Enhancing Protein Resistance of Hydrogels Based on Poly(2-hydroxyethyl methacrylate) and Poly(2-methacryloyloxyethyl phosphorylcholine) with Interpenetrating Network Structure

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Received 25 June 2010; accepted 12 December 2010
DOI 10.1002/app.33960
Published online 11 April 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: In this work, sequential interpenetrating polymer networks (IPNs) based on poly(2-hydroxyethyl methacrylate) (PHEMA) and poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC) were prepared with improved protein resistance. The bulk properties of the IPN hydrogels such as water content, ion permeability and mechanical strength were determined by the gravimetric method, ionoflux measurement technique and tensile tester respectively. The surface characteristics of the IPNs were investigated by X-ray photoelectron spectroscopy (XPS) and contact angle measurements. XPS analysis suggested that PMPC was present on the surface and in the bulk material. The IPN hydrogels possessed more hydrophilic surface than PHEMA revealed by contact angle measurements. Bovine serum albumin was used as a model protein to evaluate protein resistance by bicinchoninic acid assay method. The result revealed that the protein adsorption on the IPNs showed dramatically reduction compared to PHEMA. These results suggest that the IPNs based on PHEMA and PMPC may be further developed as ophthalmic biomaterials. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3347–3352, 2011

Key words: interpenetrating polymer network; hydrogel; 2-hydroxyethyl methacrylate; 2-methacryloyloxyethyl phosphorylcholine; protein resistance

INTRODUCTION

Poly(2-hydroxyethyl methacrylate) (PHEMA) is one of the most significant synthetic hydrogels. PHEMA hydrogel has excellent mechanical properties, nontoxicity, and favorable tissue compatibility, which leads to many biomedical applications such as contact lenses, corneal implants, ureters, cardiovascular implants, tissue repair surgery and many dental applications.[1–4] However, recent studies have reported that considerable amounts of protein easily bound to PHEMA-based hydrogels.[5,6] The adsorption and accumulation of proteins at the surface of medical devices placed in contact with the host can lead to adverse reactions. To improve the antifouling properties, PHEMA-based hydrogels can be modified by surface grafting of hydrophilic moieties, such as poly(ethylene glycol).[7,8] However, extra steps, specialized equipment and complicated procedures can increase overall manufacturing costs.

An interpenetrating polymer network (IPN) comprising two or more networks that are interlaced on a molecular scale but not covalently bonded to each other may combine networks with different properties and structures. Consisting of two or more network polymers, with at least one polymerized and/or crosslinked in the immediate presence of others,[9] the interlocked structures of the crosslinked components are believed to ensure the stability of the bulk and surface morphology.[10] The incorporation of second hydrophilic network into first network may improve the hydrophilicity significantly by the method of IPN. 2-Methacryloyloxyethyl phosphorylcholine (MPC), which is regarded as a biomimetic component of the cell membrane, is usually grafted onto the surfaces of biomaterials to improve biocompatibility. The excellent biocompatibility of MPC-containing biomaterials has been confirmed by their various characteristics such as being quite inert for biological systems, with reduced levels of protein absorption, bacterial adhesion and cellular attachment.[11–13] The grafting usually affects the surface but not the bulk properties of biomaterials.

To develop hydrogel biomaterials with improved hydrophilicity and protein resistance, PHEMA-based hydrogels were modified by the incorporation of

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Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 51073036.

poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) network onto the surface and into the bulk as well, using sequential IPN method. The host PHEMA hydrogel was first prepared using poly(ethylene glycol) dimethacrylate (PEGDMA) as the crosslinker to improve mechanical strength.[14] Then, PMPC network was incorporated into the PHEMA network to improve protein adsorption resistance and ion permeability. The bulk properties such as water content, ion permeability and mechanical strength of the IPNs were examined. Surface properties were investigated by contact angle measurements and X-ray photoelectron spectroscopy (XPS) analyses. Protein adsorption resistance of the hydrogels was studied by bicinchoninic acid assay method using bovine serum albumin (BSA) as a model protein.

EXPERIMENTAL

Materials

Ethylene glycol dimethacrylate (EGDMA) and 2-hydroxyethyl methacrylate (HEMA) were purchased from Aldrich chemical Co. and purified by distillation under reduced pressure before use. MPC was supplied from Nanjing Letian S&T Development Company. Poly(ethylene glycol) dimethacrylate (PEGDMA, number averaged molecular weight at around 598) was used as received from Yantai yunkai chemical engineering Co., Ltd. of China. Free radical photoinitiator Darocur 1173 obtained from Ciba Co. was used as received. BCA Protein Assay Reagent Kit K3000 was purchased from Shanghai Biocolor BioScience & Technology Company.

Preparation of IPN hydrogels

The monomer HEMA (20% w/w based on solvent), crosslinker PEGDMA (0.5% w/w based on monomer HEMA), and the free radical photoinitiator Darocur 1173 (0.5% w/w based on monomer HEMA) were dissolved in ethanol solvent. The mixture was introduced between two glass plates (7.5 cm × 2.5 cm) and cured under a high-pressure mercury lamp emitting overwhelmingly light at 365 nm for 1 h. Film thickness was controlled by a Teflon gasket, which gave a fairly consistent thickness of 0.25 mm. Unreacted monomer in the films was extracted by Soxhlet extraction using ethanol and then the film was hydrated to equilibrium in the distilled water. The resultant PHEMA hydrogel was dried completely and the host PHEMA film was obtained, which was weighed prior to IPN formation.

The MPC monomer (5, 10, 15, 20, 25, 30, 40, and 50% w/w based on solvent ethanol), crosslinker EGDMA (5% w/w based on monomer), and the photoinitiator Darocur 1173 (0.5% w/w based on monomer) were dissolved in ethanol. The monomer mixture was allowed to swell the host PHEMA film in vial for about 24 h until equilibrium had reached. The vials containing the films and the remaining MPC solution were placed under the UV lamp for a period of 2 h. Subsequently, the films were removed from the bulk MPC solution. The IPN hydrogels were obtained after unreacted MPC monomer was extracted using ethanol. The films were dried and weighed to determine the content of PMPC network incorporated. The PMPC network content was calculated by the following formula:

$$\text{PMPC network content (\%)} = \frac{m_{\text{IPN}} - m_{\text{PHEMA}}}{m_{\text{IPN}}} \times 100\%$$

where, m_{IPN} and m_{PHEMA} were the weights of IPN hydrogels and host PHEMA films at dry state, respectively.

Bulk characterization

The equilibrium water content was calculated as follows:

$$\text{EWC (\%)} = \frac{m_{\text{IPN}'} - m_{\text{IPN}}}{m_{\text{IPN}'}} \times 100\%$$

where, $m_{\text{IPN}'}$ and m_{IPN} were the weights of IPN hydrogels at hydrated state and dry state, respectively.

The ion permeability of the hydrogels was determined using a basic experimental set-up that involved the use of a cell with both a "donating" and a "receiving" reservoir, separated by the hydrogel under study. Ion permeability was established by measurement of the flow of ions from the donor reservoir (loaded with a solution of NaCl of known ionic concentration), across the hydrogel and into the receiver (loaded with pure water) by a conductivity electrode and meter. The diffusion coefficient (D, mm^2/min) for ion transport could then be calculated using the gradient of the resulting graph to provide the rate of ion transport (n', mol/min) and substituting into the following formula[15]:

$$D = \frac{n'}{A \times (dc/dx)}$$

where, A was the area of ion transport (mm^2), dc was the concentration difference (mol/mm^3), and dx was the thickness of hydrogel film (mm).

Stress–strain measurements of dry hydrogels were carried out using an Instron series IX materials testing system at room temperature. Dog-bone shaped samples were cut from the dry hydrogels (5 mm

wide at the narrowest point with a gage length of 15 mm). Thickness of the samples was measured with a digital micrometer having a precision of 1 μm. A crosshead speed of 10 mm/min was used and at least triplicate was tested for each sample.

Surface characterization

The surface elemental composition of the hydrogels at dry state was analyzed by X-ray photoelectron spectroscopy using a Shimadzu ESCA 750 spectrometer using MgKa radiation. The take-off angle of photoelectron was 45°.

The water contact angles of the hydrogels were measured at ambient humidity and temperature by the sessile drop method, using JC2000C1 goniometer of Zhongchen Digital Technical Co., China. The contact angle reported here was an averaged value of at least three measurements.

Protein deposition resistance

Protein deposition resistance evaluation was conducted as follows: the hydrogels were immersed in PBS overnight before immersing in 5 mg/mL BSA in PBS (pH 7.4) for 12 h at 37°C and then rinsed with 500 mL of fresh PBS twice by stirring method (300 rpm for 5 min). The adsorbed protein was detached in sodium dodecyl sulfate (SDS) 1 wt % in water by sonication for 5 h, and the concentration of protein in the SDS solution was determined by using BCA method.[16] From the concentration of protein, the amount of protein adsorbed on the surface was calculated.

RESULTS AND DISCUSSION

Preparation of IPN hydrogels

The IPN hydrogels were prepared by swelling the host PHEMA films until equilibrium in the MPC solutions with different concentrations. To optimize the PMPC network content, several fabrication parameters, including use of macromolecular crosslinker PEGDMA and solvent during the host PHEMA film curing procedure, and the effect of MPC concentration during the MPC incorporation step as well as the effect of crosslinker and initiator concentration were examined. The PMPC network content in the resultant IPN was found to be relatively unaffected by the concentration of either the crosslinker or initiator during the MPC incorporation step (results not shown). However, increasing the amount of solvent and decreasing the amount of crosslinker PEGDMA during the PHEMA film curing procedure were found to result in the host PHEMA films with greater porosity, allowing for the incorporation of larger amounts of PMPC network during the subsequent IPN formation (results not shown). In addition, increasing MPC concentration during the MPC incorporation step made the amount of PMPC network incorporated into the IPN become larger (as shown in Fig. 1).

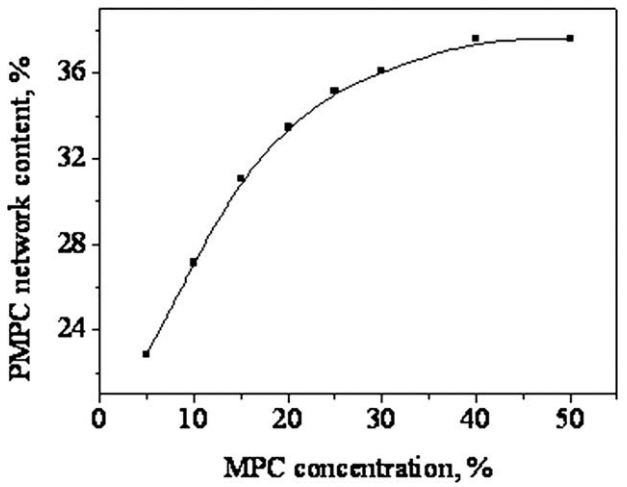

Figure 1 Influence of MPC concentration on the PMPC network content incorporated into the IPN hydrogels. The IPNs were obtained by swelling PHEMA crosslinked with PEGDMA (0.5% w/w) in the monomer mixtures with MPC concentration of 5, 10, 15, 20, 25, 30, 40, and 50%, respectively, EGDMA concentration of 5% w/w based on the weight of MPC and following photoinitiated crosslinking polymerization of MPC.

Bulk characterization

Figure 2 illustrates the relationship between PMPC network content and water content of the IPN hydrogels. The results showed that the water content of the hydrogels correlates significantly with their PMPC network content. The linear regression (R = 0.995) was positive, which indicated that increasing PMPC network content would result in an increase in water content. The phenomena may be explained that PMPC could bind more amounts of water molecules into the hydrogels due to the more hydrophilicity of PMPC than that of PHEMA.

Another property that is considered to be of importance for hydrogels if used as contact lenses is the ability to allow transport of ions and other nutrients through the lens to the cornea. One measure of the lens' ability to perform this function is the ion diffusion coefficient (D). The D values for the PHEMA hydrogel and IPN hydrogels are listed in Table I. The results revealed that all IPN hydrogels had greatly higher D values than the PHEMA hydrogel, and the D values increased with the PMPC content in the IPN.

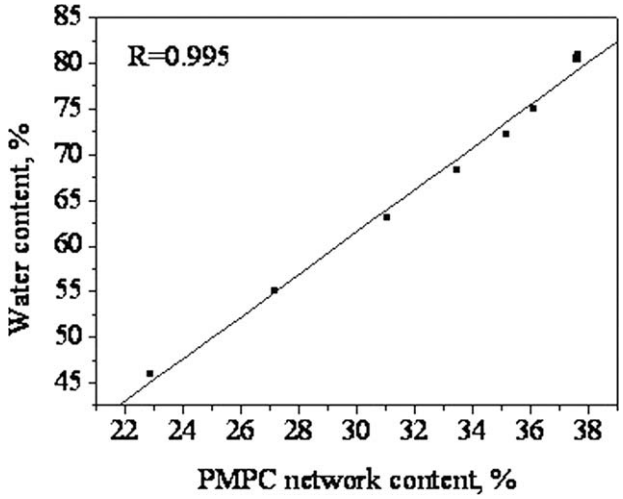

Figure 2 Dependence of water content of IPN hydrogels on PMPC network content. The IPNs were obtained by swelling PHEMA crosslinked with PEGDMA (0.5% w/w) in the monomer mixtures with MPC concentration of 5, 10, 15, 20, 25, 30, 40, and 50%, respectively, EGDMA concentration of 5% w/w based on the weight of MPC and following photoinitiated crosslinking polymerization of MPC.

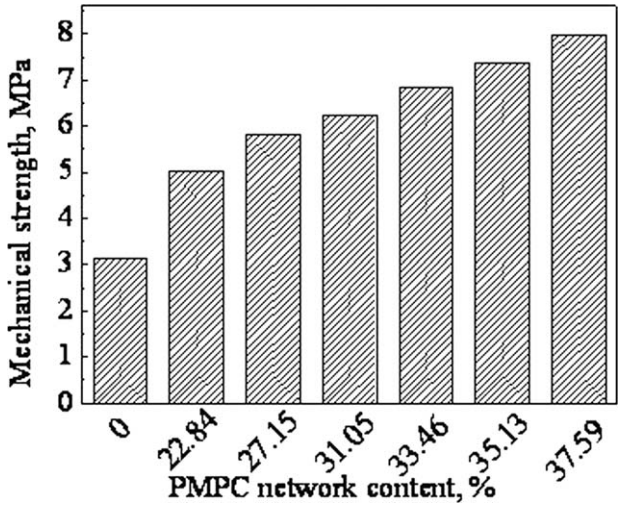

Figure 3 Mechanical strength of the PHEMA and IPN hydrogels at dry state. The IPNs were obtained by swelling PHEMA crosslinked with PEGDMA (0.5% w/w) in the monomer mixtures with MPC concentration of 5, 10, 15, 20, 25, and 40%, respectively, EGDMA concentration of 5% w/w based on the weight of MPC and following photoinitiated crosslinking polymerization of MPC.

Based on the data of the Table I and Figure 2, a linear relationship between D values and water content for the IPN hydrogels can be obtained with regression of $R = 0.999$ (figure omitted). Clearly, there was a direct proportionality between the water content of the hydrogels and ion transport capabilities. The IPN hydrogels if used as contact lenses possessed the necessary solute permeation characteristics that were believed to be required for maintenance of ocular health and on-eye movement during contact lens wear.

TABLE I
The Ionoflux Diffusion Coefficient for the PHEMA and IPN Hydrogels

Sample	Ionoflux diffusion coefficient $D \times 10^6$ (mm^2/min)
PHEMA hydrogel	56.00
IPN hydrogel 1[a]	107.64
IPN hydrogel 2[a]	115.58
IPN hydrogel 3[a]	127.50
IPN hydrogel 4[a]	129.62
IPN hydrogel 5[a]	135.50
IPN hydrogel 6[a]	141.89

[a] IPN hydrogels 1, 2, 3, 4, 5, and 6 having PMPC network content of 22.84%, 27.15%, 31.05%, 33.46%, 35.13%, and 37.59%, respectively. The IPNs were obtained by swelling PHEMA crosslinked with PEGDMA (0.5% w/w) in the monomer mixtures with MPC concentration of 5%, 10%, 15%, 20%, 25%, 40%, respectively, EGDMA concentration of 5% w/w based on the weight of MPC and following photoinitiated crosslinking polymerization of MPC.

The mechanical strength of the PHEMA hydrogel and the IPN hydrogels at dry state are summarized in Figure 3. The IPN hydrogels exhibited enhanced tensile strength compared with the PHEMA hydrogel, falling in the range from 5.36 to 8.14 MPa. Increase in the tensile strength with PMPC network content in the IPN was also found for the IPN hydrogels. It may be deduced to the fact that the crosslinked PMPC network was entrapped within the continuous PHEMA network, resulting in an effective transfer of stress between the two polymer networks in the IPN hydrogels.

Surface characterization

The elemental surface compositions of the PHEMA hydrogel and IPN hydrogels determined by XPS are summarized in Table II. The results showed that phosphorus was found on the surfaces of IPN hydrogels, whereas there was no phosphorus found on the surface of PHEMA hydrogel. The ratio of the peak area of phosphorus to that of carbon (P_{2p}/C_{1s}) was calculated and summarized in Table II. The P_{2p}/C_{1s} value of the IPN hydrogels was in the range from 0.0096 to 0.0586 and increased with the PMPC content in the IPNs. The results indicated that the content of PMPC enriched on the surface increased with the PMPC network content in the IPN.

The water contact angles of the IPN hydrogels are summarized in Figure 4. It was clear that the presence of PMPC affected the surface hydrophilicity of the IPN hydrogels. The water contact angle of the PHEMA was measured to be 55°. However, after

TABLE II
The Surface Element Compositions of the PHEMA and IPN Hydrogels

Sample	C_{1s}	O_{1s}	P_{2p}	P_{2p}/C_{1s}	PMPC content
PHEMA hydrogel	58.08	41.92	–	–	–
IPN hydrogel 1[a]	56.22	43.24	0.54	0.0096	22.84
IPN hydrogel 2[a]	50.68	48.46	0.86	0.0170	27.15
IPN hydrogel 3[a]	54.89	43.81	1.30	0.0237	31.05
IPN hydrogel 4[a]	49.96	48.56	1.48	0.0296	33.46
IPN hydrogel 5[a]	52.51	45.15	2.34	0.0446	35.13
IPN hydrogel 6[a]	49.96	47.10	2.93	0.0586	37.59

[a] IPN hydrogels 1, 2, 3, 4, 5 and 6 having PMPC network content of 22.84%, 27.15%, 31.05%, 33.46%, 35.13%, and 37.59% in the IPNs, respectively. The IPNs were obtained by swelling PHEMA crosslinked with PEGDMA (0.5% w/w) in the monomer mixtures with MPC concentration of 5%, 10%, 15%, 20%, 25%, 40%, respectively, EGDMA concentration of 5% w/w based on the weight of MPC and following photoinitiated crosslinking polymerization of MPC.

PMPC was incorporated into the IPN, the contact angle was reduced from 43° to 22°. The results revealed that water contact angles decreased when the amount of PMPC network in the IPN increased. These contact angle data agreed with the results of XPS. As determined by XPS, when the amount of PMPC network in the IPN increased, the amount on the surface would increase. Therefore, surface hydrophilicity of the IPN hydrogels increased with the PMPC network content in the IPN.

Protein deposition resistance

Proteins contained in the eye liquids in contact with implanted hydrogel lenses are expected to adsorb very rapidly onto the surface of the hydrogels. This nonspecific adsorption of proteins is uncontrolled and is thought to trigger deleterious reactions of the body, such as foreign body response and fibrous encapsulation. Therefore, *in vitro* protein adsorption was tested to estimate the protein repellency properties.

Protein adsorption resistance of the hydrogels was studied by bicinchoninic acid assay method using BSA as a model protein. The results are summarized in Table III. The amount of protein adsorption onto the IPN hydrogels was dramatically reduced compared to the PHEMA hydrogel and decreased gradually with an increase in the amount of PMPC incorporated into the resultant IPN. The protein adsorption results were in agreement with the water contact angle measurement. The smaller the water contact angle, the greater was the protein repelling ability. The amount of proteins adsorbed on the PHEMA hydrogel was originally 2.32 $\mu g/cm^2$. The lowest BSA adsorption

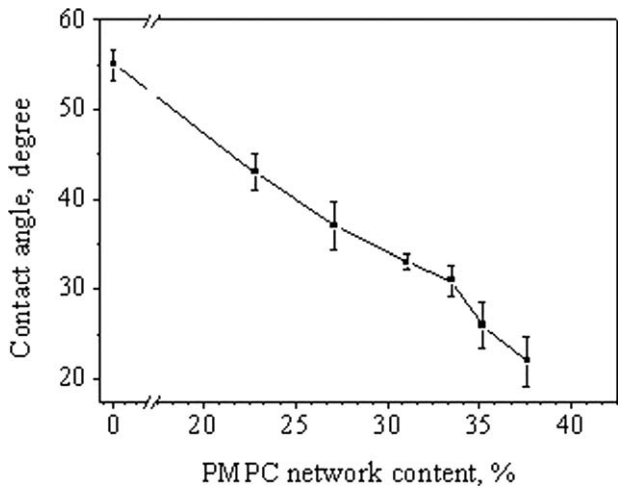

Figure 4 Influence of PMPC network content incorporated into the IPNs on the contact angle of the IPN hydrogels. The IPNs were obtained by swelling PHEMA crosslinked with PEGDMA (0.5% w/w) in the monomer mixtures with MPC concentration of 5, 10, 15, 20, 25, and 40%, respectively, EGDMA concentration of 5% w/w based on the weight of MPC and following photoinitiated crosslinking polymerization of MPC.

TABLE III
BSA Adsorption onto the PHEMA and IPN Hydrogels

Sample	BSA adsorption ($\mu g/cm^2$)
PHEMA hydrogel	2.32 ± 0.08
IPN hydrogel 1[a]	1.34 ± 0.12
IPN hydrogel 2[a]	1.02 ± 0.05
IPN hydrogel 3[a]	0.88 ± 0.09
IPN hydrogel 4[a]	0.64 ± 0.10
IPN hydrogel 5[a]	0.42 ± 0.06
IPN hydrogel 6[a]	0.35 ± 0.04

[a] IPN hydrogels 1, 2, 3, 4, 5 and 6 having PMPC network content of 22.84%, 27.15%, 31.05%, 33.46%, 35.13%, and 37.59%, respectively. The IPNs were obtained by swelling PHEMA crosslinked with PEGDMA (0.5% w/w) in the monomer mixtures with MPC concentration of 5%, 10%, 15%, 20%, 25%, 40%, respectively, EGDMA concentration of 5% w/w based on the weight of MPC and following photoinitiated crosslinking polymerization of MPC.

of 0.35 μg/cm^2 was found for the IPN hydrogel with 37.60% PMPC network in the IPN. The results may be attributed to the fact that the surface hydration, flexibility and mobility of phosphorylcholine group in the MPC played a critical role in reducing protein-surface interactions.[9–11]

CONCLUSIONS

IPN hydrogels based on PHEMA and PMPC have been prepared with improved protein adsorption resistance. The amount of PMPC network incorporated into the IPN became larger with increasing MPC concentration during MPC incorporation and the highest content of PMPC network was about 37.59%. The water content increased with the PMPC network content in the IPN. The ion permeability of IPN hydrogels was dramatically enhanced than the PHEMA hydrogel. Surface analysis, including a decrease in the water contact angle and an increase in the area of P_{2p} peak by XPS, suggested that PMPC was present on the surface of IPNs. The amount of protein adsorption onto the IPN hydrogels was dramatically reduced after incorporation of PMPC network by IPN method. The IPN hydrogels based on PHEMA and PMPC have potential applications as ophthalmic biomaterials.

References

1. Hoffman, A. S. Adv Drug Deliv Rev 2002, 43, 3
2. Jason, S. B.; Catherine, A. M.; Molly, S. S.; Miles, J.; Rajiv, M. Biomaterials 2005, 26, 1741.
3. Abraham, S.; Brahim, S.; Ishihara, K.; Guiseppi-Elie, A. Biomaterials 2005, 26, 4767.
4. Jones, D. S.; McLaughlin, D. W. J.; McCoy, C. P.; Gorman, S. P. Biomaterials 2005, 26, 1761.
5. Lord, M. S.; Stenzel, M. H.; Simmons, A.; Milthorpe, B. K. Biomaterials 2006, 27, 567.
6. Moradi, O.; Modarress, H.; Noroozi, M. J Colloid Interface Sci 2004, 271, 16.
7. Bozukova, D.; Pagnoulle, C.; De Pauw-Gillet, M. C.; Desbief, S.; Lazzaroni, R.; Ruth, N.; Jérôme, R.; Jérôme, C. Biomacromolecules 2007, 8, 2379.
8. Bozukova, D.; Pagnoulle, C.; De Pauw-Gillet, M. C.; Ruth, N.; Jérôme, R.; Jérôme, C. Langmuir 2008, 24, 6649.
9. Sperling, L. H.; Mishra, V. IPN's Around the World; Wiley press: New York, 1997.
10. Sperling, L. H. Polymeric Multicomponent Materials: An Introduction; Wiley press: New York, 1997.
11. Yao, K.; Huang, X. D.; Huang, X. J.; Xu, Z. K. J Biomed Mater Res 2006, 78A, 684.
12. Korematsu, A.; Takemoto, Y.; Nakaya, T.; Inoue, H. Biomaterials 2002, 23, 263.
13. Xu, J. M.; Yuan, Y. L.; Shan, B.; Shen, J.; Lin, S. C. Colloid Surf B 2003, 30, 215.
14. Han, Y. A.; Lee, E. M.; Ji, B. C. Fiber Polym 2008, 9, 393.
15. Willis, S. L.; Court, J. L.; Redman, R. P.; Wang, J. H.; Leppard, S. W.; O'Byrne, V. J.; Small, S. A.; Lewis, A. L.; Jones, S. A.; Stratford, P. W. Biomaterials 2001, 22, 3261.
16. Sibarani, J.; Takai, M.; Ishihara, K. Colloid Surf B 2007, 54, 88.