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ORIGINAL PAPER

Polymeric Linoleic Acid–Polyolefin Conjugates: Cell Adhesion and Biocompatibility

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Abstract To diversify edible-oil polymer composite, polymeric linoleic acid (PLina) peroxide was obtained by the auto-oxidation of linoleic acid in a simple way for use as a macroinitiator in free radical polymerization of vinyl monomers. Peroxidation, epoxidation, and/or perepoxidation reactions of linoleic acid under air at room temperature resulted in PLina, having soluble fraction more than 91 weight percent (wt%), with molecular weight ranging from 1,644 to 2,763 Da, and containing up to 1.0 wt% of peroxide. PLina initiated the free radical polymerization of ether styrene (S), methyl methacrylate (MMA), or *n*-butyl methacrylate (*n*BMA) to give PLina-g-polystyrene (PS), PLina-g-poly-MMA (PMMA), and PLina-g-poly-*n*BMA (P*n*BMA) graft copolymers. The polymers obtained were characterized by proton nuclear magnetic resonance (^{1}H NMR), thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC) techniques. Microstructure of the graft copolymers was observed by using scanning electron microscope (SEM). Graft copolymers obtained contained polymeric linoleic acid in a range between 8.5 and 19.3 mol percent (mol%). PLina-g-PS, PLina-g-PMMA and PLina-g-PnBMA graft copolymer samples were also used in cell culture studies. Fibroblast and macrophage cells were strongly adhered and spread on the copolymer film surfaces. These newly synthesized copolymers were tested for their effects on human blood protein adsorption compared with PMMA graft copolymers containing polymeric soybean oil and polymeric linseed oil; interestingly we observed a dramatic decrease in the protein adsorption on the linoleic acid graft copolymer, which is important in tissue engineering.

Keywords Polymeric linoleic acid · Macroinitiator · Graft copolymer · Styrene · Methyl methacrylate · *n*-butyl methacrylate · Fibroblast cell · Macrophage cell · Protein adsorption · Blood compatibility

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Introduction

Today, natural oils and fats are considered to be the most important class of renewable resources for the production of biodegradable polymers in two ways. The first is the production of poly(3-hydroxyalkanoates) (PHAs) as an energy reserve material by some microorganisms by using plant and fish oils [1, 2]. The second is direct polymerization of the oils; for example, a copolymerization with divinyl benzene and styrene leading to thermoset copolymers [3], or by polymerization of vinyl [4], maleic anhydride [5], glycidyl ether [6], and norbornyl [7] derivatives of the oil. We recently performed grafting reactions of monomers on naturally occurring peroxidized polymeric drying oils such as linseed oil and soybean oil [8, 9].

In tissue engineering, cell adhesion and spreading on a surface are the most indicative processes to assess the biocompatibility of a synthetic polymer. Because of their strong ability to adhere on different polymeric surfaces, L-929 fibroblast cells are the main cell type widely used in biocompatibility studies. Macrophages are important components of the mammalian immune system as professional antigen-presenting cells and nonspecific killers of a wide variety of pathogens [10]. The biocompatibility of polymeric material can be inferred by studying the protein adsorption on this polymer. The first body reaction after implanting a polymer is protein adsorption. The adsorbed proteins will determine later body reactions and finally determine whether the material will be accepted or rejected by the body. Surface chemical structures as well as surface morphology can mediate protein adsorption behavior [11].

Our latest research was on the synthesis and biocompatibility studies of graft copolymers of the naturally peroxidized edible oils [8, 9]. In a continuation of this work, a polymeric peroxide was obtained from auto-oxidation of linoleic acid, and its graft copolymers with vinyl monomers were prepared. These newly synthesized graft copolymers were tested for their biocompatibility and effects on human blood protein adsorption compared with the edible-oil graft copolymers.

Experimental

Materials

Linoleic acid (*cis, cis* 9,12 octadecadienoic acid) was supplied from Fluka as 70 weight percent (wt%) and used as received. Ether styrene (S), methyl methacrylate (MMA), and *n*-butyl methacrylate (*n*BMA) were supplied from Aldrich and freed from inhibitor by vacuum distillation over calcium hydride (CaH_2). Polymeric linseed oil (PLO) peroxide and polymeric soybean oil (PSB) peroxide graft copolymers of poly-MMA (PMMA), PLO-g-PMMA and PSB-g-PMMA, were present in our laboratory from our recent works [8, 9]. Human albumin γ-globulin was supplied by Sigma; fibrinogen was supplied from Fluka. All other chemicals were reagent grade and used as received.

Formation of Polymeric Linoleic Acid Peroxide Under Ambient Conditions

For the formation of polymeric linoleic acid (PLina) peroxide , 5.0 g of linoleic acid spread out in a Petri dish (ϕ = 5 cm) was exposed to sunlight in the air at room temperature. After a given time, polymeric linoleic peroxide, a pale-yellow viscous liquid, was formed as a mixture of soluble and gel polymer. Chloroform extraction of the crude polymeric linoleic acid for 24 h at room temperature allowed separation of a soluble part of the polymeric linoleic acid from the gel. The results and conditions of polymer formation from linoleic acid are listed in Table 1. Because gel polymer was less than 10 wt%, soluble polymeric linoleic acid (more than 90 wt%) was used throughout in this work and is referred to as PLina.

Peroxygen Analysis

Peroxide analysis of PLina fractions was carried out by refluxing a mixture of 2-propanol (50 ml)/acetic acid (10 ml)/saturated aqueous solution of potassium iodide (KI) (1 ml) and 0.1 g of the polymeric sample for 10 min and titrating the released iodine against thiosulfate solution according to the literature [12]. Peroxygen contents of the PLina samples varied from 0.5 to 1.0 wt%.

Table 1 Auto-oxidation of linoleic acid with air oxygen (O_2) at ambient laboratory conditions

Run	Linoleic acid (g)	Exposure time (day)	Polymeric linoleic acid (PLina) peroxide				
			Total yield (g)	Weight percent (wt%)	–OO–(wt%)	Molecular weight	
						M_w	MWD
65-1	5.13	30	5.13	96.70	1.0	1,869	1.15
65-2	5.00	40	5.05	95.47	1.0	1,796	1.16
65-3	5.02	50	5.02	95.64	1.0	1,644	1.15
65-4	5.01	60	5.01	91.03	0.7	1,684	1.22
65-5	5.08	82	5.02	94.28	0.6	2,096	1.39
65-6	5.01	100	5.02	96.38	0.5	2,763	1.20

–OO– peroxide group, M_w weight average molecular weight, *MWD* molecular weight distribution

Graft Copolymerization of PLina With Vinyl Monomers

A given amount of PLina and S, MMA, or *n*BMA were charged separately into a Pyrex tube. Argon was introduced through a needle into the tube for about 3 min to expel the air (Table 2). The tightly capped tube was then put in a water bath at 80°C. After the required time, the contents of the tube were coagulated in methanol. The graft copolymer samples were dried overnight under vacuum at 30 °C.

Purification of the Graft Copolymers

In a typical purification procedure via fractional precipitation [13], 0.5 g of polymer sample was dissolved in 10 ml of chloroform ($CHCl_3$). Methanol was used as a nonsolvent and kept in a 50-ml buret. Afterward, methanol was added to the polymer solution with continuous stirring until the polymer began to precipitate. At this point, γ value is simply a ratio of fractional precipitation and calculated by taking the volume ratio of the nonsolvent (methanol) consumed to the solvent (chloroform, 10 ml). The polymer fractionated was dried under vacuum at room temperature.

Polymer Characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in $CHCl_3$ at 17°C with a tetramethylsilane internal standard using a 400 MHz NMR AC 400 L. Molecular weight of the polymeric samples was determined by gel permeation chromatography (GPC) with a Waters model 6000A solvent delivery system with a model 401 refractive index detector and a model 730 data module and with two Ultrastyragel linear columns in series. Chloroform was used as the elution at a flow rate of 1.0 ml min^{-1}. A calibration curve was generated with polystyrene standards. Differential scanning calorimetry (DSC) thermograms were obtained on a Netzsch DSC 204 with CC 200 liquid nitrogen cooling system to determine the glass transition temperatures (T_g), and thermal gravimetric analysis (TGA) of the polymers obtained were performed on a PL TGA 1500 instrument to determine thermal degradation. For DSC analysis, after cooling to –100°C, samples were heated to 200°C at a rate of 10°C/min.

Scanning Electron Microscope (SEM)

Scanning electron micrographs were taken on a JEOL JXA-840A scanning electron microscope (SEM). The specimens were frozen under liquid nitrogen, then fractured, mounted, and coated with gold (300 Å) on an Edwards S 150 B sputter coater. The SEM was operated at 15 kV, and the electron images were recorded directly from the cathode ray tube on a Polaroid film. The magnification employed was varied up to 15,000×; however, 1,000, 2,000, and 3,000 magnifications were useful.

Cell Culture and Cell Adhesion Studies

The murine fibroblast cell line (L-929) and macrophage cell line (RAW 264.7) were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cell culture stock solution (RPMI-1640), which contained 10% (v/v) heat-inactivated fetal bovine serum with 100 U/ml penicillin and 100 μg/ml streptomycin, was supplied by Gibco (Invitrogen Corp, Carlsbad, CA, USA). In a typical cell culture study, a four-well chamber slide was coated with the graft copolymer film (thickness 1 mm) by means of chloroform solution casting. The polymer-film-coated chamber slide was sterilized by using ethylene oxide. A 1-mm sample of cell culture containing

Table 2 Polymerization of ether styrene (S), methyl methacrylate (MMA), and *n*-butyl methacrylate (*n*BMA) initiated by polymeric linoleic acid (PLina) (sample 65-4 in Table 1) at 80°C

Run	PLina (g)	Styrene (g)	MMA (g)	*n*-BMA (g)	Polym time (h)	Graft copolymer		Molecular weight		[PLina] in copolymer (mol%)
						Total yield, g	Soluble, wt%	$M_w \times 10^4$	MWD	
68-1	0.7520	3.0	–	–	6	1.36	98.51	17.5	2.2	
68-2	1.5004	3.0	–	–	6	1.69	98.38	10.2	2.1	10.6
68-4	3.0024	3.0	–	–	6	2.35	98.30	6.4	8.4	9.3
67-1	0.7544	–	3.089	–	6	2.14	97.29	23.5	2.1	
67-2	1.5015	–	3.089	–	6	4.22	96.93	15.7	2.0	9.7
67-4	3.0021	–	3.089	–	6	2.11	95.89	10.5	1.8	19.3
69-2	1.5035	–	–	3.0	8	3.27	97.05	18.8	1.9	10.7
69-4	3.0042	–	–	3.0	8	3.32	96.77	9.8	1.9	8.5

M_w weight average molecular weight, *MWD* molecular weight distribution

1×10^5 L-929 (or 0.5×10^5 RAW 264.7) cells was poured into the polymer-coated chamber slide and incubated at 37 °C under humidified air containing 5% (v/v) CO_2. For a given time, the cells on the polymer surface were observed with an inverted microscope at the phase contrast mode (Nikon Eclipse TE 300, Tokyo, Japan) and photographed with a Minolta Dimage 7i camera (magnification 400×).

Human Blood Protein Adsorption Test

Human albumin, γ-globulin, and fibrinogen were used to study the adsorption behavior of proteins on surfaces of polymer samples. Small disks (1.5 cm in diameter) of the polymer films were prepared using a punch and immersed in protein solutions containing 1 mg/ml of phosphate buffered saline (PBS) (pH 7.3–7.4) at 37°C for 1 h. The disks were then recovered and changed in the protein concentrations of the solution-borne proteins determined using an ultraviolet (UV) spectrophotometer [14]. Protein adsorbed on the copolymer surface was calculated. Adsorbed protein calculated (μg/ml) = 1.55 × absorbance of protein solution (λ = 280 nm) – 0.76 × absorbance of protein solution (λ = 260 nm) Adsorbed protein on the copolymer was calculated from the difference between UV absorbencies of the standard solution and the solution after protein adsorption on the polymer disk (experimental readings in each run were seven). Obtained values were divided by the disk area (μg/cm^2).

Results and Discussion

Polymeric-Linoleic-Acid-Containing Peroxide Groups

PLina peroxide was obtained from linoleic acid auto-oxidized with air oxygen at room temperature for 8 weeks. Oxidation of linoleic acid in the air involves hydrogen abstraction from a methylene group between two double bonds in a polyunsaturated fatty acid chain [9, 15]. Auto-oxidation processes are very complex, and this leads to peroxidation, perepoxidation, hydroperoxidation, epoxidation, and then cross-linking via radical recombination. The reaction pathways can be designed as in Scheme 1. PLina samples were mostly viscous liquid having cross-linked part less than 9 wt%, which was isolated with the chloroform extraction. The cross-linked polymer ranged between 3 wt% and 9 wt%. Each linoleic acid repeating unit in the PLina was estimated to have one peroxide group using peroxide content. Interestingly, gel contents of PLina graft copolymers was very low, whereas the gel contents of PSB and PLO graft copolymers [8, 9] were very high. The results and conditions of the formation of peroxidized polymeric linoleic acid are listed in Table 1. The molecular weight of the samples ranged between 1,644 Da and 2,763 Da with MWD(Molecular weight distribution):1.22 (repeating number ranged between 5 and 9). It is considerable that the molecular weights of the PLina samples increase by the exposure time, whereas peroxygen content decreased. Peroxygen contents were found to be between 0.5 wt% and 1.0 wt%.

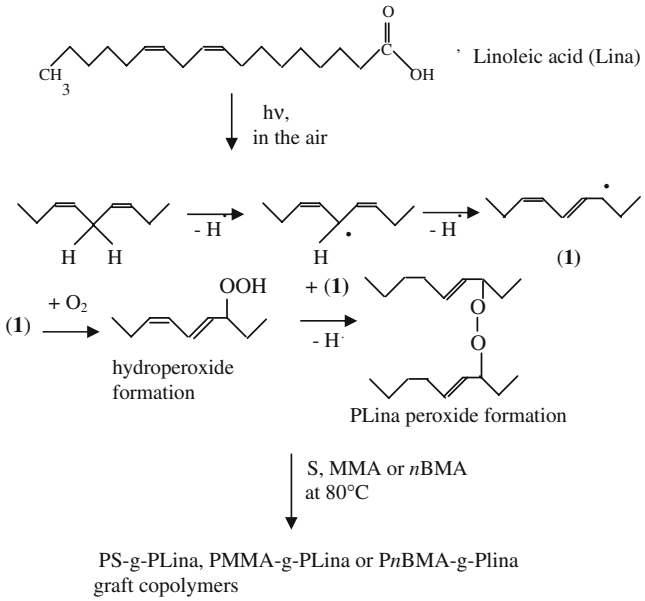
The characteristic hydroperoxide signal at δ_{ppm} = 8.0 was observed in the PLina in its ^{1}H NMR spectrum.

Graft Copolymerization

Because of their peroxide groups, PLina samples initiated the copolymerization of S, MMA, or *n*BMA at 80°C to obtain mostly soluble (ca. 95 wt%) PS-g-PLina, PMMA-g-PLina, and P*n*BMA-g-PLina graft copolymers in high yield. As can be seen in Table 2, a higher concentration of PLina gave higher yields of graft copolymers, although their molecular weights decreased. As PLina concentration in MMA solution increases, propagation and termination compete with each other and, as in the case of the highest PLina concentration (run no. 67-4), termination reactions probably dominate the polymerization reactions and results in lower polymer yield. In addition, inclusion of PLina in copolymer was the same in each run except in sample 67-4. Copolymerization conditions and copolymer analysis results are listed in Table 2. The higher concentration of PLina in monomer solution yielded similar amounts of cross-linked graft copolymer (ca. ~5 wt%), which was isolated by means of chloroform extraction and which was very low when we compare the grafting reactions with PLO and PSB [8, 9].

Soluble fractions of the graft copolymers were fractionally precipitated to determine the γ values of the graft copolymers. Homo-PS, homo-PMMA, and homo-P*n*BMA were precipitated in the γ ranges 0.7–1.0, 3.0–3.8, and 2.8–4.1, respectively, whereas PLina-g-polystyrene (PS), PLina-g-poly-MMA (PMMA), and PLina-g-poly-*n*BMA (P*n*BMA) copolymer fractions were precipitated in the ranges of 0.2–2.0, 2.9–4.8, and 2.4–4.9, respectively. Because γ values of the graft copolymers and related homopolymers were almost superimposed, fractional precipitation was useful only to determine the γ values of PLina-grafted copolymers instead of to isolate pure graft copolymers from the

Scheme 1 Formation of polymeric linoleic acid (PLina) peroxide



related homopolymers. As we will discuss below, unimodal GPC curves can be attributed to the pure graft copolymers freed from the related homopolymers. Homo-PLina in polymerization mixture, a pale yellow liquid, was already eliminated by staying in the solution during the precipitation procedure.

The ^{1}H NMR spectrum of the soluble sample of PLina-g-PS (run no: 68-2 γ: 0.2–2.0) shows characteristic peaks (δ ppm): (–CH_2–) of styrene at 6.6–7.1 and (–CH_2–, –CH_3) of linoleic acid 2.8, 2.3, 2.1, 1.7, 1.6, 0.9, the vinyl protons are detected at 5.4 ppm. When the NMR spectrum of PLina was compared with 68-2 and 68-4, for PLina-g-PS samples, PLina inclusions were found to be in the range 10.6–9.3 mol% by taking the ratio of the signals at 6.6–7.1 and 0.9 ppm, respectively (Table 2).

The ^{1}H NMR spectra of the soluble copolymer samples of PLina-g-PMMA (run no: 67-2, γ: 2.9–4.8) contained characteristic peaks (δ ppm): (–$COOCH_3$) of MMA at 3.6 and (–CH_2–, –CH_3) of linoleic acid 2.8, 2.3, 2.1, 1.7, 1.6, 0.9, the vinyl protons are detected at 5.4 ppm. When the NMR spectrum of PLina was compared with 67-2 and 67-4, for PLina-g-PMMA samples, PLina inclusions were found to be in the range 9.7–19.3 mol% by taking the ratio of the signals at 3.7 and 2.4 ppm, respectively (Table 2).

The ^{1}H-NMR spectra of the soluble copolymer samples of PLina-g-P*n*BMA (run no: 69-2 γ: 2.4–4.9) contained characteristic peaks (δ ppm): (–$COOCH_3$) of *n*BMA at 4.0 (shifted to higher field than that of PMMA) and (–CH_2–) of linoleic acid 2.8, 2.4, 1.9, 1.4, 0.9, the vinylic protons are detected at 5.3 ppm. When the NMR spectrum of PLina was compared with 69-2 and 69-4, for PLina-g-P*n*BMA samples, PLina inclusions were found to be in the range 10.7–8.5 mol% by taking the ratio of the signals at 4.0 and 2.8 ppm, respectively (Table 2).

GPC was used to determine the molecular weights and polydispersity of the copolymers. Fractionated samples of PLina-g-PS, PLina-g-PMMA, and PLina-g-P*n*BMA gave unimodal traces, which can be attributed to the graft copolymer structure without homopolymer impurities. Thermal analysis of the graft copolymers was performed by DSC and TGA. Table 3 lists the T_g), melting transition (T_m), and decomposition (T_d) temperatures. Considerable plasticizer effect of polymeric linoleic acid in the PS graft copolymers has been observed by lowering glass transition to 64°C compared with 100°C for that of homo-PS. In case of PMMA graft copolymers, glass transition lowered to 81°C compared with 110°C for that of homo-PMMA.

Table 3 Thermal analysis results of the graft copolymers and the related homopolymers

Run no.	DSC (°C)			TGA (°C)		
	T_m	T_g	T_d	T_{d1}	T_{d2}	T_{d3}
PLina	29	–	148	170	353	463
PLina-g-PS	–	64	161	170	–	445
PLina-g-PMMA	–	81	170	175	318	404
PLina-g-P*n*BMA	–	16	168	–	327	403

DSC differential scannig calorimetry, *TGA* thermal gravimetric analysis, T_m melting transition temperature, T_g glass transition temperature, T_d decomposition temperature, *PLina* polymeric linoleic acid, *PS* polystyrene, *PMMA* polymethyl methacrylate, *PnBMA* poly-*n*-butyl methacrylate

P*n*BMA graft copolymers have been observed by glass transition at around 16°C, which was the same as that of homo-P*n*BMA. Figure 1 indicates DSC traces of the polymers. At the same time, the graft copolymers obtained indicated decomposition of the peroxide residues in PLina blocks at around 170°C.

TGA traces of the graft copolymers were in the middle of the related homopolymers. When T_d of homo-PS (400°C), homo-PMMA (370°C), and homo-P*n*BMA (320°C) were compared with T_d of graft copolymers, the latter were shifted to higher values. Thermal analysis results of the PLina graft copolymers and the related homopolymers are also listed in Table 3.

SEM Analysis

SEM analysis showed the microstructure of the fractured surface of the graft copolymers obtained. Figure 2 indicates the SEM pictures of the graft copolymer samples of PLina-g-P*n*BMA, PLina-g-PMMA, and PLina-g-PS. Homogeneous structure was observed in PLina-g-P*n*BMA (sample 69-2) and PLina-g-PMMA (sample 67-2) graft copolymers with tiny holes (see Fig. 2a, b). Carboxylic ends of PLina may make the polyacrylates miscible. An interestingheterogenous view was observed in PLina-g-PS sample (68-2) (see Fig. 2c). Cones may come up because of immiscibility of PS and PLina blocks.

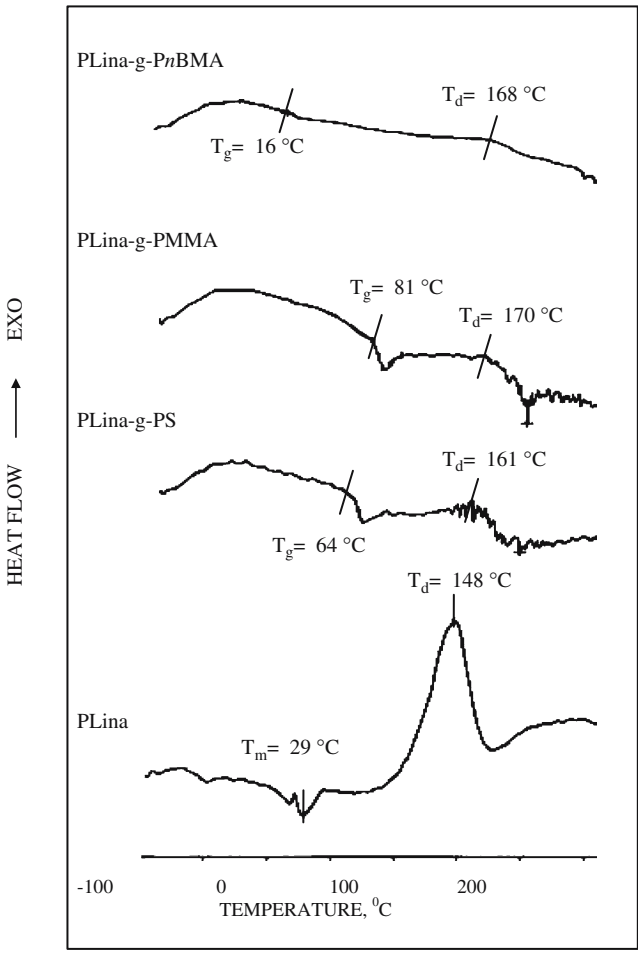


Fig. 1 Differential scanning calorimetry (DSC) traces of polymeric linoleic acid (PLina) (sample no. 65-4), PLina-g-polystyrene (PS) (sample no. 68-2), PLina-g-polymethyl methacrylate (PMMA) (sample no. 67-2), and PLina-g-poly-*n*-butyl methacrylate (P*n*BMA) (sample no. 69-2)

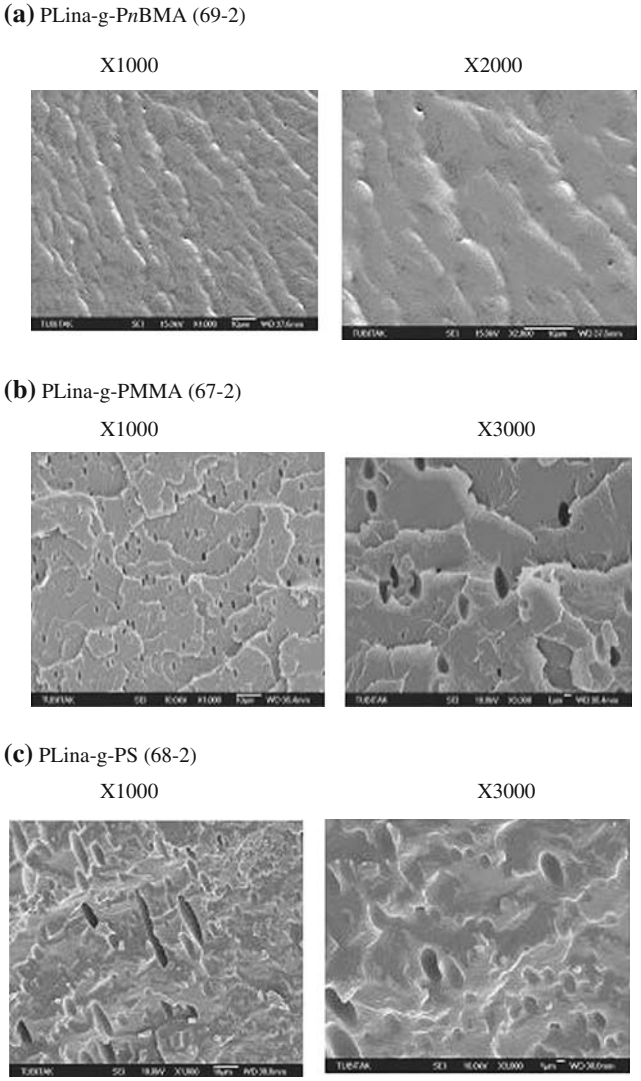


Fig. 2 Scanning electron microscope (SEM) micrographs of graft copolymers: **a** Polymeric linoleic acid (PLina)-g-poly-*n*-butyl methacrylate (P*n*BMA) graft copolymer (sample no. 69-2) (magnifications 1,000× and 2,000×); **b** PLina-g-polymethyl methacrylate (PMMA) graft copolymer (sample no. 67-2) (magnifications 1,000× and 3,000×); **c** PLina-g-polystyrene (PS) (sample no. 68-2) (magnifications 1,000× and 3,000×)

Cell Culture and Adhesion

We chose fibroblast L-929 and macrophage RAW 264.7 cells. Figure 3 shows L-929 and RAW 264.7 cell adhesion and proliferation on homopolymer and graft copolymer film surfaces. PMMA-, P*n*BMA-, PLina-g-PMMA-, and PLina-g-P*n*BMA-coated chamber slides were used for each cell type. There were abundant spherical cells at the beginning in all dishes. Early cell adhesion occurred on the PLina graft copolymers. Figure 3 indicates the cell proliferation on homopolymers and the graft copolymers of PLina. In the first hour, adhesion of L-929 and RAW 264.7 cells on the graft copolymers were clearly higher than that of homopolymers. Adhesion of the cells on the PLina graft copolymers was also higher than PSB and PLO graft copolymers reported recently [8, 9]. After 16 h and 36 h of incubation, the amounts of adhered cells significantly increased in all polymeric surfaces. Additionally, adhesion and elongation of L-929 and RAW 264.7 cells on the PLina graft copolymers were significantly higher than the homopolymers (PS, PMMA, P*n*BMA), PSB, and PLO graft copolymers. These graft copolymers indicate promising materials for use in tissue engineering.

Human Blood Protein Adsorption Test

Human albumin, γ-globulin, and fibrinogen were used as model proteins to study the surface adsorption of polymer. Protein adsorption onto polymer surfaces is important because of its possible involvement at the

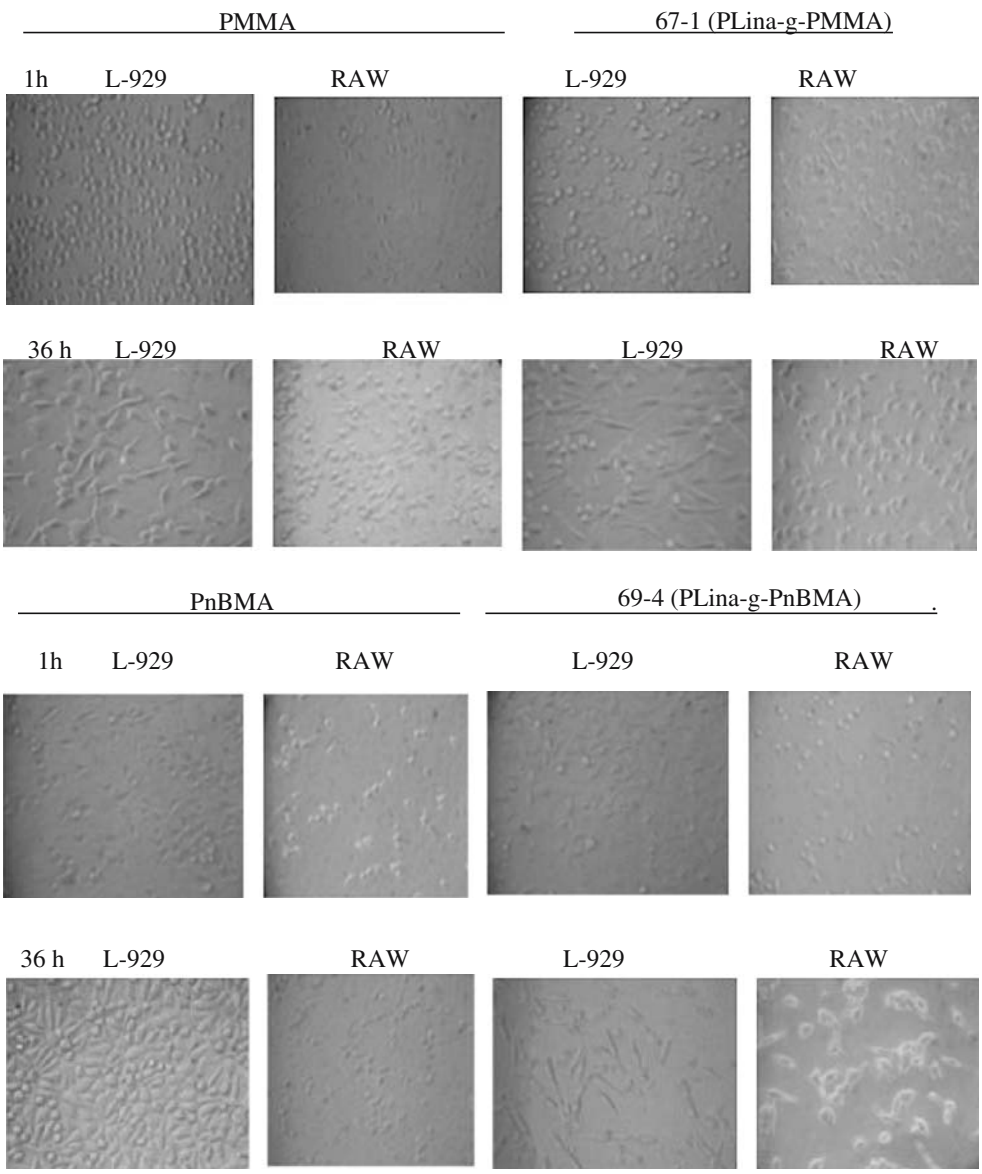


Fig. 3 Photographs of the fibroblast L-929 and macrophage RAW cells growing on the polymethyl methacrylate (PMMA), poly-*n*-butyl methacrylate (P*n*BMA), polymeric linoleic acid (PLina)-g-PMMA (sample no. 67-1), and PLina-g-P*n*BMA (sample no. 69-4) after 1 h and 36 h (magnification: 400×)

initial stage of blood coagulation. UV absorption curves of protein solutions indicated λ_{max} between 260 nm and 280 nm. Albumin is the major constituent of blood plasma (representing about 60% of plasma proteins) and is also one of the smallest proteins in the plasma. This protein is prolate ellipsoid in shape and of size 150 Å × 38 Å × 38 Å. Fibrinogen is an exceptionally elongated molecule, with an axial ratio (major axis to minor axis) of approximately 18:1. On the other hand, γ-globulin has an intermediate size and shape among the model proteins used. Fibrinogen (~340 kDa) is a large protein that is the major substrate of thrombin and the major protein in clot formation. As a fibrinogen molecule is adsorbed on a polymer surface, it undergoes structural, conformational, or orientation changes. Such changes greatly affect the binding capability of fibrinogen molecules to platelets. The surface-bound fibrinogen has an important role in thrombus formation. Therefore, understanding the molecular structures of fibrinogen molecules adsorbed on different surfaces should contribute to the understanding of platelet adhesion on such surfaces and thus the blood compatibility of these materials [11, 14]. Adsorption of the plasma proteins on PMMA and PLina-g-PMMA samples was investigated. Adsorption studies were also carried out on the recently reported PLO-g-PMMA [8] and PSB-g-PMMA [9] for comparison. Protein adsorption onto the graft copolymers was compared with that of homo-PMMA. Table 4 contains the results on the protein adsorption studies. When comparing the protein adsorption on PLO-g-PMMA and PSB-g-PMMA copolymer, adsorbed fibrinogen and γ-globulin amount on the polymer surfaces did not change, but albumin adsorption decreased. We observed a very interesting result when we investigated protein adsorption on the linoleic acid graft copolymers: there was a dramatic decrease in protein adsorption. Whereas PMMA had adsorption of albumin (7.09 μg/cm^2), γ-globulin (3.11 μg/cm^2), and fibrinogen (20.67 μg/cm^2), PLina-g-PMMA graft copolymers did not show any adsorption, except for γ-globulin with 0.71 μg/cm^2. Therefore, linoleic acid copolymers can be promising materials for medical applications where protein adsorption is unwanted.

Table 4 Comparison of protein adsorption on polymethyl methacrylate (PMMA) with its copolymers containing polymeric linseed oil (PLO), polymeric soybean oil (PSB), and polymeric linoleic acid (PLina)

Polymer	Albumin (μg/cm^2)	γ-globulin (μg/cm^2)	Fibrinogen (μg/cm^2)
PMMA	7.09	3.11	20.67
PLO-g-PMMA (39-6)	2.69	2.27	19.39
PSB-g-PMMA (56-5)	4.55	3.07	20.10
PLina-g-PMMA (67-1)	0.00[a]	0.00[a]	0.00[a]
PLina-g-PMMA (67-4)	0.00[a]	0.71	0.00[a]

[a] Ultraviolet (UV) absorbance value was slightly higher than that of original solution

To diversify edible oil polymer composites, some edible oils can be auto-oxidized under ambient conditions via peroxidation and epoxidation of the olefinic groups of mostly linoleic and linolenic acid inclusions. Pure linoleic acid can also be polymerized under sunlight and air oxygen to give peroxidized PLina. This oligomer can initiate free radical polymerization of vinyl monomers under mild conditions without any additional catalyst. Free carboxylic acid functionalities of these graft copolymers can make them very promising biomaterials by means of cell attachment and cell detachment [16]. A metal catalyst can oxidize the olefinic groups in the oils or unsaturated fatty acids; however, the catalyst used may foul the biomaterial and not be able to remove from the polymer. This polymerization system does not need any metal catalyst or any solvent that sometimes may be harmful for the environment. Therefore, this polymerization system can also be considered suitable for green chemistry [17, 18]. Apart from this, peroxidized linoleic acid polymer is a member of polymeric peroxides [19, 20] and therefore can lead to multiblock copolymer [21] synthesis. So, linoleic acid graft copolymers can initiate free radical polymerization of a second vinyl monomer to obtain triblock copolymers. In addition, some unsaturated edible oils and fatty acids can be promising materials for biomedical use. In particular, linoleic acid copolymers can be important for medical applications where protein adsorption is unwanted.

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