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Fabrication and Characterization of PU-g-poly(HEMA) Film for Clotting Time and Platelet Adhesion

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Abstract. This paper describes a fabrication of poly (2-hydroxylethyl methacrylate) poly(HEMA) grafted on polyurethane (PU) film prepared by radiation-induced grafting (RIG) copolymerization method using electron beam irradiation for the first time. This method was well known to be fast technique, clean method without involve any chemical initiator, chemically bond the materials, and at the same time is a sterile technique suitable for further potential of biomedical application. This poly(HEMA) grafted on PU film or called as PU-g-poly(HEMA) films was analysed using Fourier-transform infrared (FTIR), scanning electron microscope (SEM), water contact angle analyser (WCA), platelet adhesion and clotting time measurement. As the results, poly(HEMA) was confirmed successful grafted on PU based on the shifting of the functional group, no significant changes in surface morphology, lowering the water contact angle from 78.28° to 70.02°, nearly no platelet adhesion and no excessive disturbance of the clotting time was observed. This means that PU-g-poly(HEMA) was improved its hydrophilicity, thus significantly reduced the platelet adhesion and maintain the normal range of time taken for blood to clot. Therefore, the present PU-g-poly(HEMA) films not only improved hydrophilicity, however, was also compatible with blood. Thus, it may be potential candidates in the biomedical devices or new biomaterial useful for future tissue engineering fields.

1. Introduction

The arrangement of hard and soft segments in the PU polymer backbones make the PU confer good elasticity and biocompatible, resulting it wide application in biomedical application such as catheter [1], pacemaker insulating lead [2], hemoassess bridge fistulas [3] and vascular prosthesis [4]. The vascular graft bigger than 6 mm in diameter was successful to be use as vascular prosthesis however, smaller diameter less than 6 mm produced intimal hyperplasia and thrombosis [5]. Thus, surface modification need to be done to settle the thrombosis problem.

There are two common methods to prepare the materials either using physical blending or chemical method. Physical blending is the most common techniques where two or more components were mixed

together to form new blend polymers that supposed to improve some characteristics such as thermal stability [6], ionic conductivity, mechanical strength [7] or porous morphology [8]. Modification of polymer using chemical method was also known as graft copolymerization, can be classified into four main categories by using chemical initiator [9], photo initiator [10], plasma treatment [11] and high energy radiation [12]. Therefore, in this study, surface modification via high energy radiation using electron beam irradiation was conducted due to fast technique, clean, sterile the material with the aim to improve the hydrophilicity of materials [13].

Based on previous study, no one has reported yet the preparation of PU films modified it surface with HEMA using RIG of electron beam. In this present study, the complimentary physical characterization of membranes was performed such as percentage degree of grafting, the morphologic observation using scanning electron microscope (SEM) instrument and the interaction and functional group before and after grafted with HEMA was analysed using ATR-FTIR. Concerning of the properties of film either hydrophilic or hydrophobic, the wettability index measurement was also conducted using water contact angle analysis. Our major objective is to demonstrated the prepared modified surface film can lead to formation of hemocompatible film, so that the PU-g-poly(HEMA) could be suitable and appropriate for blood contact devices or other biomedical applications.

2. Methodology

2.1. Materials

Polyurethane (PU) pellets (SelectophoreTM, Mw: 100 kg/mol) and 2-hydroxylethyl metharcylate (HEMA) monomer (99% purity) were obtained from Sigma Aldrich. Tetrahydrofuran (THF) with 99.5% purity and N, N-dimethyl formamide (DMF) with 99.5% purity was purchased from Merck. All the chemicals were used directly without further purification. The vacutainer sodium citrate tubes were brought from BD Company, USA. Meanwhile, APTT reagent (Dade Actin SL) were purchased from Siemen Healthcare, Erlangen, Germany.

2.2. PU Films Fabrication

PU films (10wt%) was produced by mixing the PU pellets in a mixture of THF and DMF with mixing ratios (1:1) and was stirred using digital magnetic stir plate (WiseStir® SMHS, DAIHAN Scientific, Korea) for overnight at room temperature. The PU solution then was pour into glass petri dish and covered with aluminium foils and kept in fume hood at room temperature to slowly evaporate all the solvents. PU film produced was dried in an oven for 72 hours at 60 ºC to remove all residual solvent.

2.3. Surface Modification by Using Radiation-Induced Grafting

PU films produced was cut into 3x3 cm and soaked completely in 10wt% HEMA monomer for overnight in close tightly close bottle (50ml). Then, the PU film was taken out and gently dried with filter paper to remove excess monomer solution. After that, PU thin film was placed inside a thin polypropylene plastics and was sealed under vacuum. The prepared sample was put on the tray that covered with iced and then was radiated using an Electron Beam Processing System (NHV-Nissin High Voltage, EPS 3000, Cockroft Walton type, Japan) at 1 MeV an acceleration voltage, 2 mA beam current and 10 kGy per pass until total radiation dose applied was 50 kGy. After RIG process, the samples were peeled off from the polypropylene plastic and was shaken overnight with distilled water to remove excess monomer and homo-polymers**.** Finally, PU-g-poly(HEMA) film produced was dried in an oven for 72 hours at a temperature of 60°C.

2.4. Characterization of Materials

2.4.1. Percentage Degree of Grafting Analysis. The calculation for percentage degree of grafting (% DG) for modified surface films (PU-g-poly(HEMA) was described in the equation 1 below:

$$\text{Degree ofgrafting} = W_f - \frac{W_i \times 100}{W_i} \quad (1)$$

where, W_i and W_f refer to dry weight of samples before and after RIG copolymerization process.

2.4.2. Scanning Electron Microscope (SEM) Analysis. The morphology of PU film and PU-g-poly(HEMA) film was observed using scanning electron microscope (SEM) instrument (JSM-6701F, JOEL, USA). All films were coated with platinum prior to analysis for 60 second using auto fine coater instrument (JFC-1600, JOEL, USA).

2.4.3. Fourier Transform Infrared Analysis. The interaction and functional group was analysed using Fourier-transform infrared coupled with attenuated total reflectance (FTIR-ATR) (Frontier FTIR, Perkin Elmer, USA). All samples were scanned in ATR mode with a 16 scan, resolution 16 cm^{-1} and wavenumber range from 650 to 4000 cm^{-1}. The force gauge was applied in the range of 100 to 120 N and all samples were put in desiccant for overnight before analysis to reduce absorbed moisture.

2.4.4. Wettability Index Analysis. Wettability index analysis is the analysis to measure the water contact angle which indicate the characteristic of the polymer either hydrophilic or hydrophobic. Wettability index analysis for all samples were analysed using sessile drop techniques (VCA 3000S TM, AST Products Inc, USA.). The samples were put on slide for easy observation. The 100 µl of syringe size was used and 5µl of distilled water was dropped on the dry sample at room temperature. All the samples were measured ten times and average values were calculated. The contact angle value was presented as means value (n=10) with a standard deviation.

2.4.5. Clotting Time Measurement. Clotting time procedure is to measure how quickly blood clot when giving touch with foreign materials. For preparation of platelet poor plasma (PPP), around 2.7 ml of whole human blood was collected into one blood collection tube with 3.2% sodium citrate solution and was centrifuged at 3000 rpm (10 minutes). After centrifuged, the blood was separated and the plasma produced was transfer into new falcon tube which refer to platelet rich plasma (PRP). Then, the PRP was re-centrifuged at 3000 rpm for 10 minutes and PPP produced was transferred into new falcon tube without disturb the platelet pellet. The sample preparation and analysis using semi-automated blood analyser, each samples were cut into 1cm x 1cm and was put into 24 well plates. 100 µL of PPP was incubated at room temperature for 1 hour. The APTT was determined using a semi-automated blood coagulation analyser (Sysmex CA-50, Sysmex Corporation, Japan).

2.4.6. Platelet Adhesion Analysis. For the preparation of platelet rich plasma (PRP), the total of 2.7 ml of whole human blood was collected into the one blood collection tube with 3.2% sodium citrate solution and centrifuge at 3000 rpm for 10 minutes. The plasma obtained was transferred into new falcon tube which is known as PRP [14]. The unmodified and modified surface films were cut into 1 x 1 cm and were rinsed three times with normal saline and equilibrated for 30 minutes at room temperature. Then, the scaffolds were dried in oven at 60 ° C for 3 hours. The dried scaffolds were incubated with 100 µl of PRP in 24 well plates for one hour at room temperature. After that, the films were taken out and smoothly rinsed with phosphate buffer saline (PBS) to removed un-adhered platelet. Platelets attach on the samples were fixed using formalin (10%) for 30 minutes in room temperature. Then, all films were washed with PBS were dehydrated with graded ethanol from 25%, 50% to 70% for 15 minutes each and were dried overnight in oven for 60 ° C. For the morphology observation, all films were sputtered with platinum for 60 seconds before being observed using SEM instruments.

3. Results and Discussion

3.1. Percentage Degree of Grafting

The successful production of PU-g-poly(HEMA) film using RIG method was detected based on the calculation on the % DG. Therefore, modified surface film produced in this research using 10 wt% of HEMA monomer at 50 kGy absorbed dose was in the range of 16.72 - 16.26 % DG.

3.2. Scanning Electron Microscope (SEM) Analysis

The SEM images of PU films and modified surface PU film (PU-g-poly(HEMA)) were displays on Figure 1. The surface morphology images demonstrated that PU film had smooth surface whereas, PU-g-poly(HEMA) film produced showed rougher surface morphology. The same observation was reported by He et al, (2011), rougher surface morphology on modified surface PU films was produced after prepared using chemical-induced grafting method with benzoyl peroxide as initiator [9].

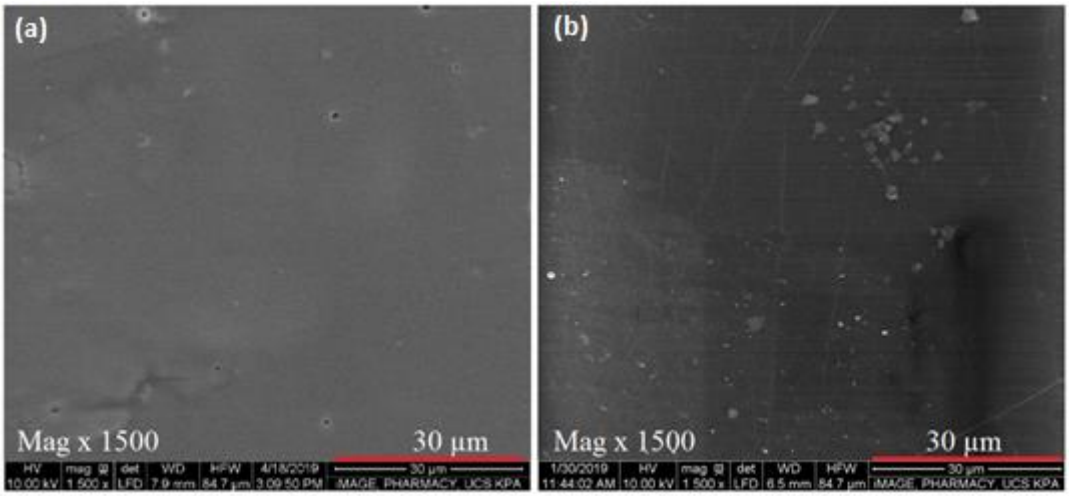

Figure 1. The SEM images shows the surface morphology of (a) PU film and (b) PU-g-poly(HEMA) 16% DG.

3.3. ATR-FTIR analysis

Figure 2 displays the ATR-FTIR images of pristine PU film, pure poly(HEMA) and the modified surface of PU films (PU-g-poly(HEMA) with 16.72% DG). For PU films, the peak at about 3321 cm^{-1} was attributed to the hydrogen bonding interaction of N-H stretching. The vibration band at 2852 cm^{-1} and 2929 cm^{-1} were corresponding to asymmetric and symmetric stretching methylene ($-CH_2$), followed by the peak at 1691 cm^{-1} correspond to the C=O stretching of the ester carbonyl group of urethane. Meanwhile, the peak at 1527 cm^{-1} and 1099 cm^{-1} were assigned to urethane N-H bending and C-O-C stretching in aliphatic the ether of soft and hard segments of PU [15]. The FTIR spectra of pristine poly(HEMA) exhibited a broad peak at 3320-3600 cm^{-1} corresponded to hydroxyl group, peak at 2848cm^{-1} for aliphatic stretching vibration of CH_2, and the sharp characteristic peak of carbonyl group was located at 1709 cm^{-1} [16]. The other two bands at 1154 cm^{-1} and 1071 cm^{-1} were attributed to carboxylic acid ester and primary alcohol –C-OH, respectively. The modified surface polymer exhibited IR peaks which quite similar to pure PU and poly(HEMA). However, the peak at 1691 cm^{-1} which responsible for urethane C=O stretching was shifted to higher wavenumber 1714cm^{-1}. Besides, the intensity of broad peak at 3320 cm^{-1} to 3600 cm^{-1} which refer to the hydroxyl group was found to become broader and increase due to cooperation of HEMA on the PU polymer backbone. This condition indicates that interaction occurred on the polymeric material surface, confirming the corporation of

poly(HEMA) on the PU film surface. As a conclusion, these results provided evidence for imparting a hydrophilic character to PU film was successfully produced using RIG method (Figure 3).

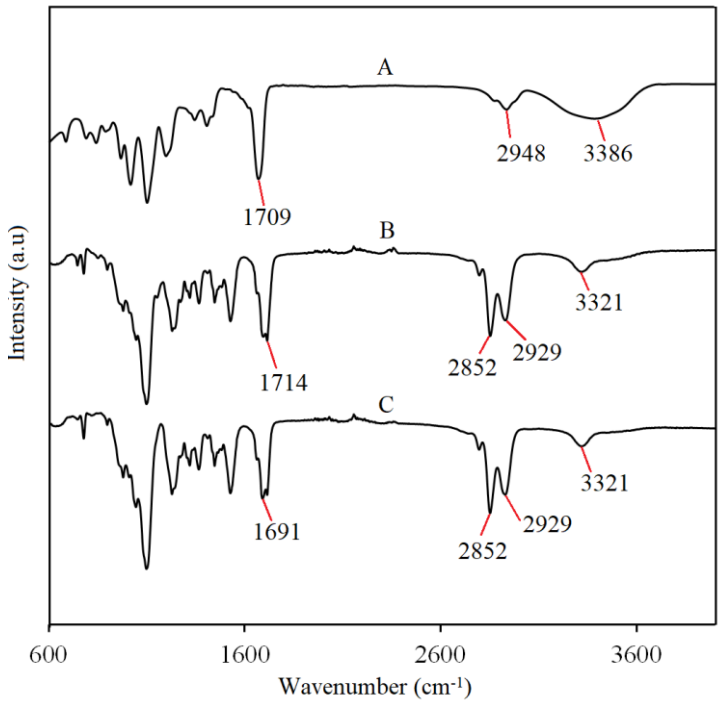

Figure 2. The ATR-FTIR spectra of: (A) PU film, (B) Poly(HEMA) and (C) PU-g-poly(HEMA) film.

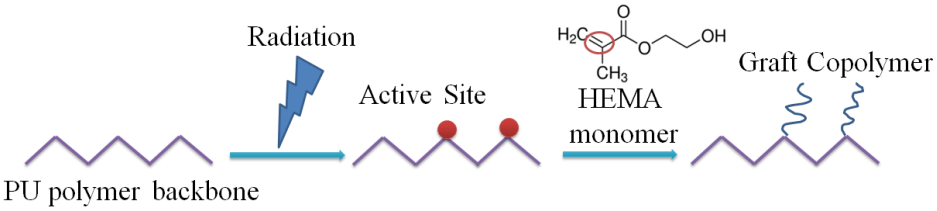

Figure 3. The production method of poly(HEMA) grafted on PU surface by using RIG copolymerization.

3.4. Wettability Index Analysis

Image of water contact angle produced using water contact angle analysis was shown in Figure 4. Smooth surface of PU film was prepared using solvent casting method was found to be 78.28° ± 0.23 and reduced to 70.02° ± 1.50 after grafted with poly(HEMA). This indicate that wettability values were significantly reduced and the hydrophilicity was risen after grafted with poly(HEMA). These findings were presented close value to previous study which is 79°, 89° and 90° for PU film and 42°,57° and 59° for poly(HEMA) grafted on surface films) [17], [9], [18]. Thus, it worth to mentioned that the hydrophilicity of PU scaffold improved after grafting with HEMA. In addition, the even surface of the materials attributed to hydrophilic nature of polymer. The reduced in wettability values was not conducive to rapid wettability of materials likely super-hydrophilic surface. However, with extended time soaked in the solution media, the material was completely wetted by liquid.

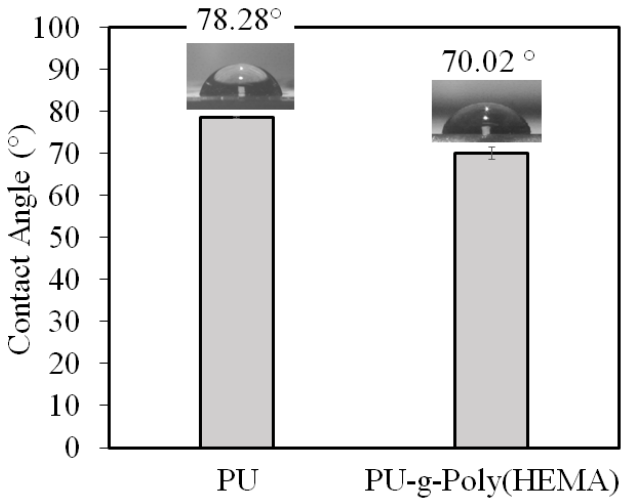

Figure 4. The water contact angle value for PU film before and after RIG surface modification.

3.5. Clotting Time Analysis

APTT mainly conducted to evaluate the effect of factors XII, XI, IX and VIII in the intrinsic pathway. PPP without scaffold was used as control and the graft for APTT results were presented in Figure 5. Based on the graft, the PU films make delay on the clotting time compared to control from 33.8 ± 0.35 to 34.1 ± 1.65 seconds and modified surface PU-g-poly(HEMA) 16.72 % DG was also increases the time taken for blood to clot from 33.8 ± 0.35 to 36.5 ± 1.1 This changes give little effect on coagulation factor where the clotting time for APTT value for normal range was in range of 30 to 40 minutes. The same trend was observed in the previous reported articles, showed that clotting time was risen when treated with bioflocculant (MBS-60) however still in normal range of APTT clotting time [19]. The result from An and co-researchers showed prolonged the clotting time with increasing degree of grafting however 30 min incubation time was conducted [20]. Longer incubation time at 37 °C will cause the loss of factor V and VIII and incubation time more than 10 minutes was not recommended. As a conclusion, the APTT value gathered was still lies in the normal range of APTT, therefore it showed that PU film and PU-g-poly(HEMA) film was compatible with blood.

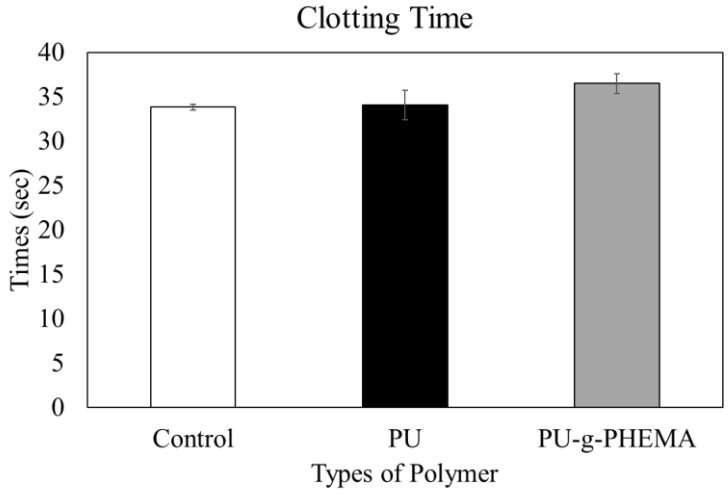

Figure 5. The APTT test for PU film before and after RIG surface modification. Control is the PPP without scaffolds.

3.6. Platelet Adhesion Analysis

Figure 6 display the SEM image for PU film, and its modified surface (PU-g-poly(HEMA) film. Unmodified surface of PU film showed abundant platelet attachment compared to the modified surface which means that PU-g-poly(HEMA) achieved highly significant reduction in platelet adhesion. These phenomena were probably due to charge repulsion since modified film contained hydrophilic surface and platelet surface are negatively charged. Thus, the platelet adhesion can be restricted by electrostatic repulsion effect between platelet and negatively charge functional groups [21]. The result reported here was in agreement with other previous published paper [22] [23]. Thus, this test demonstrated that the extent of modification was sufficient to significantly reduce platelet adhesion. A decrease platelet adhesion also could reduce not only coagulation processes, but also the possibility of early restenosis.

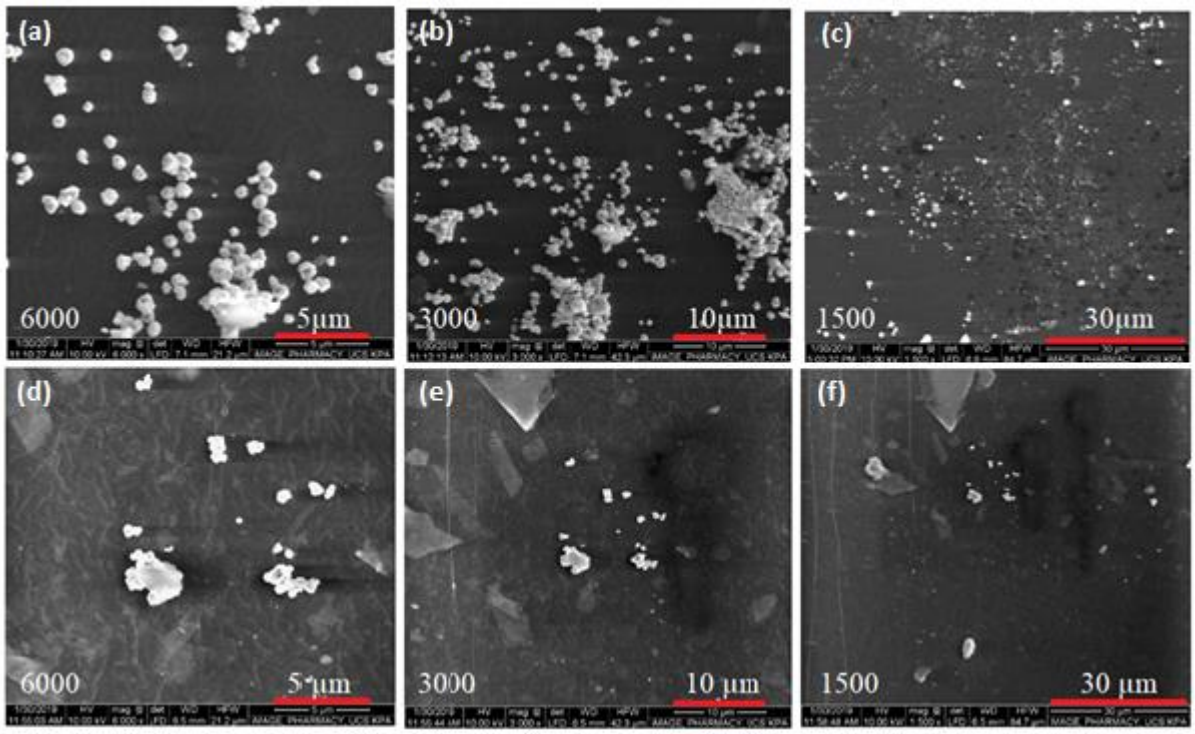

Figure 6. The SEM images of platelet adhered on pure PU film with different magnification (a-c), and on PU-g-poly(HEMA) 16.72% DG film with different magnification (d-f).

4. Conclusion

As a conclusion, the wettability index of PU-g-poly(HEMA) film was reduced from 78.28° to 70.02° revealed that the hydrophilicity of the modified film was successfully improved. Some change on the morphology and ATR-FTIR spectrum supports along the results. The clotting time analysis and platelet adhesion test indicates the film was compatible with blood. Thus, it can be concluded that PU-g-poly(HEMA) film with 16.72 % DG was successfully prepared using RIG method via electron beam radiation and was confirmed improved it hydrophilicity. Therefore, the obtained film was expected have a potential to be used as new material for tissue engineering or off-the-shelf blood contact devices.

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