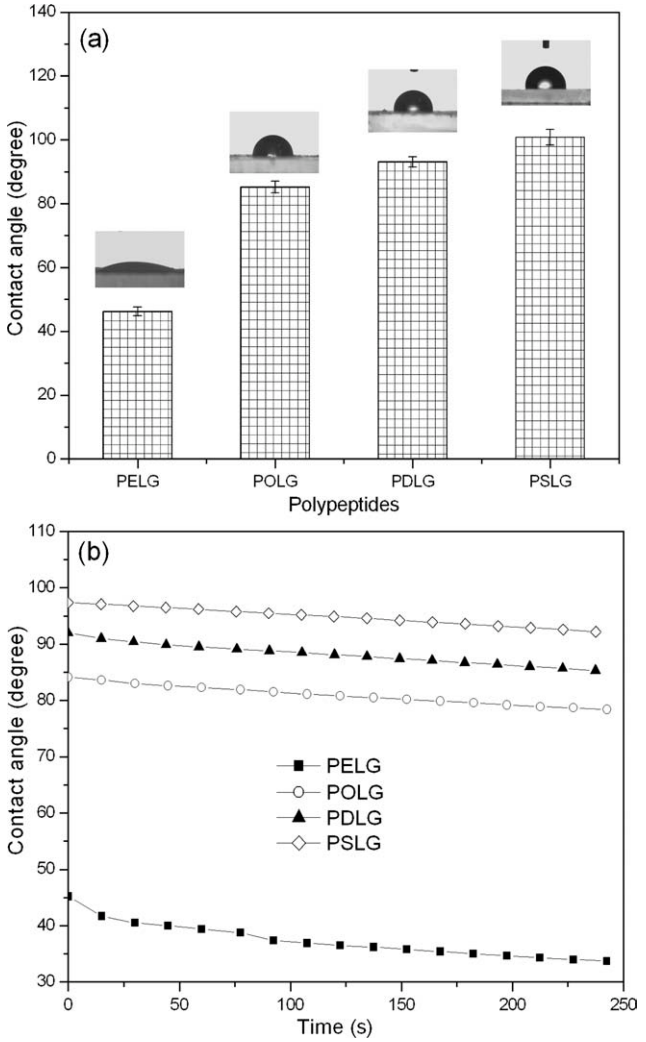


Figure 2 Static (a) and dynamic (b) water contact angles of the polyglutamate surfaces.

amide bonds of polyglutamate to form hydrogen bond.

Surface blood compatibility is an important part of biocompatibility when considering applications in tissue engineering as biomedical materials. In this work, blood compatibilities of the polypeptide films were evaluated by protein adsorption and platelet adhesion. When foreign surface is exposed to blood, a competitive adsorption of protein occurs instantaneously at the surface and a complex protein coating on the surface is formed, which affects the surface blood compatibility.[30] Therefore, it is imperative to study protein adsorption when considering blood compatibility. Table II shows the results of BSA adsorption on the surface of various polyglutamate films. BSA adsorption on the film surface increases with the hydrophobicity of the film surface. Various interactions, such as hydrophobic interaction, electrostatic interaction, acceptor-donor interaction etc., may influence protein adsorption. Among them, it is widely accepted that hydrophobic interaction between the polymer surface and protein plays a significant role in the nonselective adsorption of protein.[31–37] Usually, that is, polymers possessing hydrophilic surface show relatively low nonselective protein adsorption. Albumin is the major constituent of blood plasma and is one of the smallest plasma proteins with molecular weight of 67 kDa which adsorbs to the surface fast. Except for the nonselective interaction, the selective interaction between protein and surface polypeptide should be also considered. Albumin, like BSA or human serum albumin (HSA), is composed of a single polypeptide chain folded into three or four spherical units. The crevices between these spherical regions are supposed to be the fatty acid binding sites by hydrophobic interaction. These structures make it possible for albumin to bind to long alkyl chain selectively, especially the stearyl groups.[38–41] Therefore, the specific interaction between BSA and PSLG results in the high BSA adsorption of PSLG film. Fibrinogen is a large blood protein with molecular weight of 340 kDa, which is known to adsorb more strongly to multiple surfaces, especially polymer surfaces.[25,42] Similarly, the amounts of adsorbed fibrinogen on the surfaces increase with surface hydrophobicity (Table II). Nevertheless, due to the strong adsorption and high molecular weight, the amount of adsorbed BFg is much higher than that of BSA.

After the adsorption of proteins from blood, platelet adhesion and activation of coagulation occur,

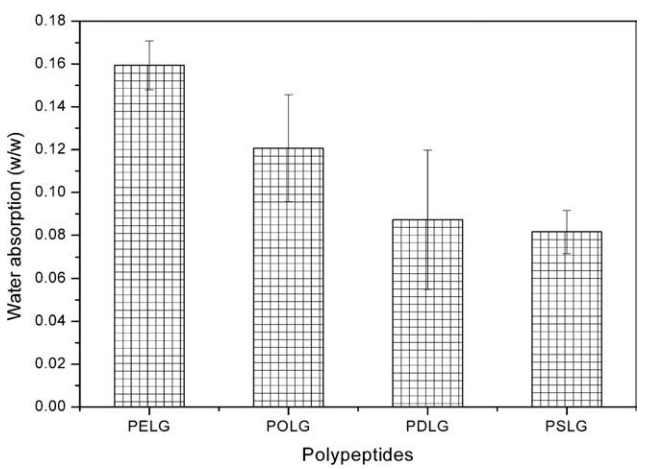


Figure 3 Water absorption of the polyglutamates.

TABLE II
Amount of Protein Adsorbed on the Surface of Various Polypeptide Films

Polyglutamate	BSA (ng/cm^2)	BFg (ng/cm^2)
PELG	24.64 ± 9.50	138.29 ± 28.48
POLG	37.42 ± 13.65	157.41 ± 15.72
PDLG	55.69 ± 16.99	171.55 ± 24.95
PSLG	80.18 ± 14.92	178.38 ± 11.66

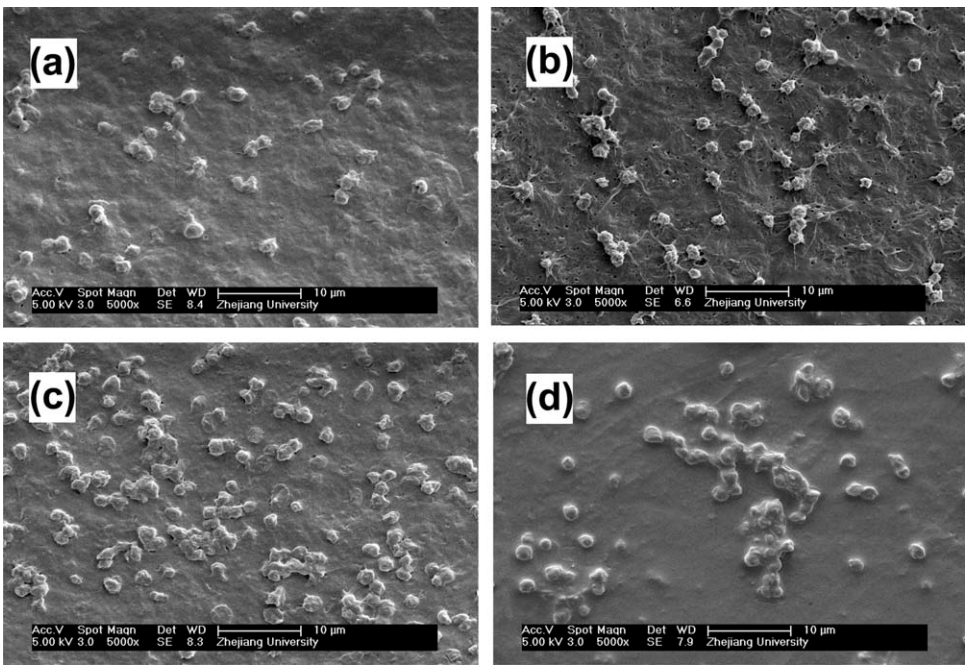


Figure 4 Surface images of platelets adhesion on polyglutamate films. (a) PELG; (b) POLG; (c) PDLG; and (d) PSLG.

leading to thrombus formation. Platelet adhesion experiment is a useful method to evaluate the preliminary blood compatibility of a polymer surface. In this study, platelets adhesion on the surface of various polyglutamate films was examined with Field Emission Scanning Electron Microscopy and evaluated based on the number and the morphology of adhered platelets. According to the arbitrary method of Ko et al.,[43] the morphology can be classified into five stages: round (unactivated), dendritic (pseudopodial but no flattening), spread-dendritic (late pseudopodial with hyaloplasm spreading), and fully spread (totally acitivated). As can be seen in Figure 4, platelets are adhered on the polyglutamate surfaces without obvious aggregation and distortion in the first stage. The number of adhered platelets is strongly dependent on the carbon number of the alkyl group. As shown in Figure 5, the amounts of adhered platelets increase in the sequence of PELG $<$ POLG $<$ PDLG. However, the amount of adhered platelets on the PSLG surface is even less than that on the POLG surface, a little more than PELG.

It has been proved that the behavior of platelet adhesion on material surfaces depends on many factors, such as wettability, surface roughness, charge density, and mechanical characteristics.[44] These factors firstly influence the adsorption of HSA and fibrinogen in plasma. Fibrinogen is reported to be a key protein relating to platelet adhesion and activation by binding to the platelet receptors (GPIIb/IIIa, GP Ib, and possibly $\alpha_x\beta_3$), leading to thrombus formation, while HSA is passive for the adhesion of platelets.[33–37,45] When the polypeptide film is exposed to the PRP, the low molecular weight proteins like HSA arrive firstly and adsorb at the surface. However, these adsorption is easily displaced by the later arrived, high molecular weight proteins with high affinity to the surface (so-called Vroman effect).[46,47] As has been indicated earlier, albumin is a small protein while fibrinogen is a larger protein with high affinity. For PELG, POLG, and PDLG films, the first adsorbed HSA would be replaced easily by fibrinogen due to the strong absorption of fibrinogen, leading to the suppressing of HSA adsorption. The amounts of adsorbed fibrinogen increased with the hydrophobicity of surfaces. As

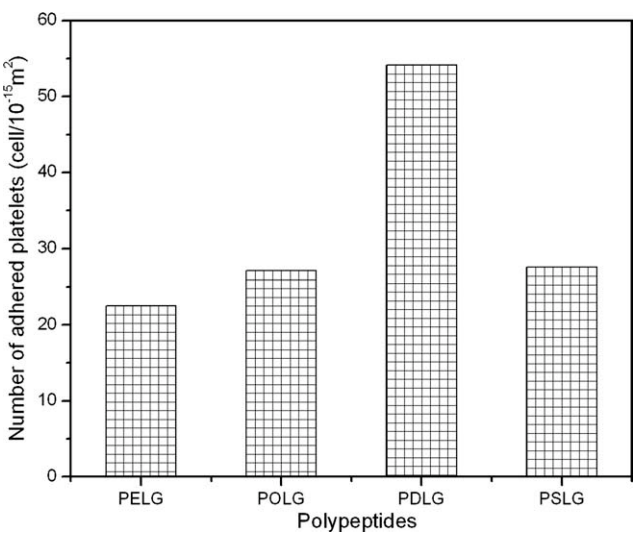


Figure 5 Platelets adhesion on the polyglutamate surfaces.

a result, platelets adhered to the polyglutamate surface increase with surface hydrophobicity as well as the increasing of adsorbed fibrinogen. For PSLG, however, the specific interaction between the stearyl groups and the alkyl-binding cites of albumin makes albumin adsorption strong enough to resist the replacement by fibrinogen.[38–41] Therefore, the amount of platelets adhered on the PSLG surface is as few as PELG.

CONCLUSIONS

In this article, surface characteristics of PALGs with different alkyl groups are described in relating to wettability and biocompatibility. PELG is hydrophilic and POLG, PDLG, and PSLG are hydrophobic. The main reason for PELG is the hydrophilic polyglutamate backbone, whereas that for POLG, PDLG, and PSLG is ascribed to the long hydrophobic alkyl groups. For surfaces of PELG, POLG, and PDLG films, hydrophobicity is a decisive factor for the nonselectively adsorption of proteins and platelets. As a result, the amounts of BSA and BFg adsorbed to the polyglutamate surfaces increase with the surface hydrophobicity, so does the platelets adhesion. For the PSLG surface, however, the specific interaction between stearyl groups of polypeptide and albumin predominates, which inhibits the competitive adhesion of fibrogen. As a result, the amount of platelets is even less than that on the POLG surface.

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References

1. Bamford, C. H.; Eliot, A.; Hanby, W. E. Synthetic Polypeptides; Academic Press: New York, 1956.
2. Kricheldorf, H. R. α-Amino Acid-N-Carboxyanhydrides and Related Materials; Springer: New York, 1987.
3. Kricheldorf, H. R. Angew Chem Int Ed 2006, 45, 5752.
4. Deming, T. J. J Polym Sci A Polym Chem 2000, 38, 3011.
5. Oh, S. B.; Choi, Y. K.; Cho, C. S. J Appl Polym Sci 2003, 88, 2649.
6. Hellaye, M. L.; Fortin, N.; Guilloteau, J.; Soum, A; Lecommandoux, S; Guillaume S. M. Biomacromolecules 2008, 9, 1924.
7. Deming, T. J Adv Drug Deliver Rev 2002, 54, 1145.
8. Deng, C.; Chen, X.; Yu, H.; Sun, J.; Lu, T; Jing, X. Polymer 2007, 48, 139.
9. Barbosa, M. E. M.; Montembault, V.; Cammas-Marion, S.; Ponchel, G.; Fontaine, L. Polym Int 2007, 56, 317.
10. Yan, G. P.; Li, H.; Cheng, S. X.; Bottle, S. E.; Wang, X. G.; Yew, Y. K.; Zhuo, R. X. J Appl Polym Sci 2004, 92, 3869.
11. Bhaw-Luximon, A.; Jhurry, D.; Belleney, J.; Goury, V Macromolecules 2003, 36, 977.
12. Wieringa, R. H.; Siesling, E. A.; Geurts, P. F. M.; Werkman, P. J.; Vorenkamp, E. J.; Erb, V.; Stamn, M.; Schouten, A. J Langmuir 2001, 17, 6477.
13. Kim, G.; Seo, M.; Sohn, D.; Han, D. Y.; Lee, Y. Polymer 2001, 42, 8469.
14. Watanabe, J; Ono, H.; Uematsu, I.; Abe, A. Macromolecules 1985, 18, 2141.
15. Gitsas, A.; Floudas, G. Macromolecules 2007, 40, 8311.
16. Liu, Z. M.; Xu, Z. K.; Ulbricht, M. Chinese J Polym Sci 2006, 24, 529.
17. Liu, Z. M.; Xu, Z. K.; Wang, J. Q.; Yang Q.; Wu, J; Seta, P. Eur Polym Mater 2003, 39, 2291.
18. Wu, J; Hu, X. P. Polym Adv Technol 2002, 13, 201.
19. Hu, X. P.; Wu, J.; Xu, Z. K.; Feng, L. X. Chinese J Polym Sci 2000, 18, 369.
20. Shao, L. J.; Wu, J.; Xu, Z. K. Chinese J Polym Sci 2009, 27, 115.
21. Poche, D. S.; Moore, M. J.; Bowles, J. L. Synth Commun 1999, 29, 843.
22. Poche, D. S.; Daly, W. H.; Russo, P. S. Macromolecules 1995, 28, 6745.
23. Daly, W. H.; Poche, D. Tetrahedron Lett 1988, 29, 5859.
24. Daly, W. H.; Poche, D. Polym Prepr 1989, 30, 107.
25. Han, D. K.; Park, K. D.; Ryu, G. H.; Kim, U. Y.; Min, B. G.; Kim, Y. H. J Biomed Mater Res 1996, 30, 23.
26. Stenberg, E.; Persson, B.; Roos, H.; Urbaniczky, C. J Colloid Interface Sci 1991, 143, 513.
27. Bergstrand, A.; Rahmani-Monfared, G.; Ostlund, A.; Byden, M.; Holmberg, K. J Biomed Mater Res A 2009, 88A, 608.
28. Mori, H.; Iwata, M.; Ito, S.; Endo, T. Polymer 2007, 48, 5867.
29. Jeon, S.; Choo, J.; Sohn, D.; Lee, S. N. Polymer 2001, 42, 9915.
30. Lee, J H.; Ju, Y. M.; Kim, D. M. Biomaterials 2000, 21, 683.
31. Sigal, G. B.; Mrksich, M.; Whitesides, G. M. J Am Chem Soc 1998, 120, 3464.
32. Bajpai, A. K. Polym Int 2007, 56, 231.
33. Rodrigues, S. N.; Goncalves, I. C.; Martins, M. C. L.; Barbosa, M. A.; Ratnre, B. D. Biomaterials 2006, 27, 5357.
34. Welle, A.; Grunze, M.; Tur, D. J Colloid Interf Sci 1998, 197, 263.
35. Nagahama, K.; Nishimura, Y.; Ohya, Y.; Ouchi, T. Polymer 2007, 48, 2649.
36. Tzoneva, R.; Heuchel, M.; Groth, T.; Altankov, G.; Albrecht, W.; Paul, D. J Biomater Sci Polym E 2002, 13, 1033.
37. Bayramoglu, G.; Yilmaz, M.; Batislam, E.; Arica, M. Y. J Appl Polym Sci 2008, 109, 749.
38. Spector, A. A. J Lipid Res 1975, 16, 165.
39. Grasel, T. G.; Pierce, J. A.; Cooper, S. L. J Biomed Mater Res 1987, 21, 815.
40. Zhao, G. W.; Chen, Y. S.; Wang, X. L. Appl Surf Sci 2007, 253, 4709.
41. Ji, J; Feng, L.; Shen, J.; Barbosa, M. A. J Biomed Mater Res Part A 2002, 61, 252.
42. Martins, M. C. L.; Wang, D.; Ji, J.; Feng, L; Barbosa, M. A. Biomaterials 2003, 24, 2067.
43. Ko, T. M.; Lin, J. C.; Cooper, S. L. Biomaterials 1993, 14, 657.
44. Park, J B. Biomaterials Science and Engineering; Plenum Press: New York, 1984, p 1.
45. Beguin, S.; Kumar, R. Thromb Haemostasis 1997, 78, 590.
46. Vroman, L.; Adams, A. L. J Colloid Interface Sci 1986, 111, 391.
47. Wojciechowski, P.; Tenhove, P.; Brash, J. L. J Colloid Interface Sci 1986, 111, 455.