Journal Name

Dynamic Article Links►

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Supporting Information

An Extremely Simple Method for Fabricating 3D Protein Microarrays with an Anti-Fouling Background and High Protein Capacity

Zhifeng Lin[a], Yuhong Ma[b], Changwen Zhao*[a], Ruichao Chen[a], Xing Zhu[a], Lihua Zhang[a], Xu Yan[a], Wantai Yang*[a,b]

[a]State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, 100029, China

[b]Key Laboratory of Carbon Fiber and Functional Polymers, Ministry of Education, College of Materials Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China

Surface Characterization.

Surface characterization by UV. In order to confirm the forming of LDPE surface photo-initiators and their still existence on the surface after first grafting polymerization, the qualitative analysis by UV spectroscopy was conducted as shown in Figure 1S. The blank LDPE presented no absorption bands in the UV-visible region. After immobilized the photo-initiators which with semipinacol dormant species onto the LDPE films, the UV-visible spectra of LDPE-ITXSP presented two absorption bands, one was extended from 250 - 330 nm in the ultraviolet rays range, another was extended from 380 - 410 nm in the visible light range. These absorption bands were related to the semipinacol dormant groups on the LDPE films. After grafting PEGMEMA on the LDPE-ITXSP, the absorption bands on the same regions still existed but the absorption intensity decreased, which meant the LDPE-g-(PEG brushes) could be re-initiated in lower surface photo-initiators concentrations.

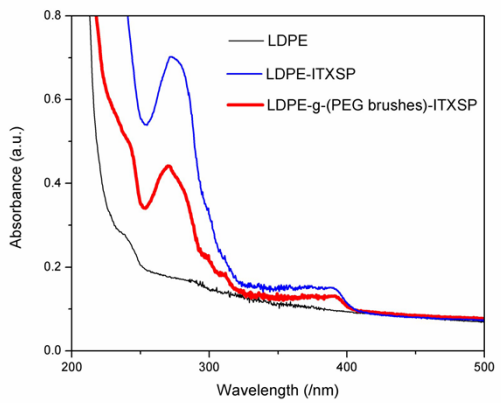

Figure 1S. UV-visible absorption spectra of the blank LDPE, ITX modified LDPE, and PEGMEMA grafting modified LDPE.

Surface characterization by FTIR. To investigate the chemical characteristics of LDPE, LDPE-ITX, LDPE-g-(PEG brushes) and LDPE-g-(PEG brushes)-g-(functional gel), ATR-FTIR spectroscopy was employed. As show in Figure 2S, four films displayed three common absorption bands at around 1460cm-1, 2850cm-1, 2900cm-1 which were attributed to C-H bonds from LDPE support. Two spectra of LDPE and LDPE-ITXSP presented little difference due to the low level of ITXSP coupled on LDPE. Two spectra after polymer grafting, which were LDPE-g-p(PEGMEMA) and LDPE-g-p(PEGMEMA)-g-p(PEGDA/GMA) films, have two common absorption bands at around 1730cm-1 which is attributed to carbonyl bonds(C=O) and around 1200cm-1 which is attributed to C-O-C stretching bonds. LDPE-g-p(PEGMEMA)-g-p(PEGDA/GMA) film showed higher intensity of these bands than LDPE-g-p(PEGMEMA). Besides, it appeared a clear peak at around 900cm-1 which is attributed to epoxy bond. Based on these results, it was demonstrated that PEG brushes were grafted onto LDPE and functional gels were grafted onto the surface of LDPE-g-(PEG brushes) successfully.

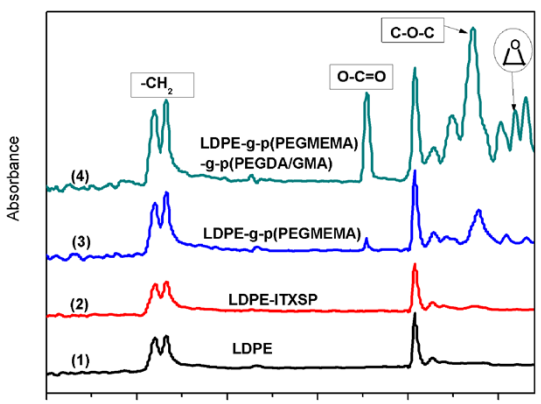

Figure 2S. ATR-FTIR spectra of (1) blank LDPE film, (2) LDPE film coupled with semipinacol radicals, (3) PEG brushes functionalized LDPE film by visible light-induced grafting 0.3M PEGMEMA polymerization for 30min, (4) double-modified LDPE film by visible light-induced grafting 0.6M PEGDA/GMA copolymerization for 60min on LDPE-g-(PEG brushes).

Surface characterization by XPS. The elemental composition of surface was investigated by measuring the XPS spectra. Table 1S showed the atomic concentration of C, O, N (%) and peaks components percent of C 1s core-level spectra. The [C/O] ratio of LDPE-g1-g2-protein is 3.04, it also appears the signal of N(398.4) and the concentration of it increase to 2.7 % from 0 %, indicating the protein was immobilized onto the surface successfully. Meanwhile, the components changed significantly after protein was immobilized. The O-C=O species content increases to 7.3 %, O-C species content decreases while C-C species content increases, also indicating the immobilization of protein.

Table 1S. Atomic concentration of C, O, N (%) and peaks components percent of C 1s core-level spectra (%) by XPS

XPSsignals/peaks(eV)	LDPE	LDPE-ITXSP	LDPE-*g*1	LDPE-*g*1-*g*2	LDPE-*g*1-*g*2-protein
C(285)	79.9	85.03	70.88	72.32	73.2
O(533)	20.1	14.97	29.12	27.68	24.1
N(398.4)	0	0	0	0	2.7
C-Si(282.4)	6.75	1.21	3.01	2.13	1.0
C-C(285)	66.41	83.93	34.97	39.38	52.8
C-O(286.5)	23.61	13.91	61.61	54.56	38.9
O-C=O(289)	3.23	0.95	0.42	3.93	7.3

Surface characterization by water contact angle and grafting yield. Water contact angles and grafting yields of these films were measured and shown in Table 2S. The LDPE and LDPE-ITXSP films were hydrophobic with water contact angles of 103.0 ° and 102.0 °, and the weights difference of two films could not be measured precisely since only a small amount of ITX was coupled. After 30 minutes of irradiation by visible light, the LDPE-g1 film is hydrophilic with a contact angle of 37.3 °, and the grafting yield is 33.33μg/cm^2. Then, under the irradiation of visible light for 60 minutes, the LDPE-g1 film was reinitiated and grafted with func-tional hydrogel. And the LDPE-g1-g2 film is hydrophilic with a contact angle of 80.6 °, and the grafting yield is 339.80μg/cm^2. The significant change in the water contact angle and film weight also indicated the successful grafting of p(PEGMEMA) and p(PEGDA/GMA) on LDPE surface.

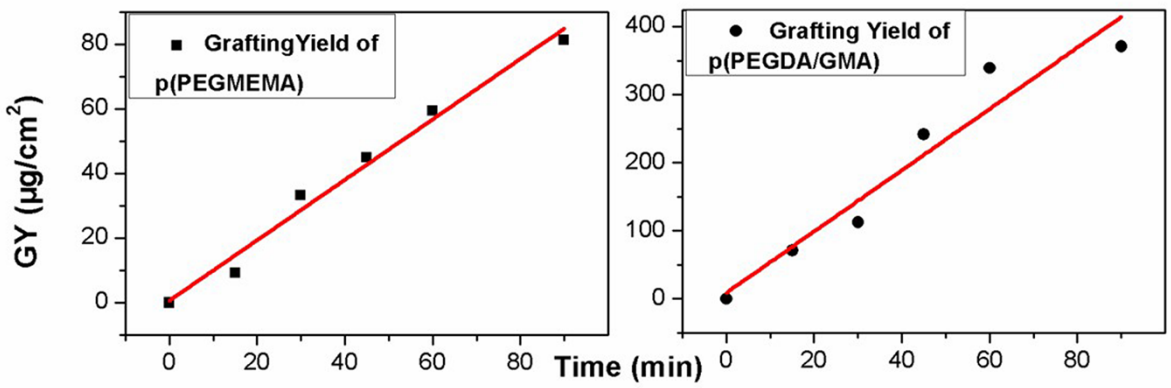

Figure 3S. Grafting yields of p(PEGMEMA) on LDPE-ITXSP film and p(PEGDA/GMA) on LDPE-g1 films at different surface-initiated grafting time. Grafting yield (GY) is defined as GY=(Wb-Wa)/A, where Wa and Wb represent the weight of the dry films before and after grafting, A represents the film area(9cm^2).

The growth kinetics of polymer or copolymer. The growth kinetics of polymer or copolymer from the LDPE-ITXSP or LDPE-g1 films via surface-initiated grafting polymerization was investigated by grafting yield. Approximately liner increases in GY of p(PEGMEMA) and p(PEGDA/GMA) grafted onto LDPE were shown in Figure 3S. The grafting yield of p(PEGMEMA) growth from 9.38 μg/cm^2 to 81.33 μg/cm^2 with the time increased from 15 min to 90 min, the grafting yield of p(PEGDA/GMA) growth from 70.81 μg/cm^2 to 370.10 μg/cm^2 with the time increased from 15 min to 90 min, which suggest that the chain growths from LDPE and LDPE-g1 films were consistent with a controlled/living process. The growth of p(PEGDA/GMA) is much faster than p(PEGMEMA) since PEGDA and GMA have much higher reactivity than PEGMEMA.

Table 2S. Reaction Time, Grafting Yield (GY), Static Water Contact Angle (C.A) of the LDPE, LDPE-ITXSP, LDPE-g1, LDPE-g1-g2 films.

Sample	Step 2		Step 3		C.A(°)
	T(min)	GY(μg/cm^2)	T(min)	GY(μg/cm^2)	
LDPE	-	-	-	-	103
LDPE-ITXSP	-	-	-	-	102
LDPE-*g*1^1	15	9.38	-	-	54.6
LDPE-*g*1^2	30	33.3	-	-	37.3
LDPE-*g*1^3	45	44.99	-	-	36.4
LDPE-*g*1^4	60	59.53	-	-	38.2
LDPE-*g*1^5	90	81.3	-	-	37.9
LDPE-*g*1-*g*2^1	30	33.3	15	70.81	42.4
LDPE-*g*1-*g*2^2	30	33.3	30	112.82	46.3
LDPE-*g*1–*g*2^3	30	33.3	45	241.96	72.1
LDPE-*g*1-*g*2^4	30	33.3	60	339.8	80.6
LDPE-*g*1-*g*2^5	30	33.3	90	371.1	83.1

Research of Protein incubation time.

The protein was immobilized onto the substrate as Scheme1 showed. The immobilized kinetics of protein (IgG soultion, 0.5 mg/mL) on substrate was investigated by florescence intensity. 6 groups of protein microarrays which respectively incubated for 0 h, 1h, 2 h, 4 h, 6 h and 12 h were carried out. As Figure 4S showed, the florescence intensity growth from 0 to 120 with the incubate time increased from 0 to 2 h. After the proteins were incubated for 1 h, the value of florescence intensity was 63 and it was not the biggest value, indicating protein was immobilizing onto the substrate and a part of epoxy groups participated in the reaction. The florescence intensity maintained a top value with incubate time increased from 2 h to 12 h, indicating all of epoxy groups thoroughly combined with protein. According to the above, two hours' protein incubation time is long enough for the protein immobilization.

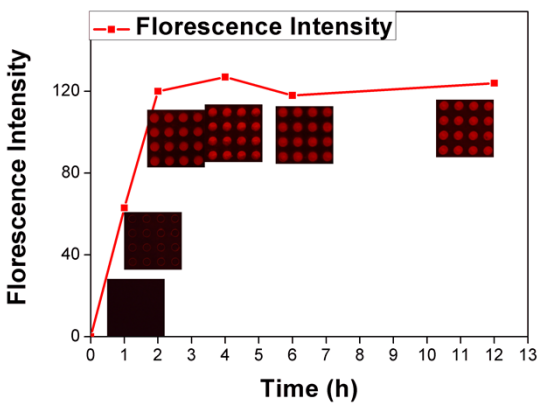

Figure 4S. Florescence intensity of protein microarrays with different protein incubation time.