Supporting Information for

A Facile Bifunctional Strategy for Fabrication of Bioactive or Bioinert Functionalized Organic Surfaces via Amides-initiated Photochemical Reactions

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Experimental Section.

Materials. Commercial PET, BOPP and LDPE films were subjected to ultrasonic cleaning with methanol, followed by Soxhlet extraction with acetone (or methanol) for 12 hr to remove impurities and additives before use. N-Acetylethylenediamine (AED) was obtained from J&K. AEC Staining Kit to detect HRP, SAv, OEGMA (average Mn~300, containing 100 ppm MEHQ and 300 ppm BHT as inhibitor) and HEMA were purchased from Sigma-Aldrich. Before graft polymerization, these monomers were passed through a SiO_2 column to remove the inhibitors. The monomers CBAA and HPMA were synthesized and purified based on the procedures described elsewhere,[2b] and used directly for graft polymerization. The FITC conjugated streptavidin (FITC-SAv) and Cy5 conjugated streptavidin (Cy5-SAv) were obtained from GeneTex and Life Technologies respectively. HRP was purchased from Shanghai Linc-Bio Science. Glutaraldehyde (GA, 25%-28% solution, BR grade) was obtained from Shanghai Shanpu Chemical. PBS and HEPES buffer (pH=7.2~7.4, sterilized) were obtained from Solarbio. Ultrapure water was used in all experiments and was supplied by Milli-Q Advantage A10 (Millipore, USA).

The Synthesis of Bioactive Surfaces by Introducing Primary Amine Groups on Polymer Substrate. The confined photochemical reaction setup was illustrated elsewhere.[4] The main procedures were as follows. A predetermined amount of acetone solution of AED was deposited on the bottom polymer (PET, BOPP or LDPE) film with a microsyringe. The top BOPP film covered this solution and the drop of solution was spread into an even and very thin liquid layer under suitable pressure from a quartz plate. For micropatterning, a metallic photomask was covered onto BOPP film surface to control the irradiation area. This assembly was then irradiated by UV light at room temperature from the topside (a high-pressure mercury lamp, 1000 W). After the irradiation, the bottom polymer film was taken out, and subjected to Soxhlet extraction with acetone for 8 hr to remove surface small molecular reaction products. The resultant substrate was recognized as the aminated surface. In order to measure the surface density of grafted amine groups on the aminated surface, the 2×2 cm modified film was immersed in a freshly prepared 150 μl ninhydrin testing solution (formulated as 6% ninhydrin/ethanol solution containing water and pyridine at the ratio of 1:49 v/v) for 1 min and then heated to 80 °C for 15~20 min. After the solution adequately displayed blue, the blue solution was diluted by 1, 4-dioxane to 5 ml, followed by adding another 5 ml 2-propanol to stabilize the resultant compound. The absorbance at 450-650 nm of this mixture was scanned by a UV-vis spectrophotometer, and the absorbance value at 538 nm was used to correlate with the calibration curve for the determination of surface amine group density (the calibration curve was directly referred to that in previous work, Y. Zhu, C. Gao, X. Liu, and J. Shen, *Biomacromolecules* **2002**, *3*, 1312-1319).

GA Coupling on the Aminated Surface and Subsequent Protein Immobilization. GA was used to react with the aminated surface to prepare an aldehyde-modified surface. Specifically, the aminated surface was incubated in the GA aqueous solution (5% wt in PBS, pH 6.2) at room temperature for 12 hr. The resulting film was then immersed in HEPES buffer of target protein (1 mg/ml) and incubated at room temperature for 2

hr. Thorough washing with HEPES buffer (pH 7.4) (5 min × 3) was performed to remove the non-specific adsorption. The immobilized HRP was revealed under optical microscope after AEC staining and the immobilized FITC-SAv was observed directly under fluorescent microscope.

DMF-initiated Photografting of Anti-fouling Polymers on Polymer Film. The apparatus used to perform the photograft polymerization was similar to the aforementioned setup used in the amination on polymer surface. The main procedures were as follows. A predetermined amount of DMF solution of anti-fouling monomers including OEGMA, HEMA, CBAA or HPMA was deposited on the bottom polymer (PET, BOPP or LDPE) film with a microsyringe. The top film (BOPP or LDPE) covered this solution and the drop of solution was spread into an even and very thin liquid layer under suitable pressure from a quartz plate. Then such a sandwich assembly was irradiated by UV light at room temperature from the topside (a high-pressure mercury lamp, 1000 W). For micropatterning, a metallic photomask was placed on the BOPP film surface to control the photografting area. After the irradiation, the two films were taken out, separated, dried to constant weight, and then subjected to Soxhlet extraction with methanol overnight to remove homopolymers. Four parameters, grafting percent (GP), grafting efficiency (GE), total monomer conversion percent (CP) and grafting conversion percent (CG) were determined according to the following equations, where M_G is the mass of the grafted polymer, M_P is the mass of the polymer formed (the total mass of the graft polymer and homopolymer), M_S is the mass of blank polymer substrate, M_M is the mass of the monomer.

$$GP = (M_G/M_S)\ 100\% \quad (1)$$

$$GE = (M_G/M_P)\ 100\% \quad (2)$$

$$CP = (M_P/M_M)\ 100\% \quad (3)$$

$$CG = (M_G/M_M)\ 100\% \quad (4)$$

Evaluation of Protein-resistant Behaviors on Anti-fouling Polymer Grafted Surfaces. A micropatterned anti-fouling polymer grafted surface was firstly achieved by using a photomask-controlled photografting polymerization on polymeric substrate. The resultant micropatterned grafted surface was then incubated in a protein solution (1 mg/ml in HEPES, pH 7.4) to check its anti-fouling features. In order to remove any non-specific adsorption, the surface after incubation was rinsed with HEPES buffer (5min × 3). The possible adsorption of HRP onto the surface background was checked through AEC staining followed by observation under optical microscope, on the other hand, the possible adsorption of Cy5-SAv on the surface background was checked directly under fluorescent microscope.

Characterization. X-ray photoelectron spectra (XPS) were obtained with AXIS ULTRA from Kratos Analytical Ltd., the energy resolution is 0.48eV/(Ag3d5/2), 0.61eV(C1s). Optical observations including bright field and fluorescent modes were carried out on a Nikon T*i*-U (Tokyo, Japan) and the laser scanning confocal microscope TCS SP5 (Leica, Germany) respectively. The FITC fluorophor was excited by the blue light excitation on Nikon T*i*-U, and the Cy5 fluorophor was excited by the 633 nm laser from TCS SP5 (633 nm, 33%; the channel voltage, 806.1V; zoom 1.5). The intensities of the fluorescence microscopic images along a line across the bright and dark regions were recorded by the integrated software equipped in Nikon T*i*-U (the pixel intensity for FITC-SAv) and Image J (gray value for Cy5-SAv) respectively. ATR-FTIR spectra were recorded on a Vetex 70v (Bruck).

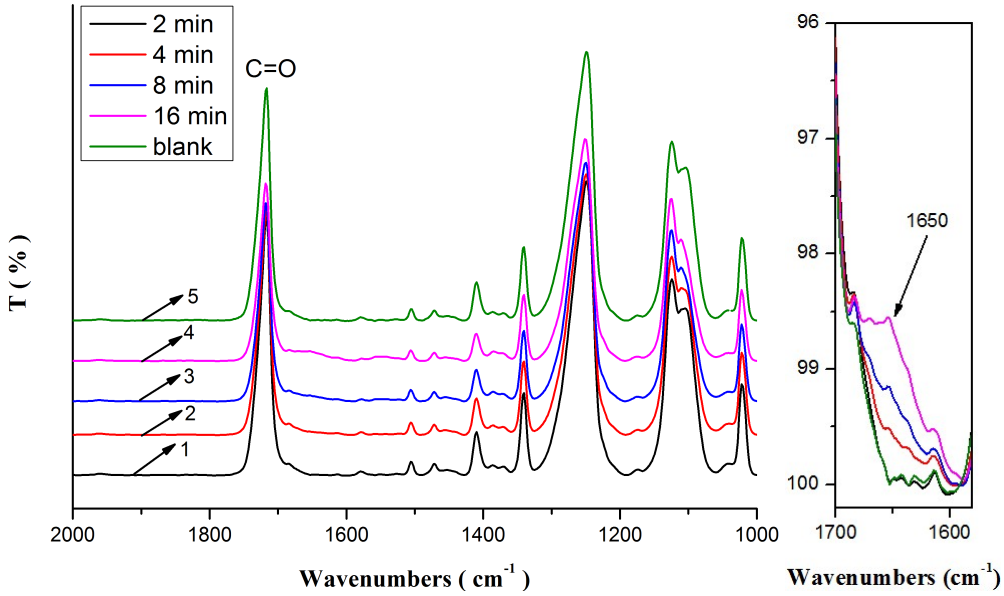

Figure S1. The ATR-FTIR spectra on the aminated PET substrates with different irradiation times: (1) 2 min (2) 4 min (3) 8 min (4) 16 min (5) virgin PET before irradiation. With increasing the irradiation time (e.g. from 2 min to 16 min), the intensity of the absorption band at 1650 cm^{-1} assigned to C=O group enhanced gradually, which reflected that more amide/amine structures were formed.

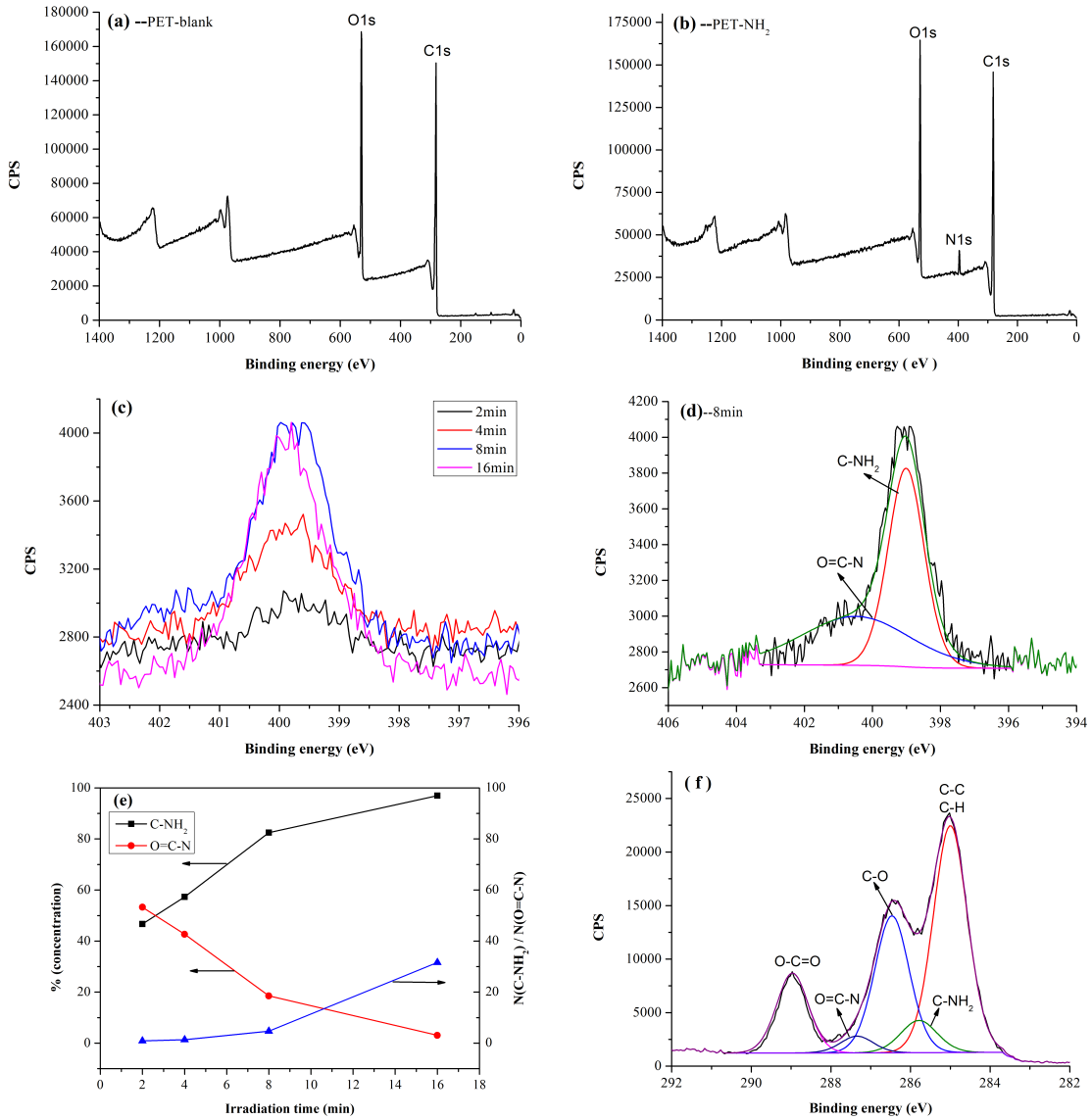

Figure S2. The XPS spectra of the aminated PET surfaces. (a) the survey on the virgin PET substrate before the modification; (b) the survey on the aminated PET substrate; (c) a collection of high-resolution N1s regional scans with different irradiation times; (d) a typical deconvoluted N1s regional scan at the irradiation time being 8 min to show two fine structures as N (O=C-N) and N (C-NH_2); (e) the effect of irradiation time on the concentration of the two fine structures analyzed from (d) as N (O=C-N) and N (C-NH_2), for clarity, the concentration ratio of the two fine structures was also plotted; (f) a typical deconvoluted C1s regional scan at the irradiation time being 8 min to show five fine structures as C (O-C=O), C (O=C-N), C (C-O), C (C-NH_2), and C (C-C, C-H).

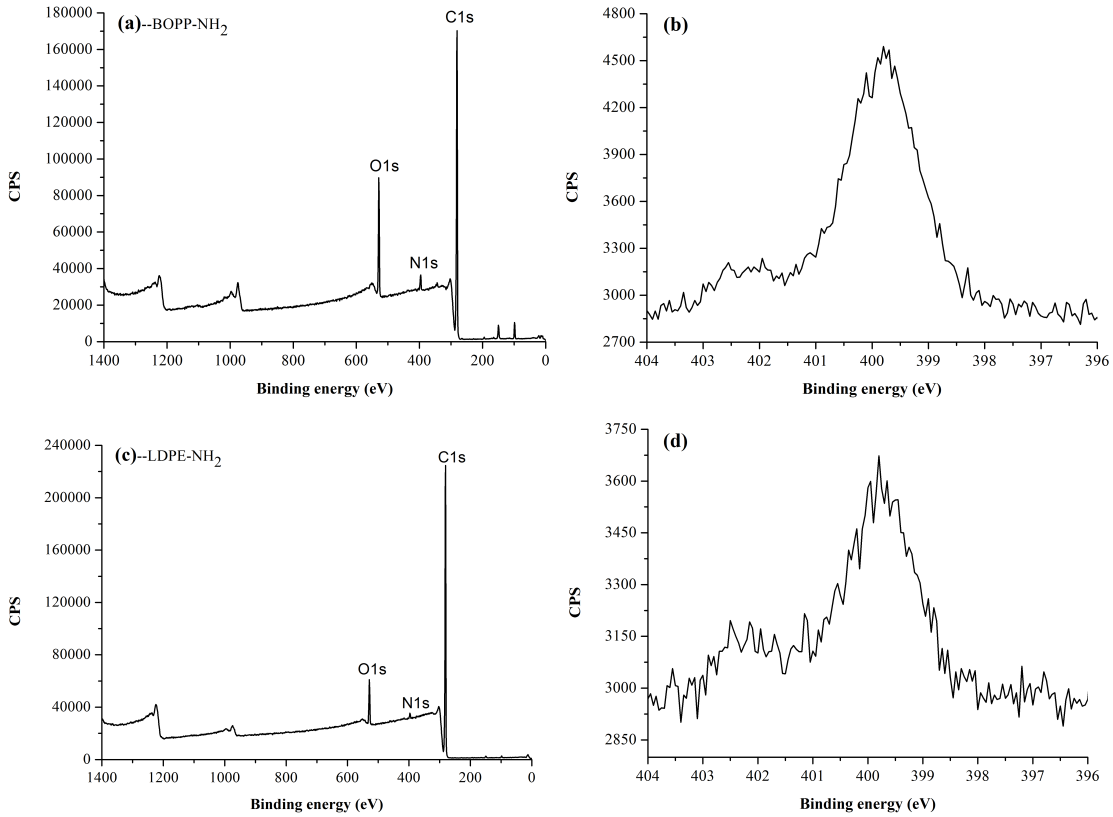

Figure S3. The XPS spectra of the aminated BOPP (a, b) and LDPE (c, d) surfaces. (a) and (c) the survey spectra; (b) and (d) the high-resolution N1s regional scan. The nitrogen concentration on the modified BOPP and LDPE surfaces were 2.3% and 0.81% respectively.

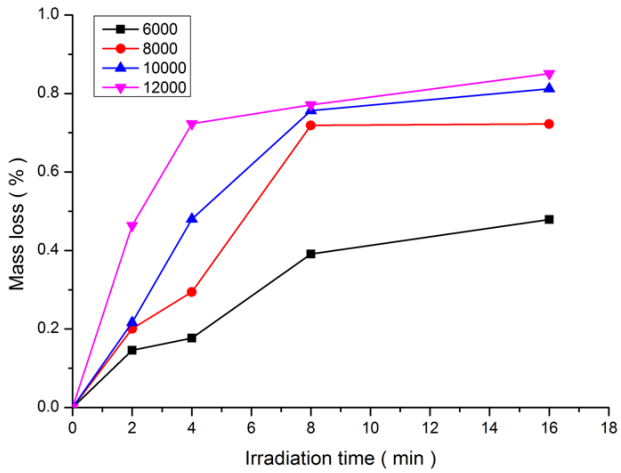

Figure S4. The monitoring on the amination process of PET surface through investigating the effect of irradiation time and UV intensity ($\mu W/cm^{-2}$) on the mass loss of PET film.

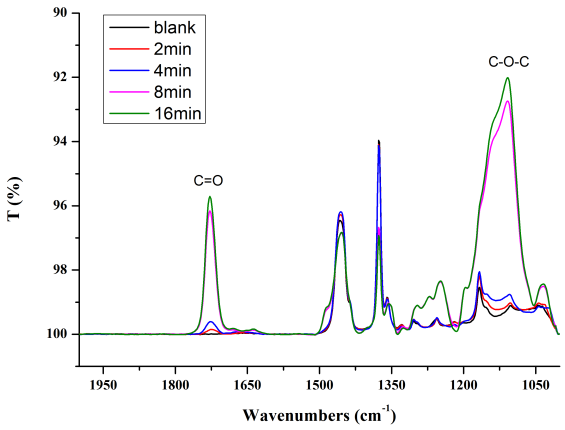

Figure S5. The ATR-FTIR spectra on the BOPP surface before and after photografting of POEGMA layer with different irradiation time. The peak intensity of ester bond (C=O and C-O-C) increased with prolonging the irradiation time.

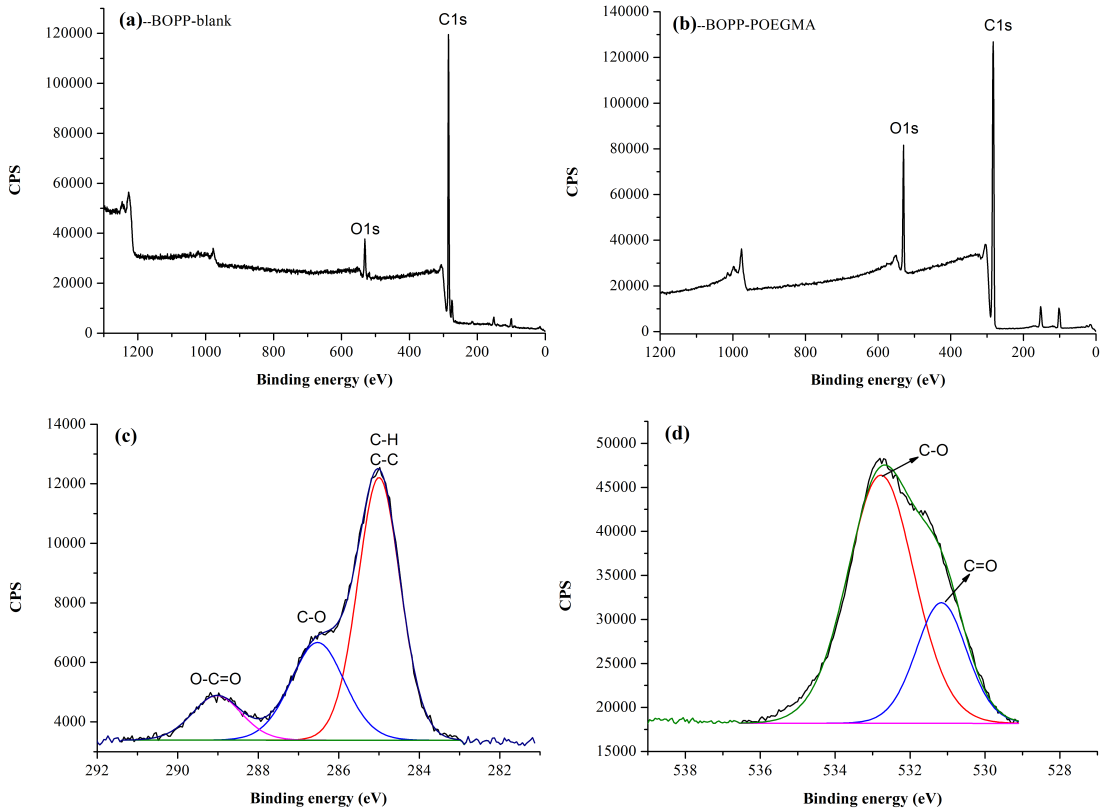

Figure S6. The XPS spectra on the BOPP surface before and after photografting of POEGMA layer. (a) the survey on the virgin BOPP before photografting; (b) the survey on the POEGMA grafted BOPP surface; (c) the deconvoluted high-resolution C1s regional scan to show three fine structures as C (O-C=O), C (C-O) and C (C-C/C-H); (d) the deconvoluted high-resolution O1s regional scan to show two fine structures as O (C-O) and O (C=O).

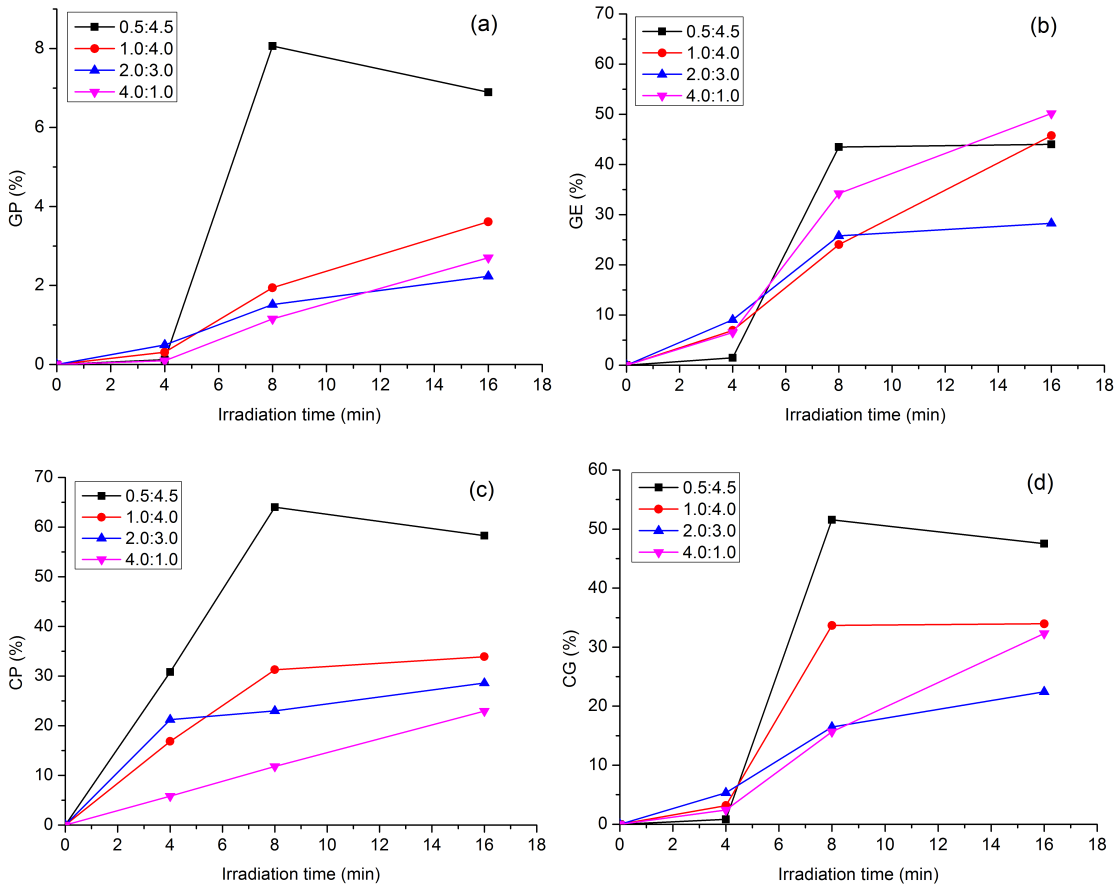

Figure S7. The controlling on the photografting parameters as GP (a), GE (b), CP (c) and CG (d) by UV irradiation time and the ratio of DMF to OEGMA. The substrate used was PET.

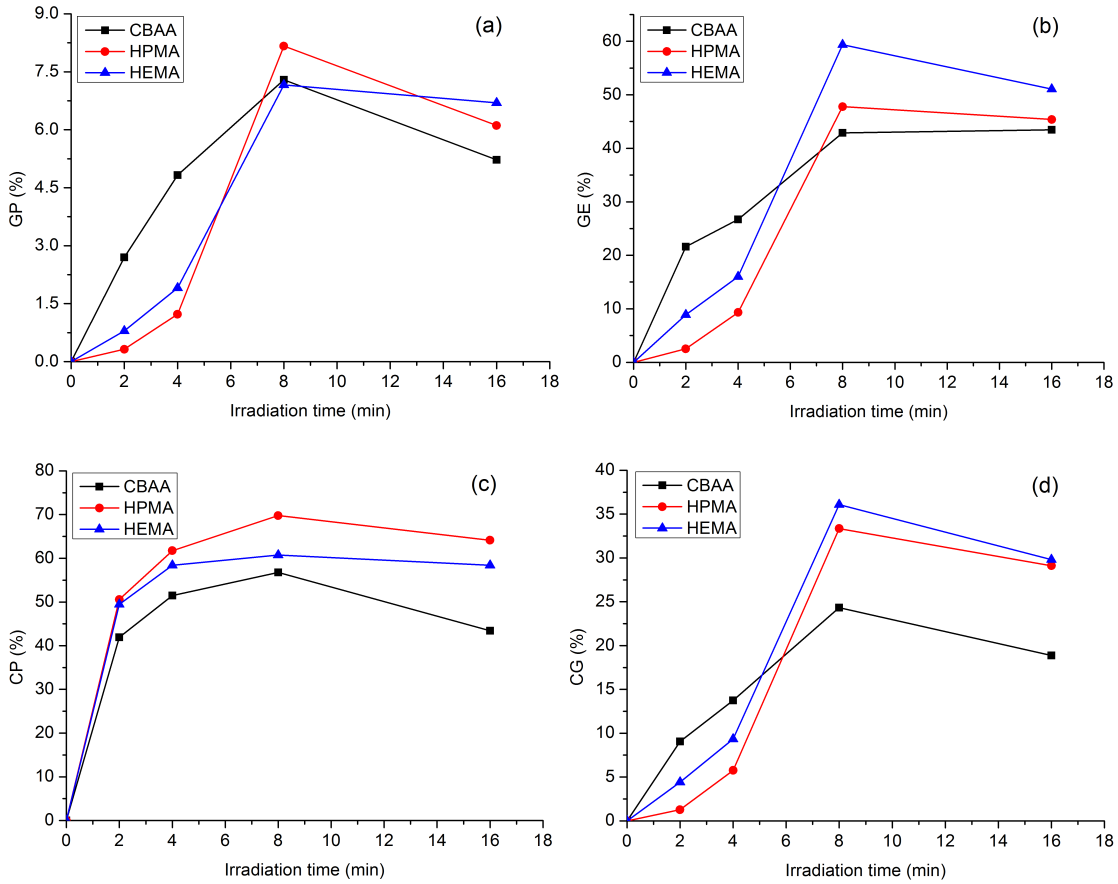

Figure S8. The controlling on the photografting parameters as GP (a), GE (b), CP (c) and CG (d) by UV irradiation time (the ratio of DMF to monomer is 0.5:4.5). The substrate used was PET, and the monomers used were CBAA, HPMA and HEMA respectively.

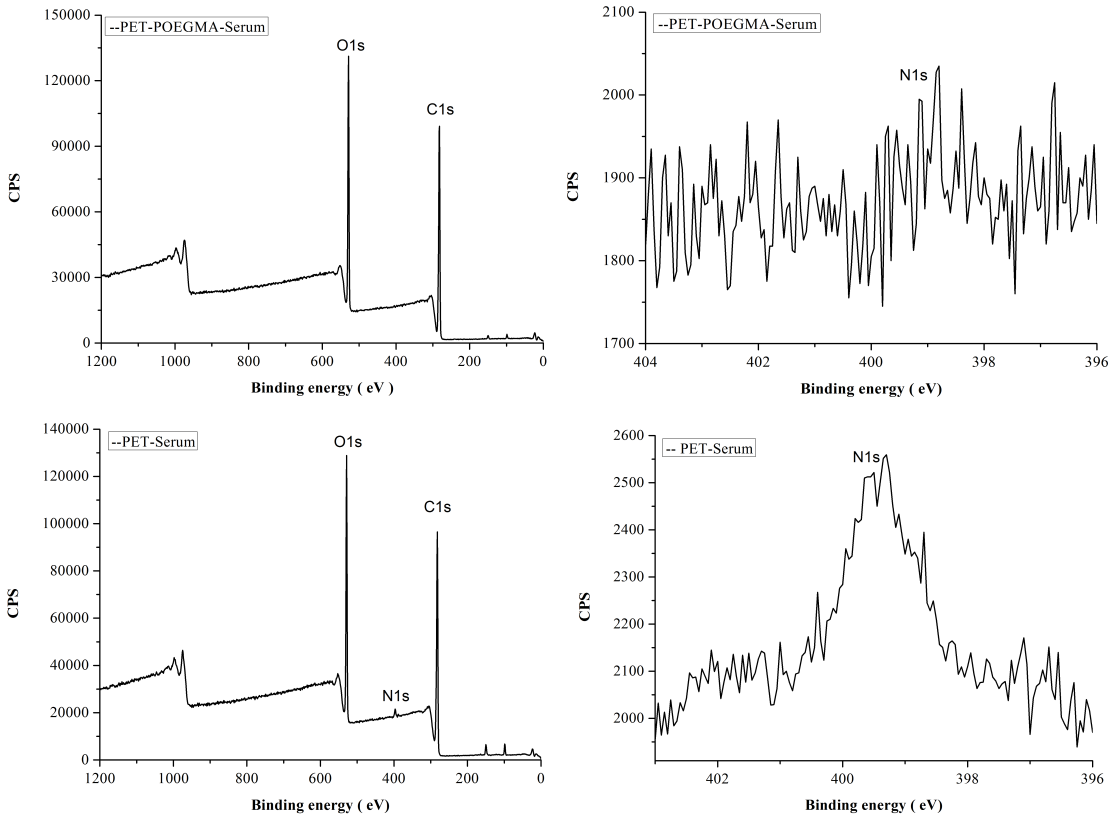

Figure S9. The XPS spectra on the PET surfaces with and without POEGMA grafts after incubation in undiluted serum. The top two figures showed the full scan and high-resolution N1s signal from POEGMA-grafted PET surface after incubation in undiluted serum, and the bottom two figures showed the full scan and high-resolution N1s signal from naked PET surface without POEGMA grafts after incubation in undiluted serum. The comparisons clearly reflected an excellent non-fouling capability on POEGMA-grafted PET surface, which did not show any detectable N1s signal from full scan and high-resolution spectra.